Night fish avoidance of *Microcystis* bloom revealed by simultaneous hydroacoustic measurements of both organisms

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Abstract

Simultaneous observations of fish and cyanobacteria were conducted in the shallow Sulejów Reservoir (Poland) during the occurrence of *Microcystis* bloom. A Simrad EY60 split beam echosounder with a 200 kHz transducer beaming horizontally was applied to assess fish and cyanobacteria spatial distribution. Additionally, fish size distribution and species composition were evaluated with gillnets, and cyanobacterial biomass was determined by using an online phycocyanin fluorescence probe. Physico-chemical parameters and water samples for biological analyses were collected at 14 fixed stations situated along the acoustic transects. We found cyanobacteria represented by the genus *Microcystis*, with their toxigenic genotypes in all analyzed samples. The hydroacoustic results provided direct evidence for fish night avoidance of the bloom. The biomass of fish and cyanobacteria demonstrated opposing trends and their peak values spatially mismatched. The number of fish caught in gillnets within the bloom area was about half that caught outside the bloom area. In spite of the presence of

intracellular microcystins (hepatotoxin) at all stations, no extracellular microcystins were identified in water samples and in fish tissues.

Key words: hydroacoustics; fish spatial distribution; microcystins; toxic bloom; eutrophic reservoir

1. Introduction

Deterioration of water quality is a world-wide problem. In the past decades, increasing eutrophication has led to frequent outbreaks of cyanobacterial blooms in many lakes around the world (Anderson et al., 2002; Briand et al., 2003; Chorus, 2005; Gkelis et al., 2006; Kobus et al., 2013; Meriluoto et al., 2017; Tarczyńska et al., 2001). In addition, recent climate change observations and scenarios suggest that the probability of the occurrence of cyanobacterial blooms will be even higher in the near future (Jöhnk et al., 2008; Paerl and Huisman, 2008; Wagner and Adrian, 2009). Cyanobacterial dominance in water systems can have serious economic and societal consequences, as it limits the range and value of important ecosystem services of inland waters, including recreational use, aquaculture and drinking water usage (Carmichael, 1992; Huisman et al., 2005; Ibelings et al., 2014; Paerl and Huisman, 2009; Paerl et al., 2001). *Microcystis*, one of the major components of cyanobacterial blooms, produces metabolites such as microcystins (MCs). These can be toxic to many aquatic organisms, including zooplankton and fish (Babica et al., 2007; Hansson et al., 2007; Sotton et al., 2014, 2012a, 2012b; Sun et al., 2012, 2011; Tellenbach et al., 2016; Trinchet et al., 2013). MCs are mainly retained within producer-cells during cyanobacterial bloom development and might be released into water after lysis of cyanobacterial cells during bloom collapse. The released toxins can then affect a wide range of aquatic organisms and have deleterious effects on them, including accumulation in animal tissues (Papadimitriou et al., 2009; Sierosławska et al., 2012; Sotton et al., 2012a, 2012b; Xie et al., 2007b, 2005).

Cyanobacteria have gas vesicles responsible for the adjustment of cell position in the water column to get optimal position for photosynthesis and growth. Usually the shape of the gas vesicles is spherical, but *Microcystis aeruginosa* have a form of a hollow cylindrical tube with a diameter of 60–70 nm and maximum length of ca. 600 nm (Dunton and Walsby, 2005). Additionally, *M. aeruginosa* forms large colonies ranging from 100 μm to even 2 cm in size (Kaczkowski et al., 2017). These properties make cyanobacteria effective sound scatterers. Usually, signals from cyanobacteria recorded by an echosounder are treated as unwanted noise and removed during analysis of hydroacoustic survey data. We did, however, measure fish and cyanobacteria spatial distribution simultaneously, which enabled us to observe them with the same resolution in space and time.

In spite of the extensive literature concerning the negative effects of cyanobacteria on fish, there are only a few studies related to natural ecosystems, which deal with the spatial distribution of fish during toxic cyanobacterial bloom events (Ernst, 2008; Godlewska et al., 2016; Kaczkowski et al., 2017; Potter et al., 1983; Sotton et al., 2011; Wojtalik et al., 2006). In all published studies so far, fish spatial distribution has been measured continuously with an echosounder, while cyanobacteria have been measured at fixed locations along the transects. The distributions of both fish and bloom are highly variable and change continuously in time and space (Izydorczyk et al., 2005), hence, non-point rapid measurement methods are required to enable a deeper understanding of the complex relationships between them. However, to our knowledge, no research presenting simultaneous and continuous spatial measurement of cyanobacteria and fish occurrence with an echosounder has yet been published.

The study is a part of the project “Do fish adapt to cyanobacterial blooms” financed by the Polish National Science Centre (http://www.erce.unesco.lodz.pl/story/national-projects), and this fraction aims at an assessment of fish spatial distribution in the Sulejów Reservoir in

relation to cyanobacterial bloom and its toxicity. We hypothesize that fish avoid those areas covered by cyanobacterial blooms, and in this way decrease their exposure to toxic MCs, and avoid or substantially limit their negative impact.

2. Materials and methods

2.1. Study area

Measurements were performed in the Sulejów Reservoir (51°22′-51° 28′ N, 19°51′-20°01′ E), central Poland, on two consecutive nights (September 2 and 3, 2015) during the occurrence of a cyanobacterial bloom. The Sulejów Reservoir is a typical lowland shallow reservoir, with an area of approximately 2,700 ha and an average depth of 3.3 m, in which cyanobacterial blooms are observed regularly at the end of the vegetation season (Izydorczyk et al., 2008a, 2008b; Tarczyńska et al., 2001). The dominant species of bloom-forming cyanobacteria is usually *M. aeruginosa*, which produces the microcystins MC-LR, MC-YR and MC-RR (Gągała et al., 2013; Jurczak et al., 2004; Kaczkowski et al., 2017; Mankiewicz-Boczek, 2006). The dominant fish species of the reservoir assemblage include roach (*Rutilus rutilus*), bream (*Abramis brama*), ruffe (*Gymnocephalus cernua*), white bream (*Blicca bjoerkna*), perch (*Perca fluviatilis*), pikeperch (*Sander lucioperca*) and pike (*Esox lucius*). The average sizes of the most common cyprinids are 20–30 cm total length, but larger specimens of bream and piscivorous fish, up to more than 50 cm, are quite common (Frankiewicz and Świerzowski, 2004; Kaczkowski et al., 2017).

2.2 Hydroacoustic measurements

Hydroacoustic measurements were performed from a boat sailing at a constant speed of approximately 1.5 m s^{-1} along 10 pre-determined parallel transects (Tr1–Tr10, Fig. 1). The transects were separated by a distance of approximately 500 m. Data were collected on September 2 and 3, 2015, starting one hour after sunset, when fish are dispersed in the open

water, and were completed at least one hour before sunrise. A Simrad EY60, 200 kHz split beam echosounder, equipped with an ES200-7x7 (opening at -3 dB) composite transducer beaming horizontally was used to record the data. The transducer was mounted to the side of the boat at a depth of 0.5 m, tilted down by 3.5° and panning 90° to the boat's along-ship axis. The pulse duration was 0.128 ms, and the repetition rate was 10 pings per second. The echosounder was calibrated beaming vertically in the deepest part of the lake at the beginning of the study, following the standard calibration procedure (Foote et al., 1987). Data were stored on a computer and later processed by the Sonar5-Pro (S5) software (Balk and Lindem, 2014).

Fish and cyanobacteria were separated using the Cross-Filter Detector (CFD) module in S5, set up for target-noise separation. The CFD method and module were originally developed to overcome tracking problems in noisy environments, where missing single echo detections from fish and false detections from noise tend to fool ordinary trackers. The CFD uses filters to obtain an adaptive threshold of the echogram. The regions found by the adaptive thresholder are tested with respect to features such as echo-length and track-length to ensure that only single fish tracks are detected. A classical single echo detector is applied to the detected tracks to obtain correct target strength, range and beam position.

When fish targets have been detected, these detections can either be removed from the echogram as noise or kept while removing everything else. We could have set up the CFD to directly do the fish tracking and cyanobacteria estimation on the original echograms, but in order to verify the detection process we let the CFD produce a set of echogram-files with fish-tracks and another set of echogram-files with cyanobacteria and with the detected fish-tracks removed. Studying these two different echogram-sets before carrying out the final processing provided us with a good way of verifying that the CFD had separated fish and cyanobacteria well.

The same CFD detector parameters were applied to produce the two echogram-file sets, except for a growing operation applied for the production of the cyanobacteria echograms. The growing operator has a similar function as the margin has for the bottom detection. It reduces the risk the cyanobacteria data being infested by higher intensity fish echoes. For detecting the fish echogram set, the CFD's adaptive thresholder was set up with foreground filter [1, 3], background filter [55, 1], and offset=7dB. The notation [x, y] indicates the number of samples in the range domain followed by the number of samples in the ping domain. The CFD's evaluator was set up to remove targets smaller than 5 samples and longer than 125 samples in the ping domain. No other features appeared to be needed for correct detection. TS for individual echoes in detected fish tracks were found using the CFD's built in classical single echo detector setup with Echo length=[0.7.. 1.3], Multiple peak suppression=medium, Max gain comp=3 (one way), and Max Phase dev=0.8. Threshold for the SED was set to -45 dB in order to match the applied -51dB echogram threshold with respect to the split beams off-axis compensation (6 dB for 2-ways).

For detecting the cyanobacteria echogram-file set, the CFD was set up in the same way except for an additional growing operator applied after the track length evaluator. The operator was set to add 5 samples before and after the track detections in the ping domain and to add 3 samples above and below in the range domain. The regions covered by these grown detections of fish were then removed from the echograms resulting in cyanobacteria echograms without fish tracks.

The final analysis was then performed on the two echogram sets using the same positioned analysis cells and the same threshold (-51dB). The applied threshold models where 40LogR for the fish-echograms and 20logR for the cyanobacteria echograms.

152 The areal sound backscattering coefficient Sa (m^2ha^{-1}) (MacLennan et al., 2002) was
153 considered as a proxy for fish and cyanobacterial biomass (Simmonds and MacLennan,
154 2005).

155 Maps of fish and cyanobacteria spatial distributions were based on an Elementary Sampling
156 Distance Unit (ESDU) of 100 m and were produced using the kriging interpolation method in
157 Surfer 8 software. The t-test for unequal sample sizes was used for comparison of the acoustic
158 measurements, with cyanobacterial biomass as the discriminating factor.

159 2.3 Fish gillnet catches

160 Fish sampling was performed with multi-mesh gillnets during the night on September 2,
161 2015. Gillnets were used only as an additional tool to obtain information on species
162 composition and size distribution. The gillnet effort had to be limited to adopt our fishing
163 effort to the annual gillnet yield level used by the fishery manager at the reservoir, i.e. 500 kg
164 per year. One set of the gillnets was 77 m long and 3 m deep with 11 different mesh-sizes,
165 ranging from 11 mm to 80 mm knot to knot. Fishing gear was described in detail in
166 Kaczkowski et al. (2017). Based on information on historical cyanobacterial bloom
167 distributions in the Sulejów Reservoir and daytime visual screening, one set of nets was
168 located in the area of Tr2 (downstream part of the reservoir), and a second one was located in
169 the area of Tr9 (upstream part of the reservoir) (Fig. 1). Each gillnet was set perpendicular to
170 the shoreline and was exposed at the same time as the hydroacoustic survey, i.e. from 9 PM to
171 12 PM (sunrise was at 6:40 AM and sunset at 6:46 PM local time). There was a 45-minute
172 difference between the setting and collecting of the two gillnets, i.e. the gillnet that was set
173 first was also collected first to ensure equal fishing effort. After sampling, catch data were
174 recorded, including the determination of the taxonomic classification of fish, and the total
175 length and weight for each specimen. None of the activities in this study involved endangered

or protected species. Sampling *via* gillnetting was performed in accordance with the Inland Fishery Act with the permission of the Lodz Voivodeship Marshal (No. RŚI.7143.3.2015.PP).

2.4 Phycocyanin fluorescence

The measurements of phycocyanin fluorescence were synchronized in time with the hydroacoustic measurements. They were recorded *in situ* applying a Turner 10-AU-005 fluorometer using the Phycocyanin Optical Kit (P/N: 10–305). The fluorometer was operated in continuous flow mode, pumping the water from a depth of about 0.5 m, and letting it flow through a 25 mm cell. During the flow, measurements were taken at 2 s intervals and recorded by a data logger. To calibrate the measurements for the phycocyanin fluorescence and cyanobacterial biomass, subsamples of surface water were collected from the pump during the continuous fluorescence measurements (for details, see Godlewska et al., 2016). Based on all records from 2 September, a regression was produced between cyanobacterial biomass assessed by fluorometer (in mgL^{-1}) and cyanobacterial biomass assessed by an echosounder (in $m^2\ ha^{-1}$). The relationship was used to express hydroacoustic data in the same units as those from the fluorometer i.e. mgL^{-1}.

The cyanobacteria biomass derived from the fluorescence measurements and acoustic survey was compared with the t-test analysis.

2.5 Physico-chemical parameters of water

On September 3, 2015 physico-chemical parameters at 14 stations situated along the acoustic transects were measured simultaneously with the hydroacoustic survey, i.e. during night time (Table 2), including temperature and oxygen profiles. The temperature profile was applied as input to the S5 ray tracer to verify how the acoustic beam behaved and determine if it covered the whole water column.

At the same time, integrated water samples were collected using a 5-liter sampler from each meter of the entire water column. These samples were used for biological analyses: microscopic identification of cyanobacteria, chlorophyll *a* and cyanobacterial chlorophyll *a* concentration, the number of 16S rRNA and the *mcy*A gene copies, and microcystins concentrations.

2.6 Genetic assessment of *Microcystis*

DNA assays were prepared according to Mankiewicz-Boczek et al. (2006). Always 100 mL of water was used for filtering. Next, filter with cyanobacterial material (0.45 μm, Millipore, USA) were put into lysis buffer (40 mM EDTA, 400 mMNaCl, 0.75 M sucrose, and 50 mM TRIS-HCl, pH 8.3) and kept at −20°C until further analysis. Genetic material was extracted from the filter according to Giovannoni et al. (1990) with some modifications: for the centrifugation, a speed of 13,000 × *g* was used; for the enzymatic lysis step, a final concentration of proteinase K of 275 μg mL^{-1} was used; and during the phenol/chloroform step, a volume of chloroform/isoamyl alcohol (24:1) equal to the volume of supernatant was applied.

Extracted DNA was used as the template for quantitative (qRT-PCR, quantitative real-time PCR) determination of: 16S rRNA (250 bp) gene fragment specific for the genus *Microcystis* and *mcy*A (395 bp) gene fragment specific for toxigenic *Microcystis* genotypes. The analysis of qRT-PCR was made by using Maxima™ SYBR Green/ROX qPCR Master Mix (MBI Fermentas) and a real-time PCR system (7900HT, Applied Biosystems).

The qRT-PCR analyses for 16S rRNA and *mcy*A were prepared according to Gągała et al. (2010). In order to prepare the calibration standards for 16S rRNA/*mcy*A, the genomic DNA of *M. aeruginosa* strain PCC7820 was used as a template for PCR amplification of reference sequences.

2.7 Microcystins in cyanobacteria, water and fish tissue

Water samples for determination of microcystin concentrations were collected from 14 stations in a volume of 1 L and were filtered immediately after sampling with GF/C Whatmann filters (0.45 µm) for separation of cyanobacteria from water. Extracellular microcystins were extracted by solid phase extraction according to the method described by Kaczkowski et al., (2017). Filters with cyanobacterial wet biomass were sonicated (sonicator model XL 2020, Misonix Inc. USA) in 75% of aqueous methanol for extraction of microcystins into solvent.

In turn, biological material (organs isolated separately from *A. brama* and *R. rutilus*) collected from the Sulejów Reservoir was frozen and then lyophilized using an Eppendorf lyophilizer. A total of 1–2 g of lyophilized material (livers and kidneys) was extracted according to a modified method described by Xie and Park (2007a), using 75% aqueous methanol and a sonication process for extraction MCs from tissue. Crude extracts were filtered using GHP Acrodisc minispike filters and these were then analyzed using the HPLC method with diode array detection at 238 nm according to Jurczak et al. (2005).

3. Results

3.1 Hydroacoustic survey

The area occupied by cyanobacterial bloom was limited in space and had a clear boundary between bloom-occupied and bloom-free areas. Hydroacoustic transects passed several times through the border of the cyanobacterial bloom. An example of a strong bloom edge is presented in Fig. 2. The echogram shows, in a spectacular way, great numbers of fish crowded just at the bloom edge, while very few fish were present within the bloom. Detailed investigation of transects passing through the bloom border with the selected ESDU resolution of 100 m indicated clearly that cyanobacterial and fish biomass adopted opposite

248 trends in their spatial distribution (Fig. 3). The most dramatic slope was observed at the edge
249 of the bloom. The abundance of fish was the highest just next to the border and decreased
250 further with increasing distance from the edge of the bloom. A map of fish spatial distribution
251 (Fig.4) shows maximum fish concentrations in the middle part of the study area where bloom
252 was present. Indeed, in two out of three transects with high concentrations of cyanobacteria,
253 peak values of cyanobacteria and fish were observed along the same transects (7 and 8).
254 However, analyses at a scale of 100 m showed that these maxima mismatched in space.
255 Analyses of fish areal abundance, fish biomass (expressed as a sound scattering coefficient,
256 Sa in m^2ha^{-1}, which is often used as a proxy for fish biomass), average fish size TS in dB, and
257 the number of single echo detections (SED) along the gradient of cyanobacterial biomass
258 show that all these parameters change their characteristics at the same border line of about 7
259 mgL^{-1} of cyanobacteria biomass (Fig. 5a,b,c,d). Therefore, based on Fig. 5 we assumed that 7
260 mgL^{-1} was the threshold in cyanobacterial biomass causing fish avoidance in the Sulejów
261 Reservoir in September 2015. Statistical analyses confirmed that all fish parameters, i.e. fish
262 abundance, fish biomass, number of SED and average fish size differed significantly above
263 and below 7 $mgL^{-1}$of cyanobacterial biomass (Table 1).

264 3.2 Gillnet catches

265 Based on cyanobacteria distribution (Fig. 4), Tr2 in our study was in the area without bloom,
266 while Tr9 was inside the bloom (at its periphery). The total number of fish caught at Tr2 was
267 more than twice as high as at Tr9 (Fig. 6). Near Tr2, we captured 8 species with an average
268 length and weight of 21.2 ± 8.5 cm, 174 ± 197 g. Near Tr9, 5 species were captured, with an
269 average size of 24.9 ± 9.7 cm, 292 ± 338 g.

270 At both sites, one species made up over 60% of the catch. *B. bjoerkna* was the dominant fish
271 species at the downstream site (Tr2), while it was *R. rutilus* at the upstream gillnetting site
272 (Tr9). Both species were also the second most abundant taxa, *B. bjoerkna* and *R. rutilus* near

Tr9 and Tr2, respectively, and formed equally 20% of the catch (Table 2). Other fish species observed at both sites were *P. fluviatilis* and *G. cernua*. *A. brama, A. alburnus* and *S. lucioperca* were only near Tr2, and Wels catfish *S. glanis* only near Tr9.

3.3 Cyanobacteria distribution

There was a strong correlation between cyanobacterial biomass recorded by an echosounder and by fluorometer, with the following regression line:

cyanobacterial biomass (mgL^{-1})=0.69 x cyanobacterial biomass (m^2ha^{-1}) - 6.25, R^2=0.895.

Maps of the cyanobacterial biomass distribution, based on phycocyanin fluorescence measurements and hydroacoustics received on September 2, 2015 (the day with most favorable measurement conditions), are presented in Fig. 4 along with the fish spatial distribution. During measurements, the cyanobacterial bloom was concentrated in the central part of the study area between Tr5 and Tr7, and the highest cyanobacterial biomass reached 33.8 mgL^{-1}.

Comparison of cyanobacterial biomass results based on phycocyanin fluorescence (mean value = 4.08 mgL^{-1}, SD = 3.39) and acoustics (mean value = 4.10 mgL^{-1}, SD =3.19) shows no statistical differentiation ($t = 0.97$, $df = 207$, $p > 0.05$).

3.4 Physico-chemical parameters

Physico-chemical parameters for 14 sampling stations are summarized in Table 3. Water temperature was about 20°C and this was stable both between the sampling stations and with depth (max difference between surface and the bottom was 1.7°C). Thus, the water had a fairly homogenous temperature profile, which did not cause any significant refraction and misshaping of the acoustic beam. Also, pH was practically constant, with an average value equal to 8.57±0.18. The whole water column was characterized by relatively good oxygen

conditions. Only at 4 stations did the oxygen concentration drop below 4 mgL^{-1} at the very bottom. At all other stations and depth levels, concentrations were close to or above 6 mgL^{-1}.

3.5 Toxigenic *Microcystis*

Cyanobacteria of the genus *Microcystis* occurred at all 14 stations (Fig. 7). The greatest number of copies of 16S rRNA (1.94×10^5) were observed at station7 (Tr5). The average amount of *Microcystis* (calculated from 14 stations) was equal to 1.09×10^5 gene copy number. In turn, the highest amount of toxigenic genotypes of *Microcystis* (8.49×10^4gene copy number based on *mcy*A) was observed at station 11 (Tr8). The average amount of toxigenic genotypes was equal to 2.8×10^4. An increase was observed from the dam to the center of the reservoir in terms of the amount of toxigenic genotypes (*mcyA* gene) with the potential for microcystins production, relative to the whole population of *Microcystis* (16S rRNA gene) (Fig. 7).

3.6 Toxins in cyanobacteria, water and fish tissue

At all analyzed stations three microcystins (MC-RR, MC-YR and MC-LR) were identified in cyanobacterial biomass, but only intracellular variants. The average concentration of total microcystins was 1.71 μgL^{-1}, with a maximum of 4.34 μgL^{-1}. The highest concentrations of MCs were found at stations 10, 11, 12, 13, and 14 (Fig. 8). No extracellular MCs were found in any water or fish-tissue samples.

4. Discussion

The present study provides the results of *in situ* investigations of the relationship between spatial distributions of fish and cyanobacterial bloom dominated by *Microcystis* in a lowland eutrophic reservoir. Only few studies exist on fish distribution during phytoplankton bloom events in natural environments. As early as in the1980s evidence was presented, which indicated that dense blooms of blue-green algae *Nodularia spumigena* had affected fish and

crab populations in a large estuarine system of south western Australia (Potter et al., 1983). The numbers of fish were very low at sites where chlorophyll *a* levels were>100 μgL^{-1}, while more active species moved into regions where *N. spumigena* was virtually absent. Two of the field studies (Ernst, 2008; Sotton et al., 2011) were related to peri-alpine oligotrophic lakes, where coregonids are among the dominant species of the ichthyofauna with a dominance of whitefish (*Coregonus lavaretus*) and simultaneous presence of *Planktothrix rubescens*, which belongs to the most ubiquitous cyanobacterial species. Their results, however, were contradictory. Thus, Sotton detected a spatial match between *P .rubescens* and whitefish in the mesotrophic Lake Bourget (France), while Ernst observed a spatial mismatch between the two taxa in the oligotrophic Lake Ammersee (Germany).

Thus, none of the previous papers referred to fish behavior relative to *Microcystis* bloom. Our paper (Godlewska et al., 2016), which studied such relationships in the Sulejów Reservoir in 2013, did not provide direct evidence for fish avoidance. However, in 2013 the *Microcystis* bloom was observed only in the downstream part of the reservoir, and moreover, it was distributed in parallel to acoustic transects, so that the boat never passed the border of the bloom.

The results of the present study thus provide the first published direct evidence for night fish avoidance of *Microcystis* bloom. The horizontal recordings from the boat passing in and out of the bloom clearly showed aggregation of fish at the edge outside the bloom. The fish abundance was low inside the bloom, it increased dramatically after crossing the border of the bloom and it declined again with increasing distance away from the bloom edge. There may be different reasons for the higher concentration just outside the bloom. Either the aggregations of fish had not yet had time to diffuse further into the non-infested waters or the fish found some attraction near the bloom and thereby preferred to stay there. Of course, avoidance is only possible if the bloom does not cover the entire water body and fish are able

345 to detect “the edge”. The fact that fish were crowded just outside the bloom border and then
346 slowly dispersed suggests that the factor causing fish avoidance was present only within the
347 bloom and disappeared as soon as fish passed the border. The present paper does not provide
348 the answer to the question of what exactly the factor is that causes fish avoidance. Avoidance
349 behavior could be due to the clogging of the gills (Engström-Őst et al., 2006) or lowered
350 vision caused by turbidity (Gray et al., 2014; Utne-Palm, 2002). The latter is difficult to
351 resolve in our case because of the darkness during the data collection. Mayr (2002) also
352 reported spatial mismatch between whitefish abundance and turbidity, caused by high flows
353 of the main tributary. Generally, turbidity may have many detrimental effects on fish,
354 including problems with feeding success (Mayr, 2002), social behavior (Borner et al., 2015),
355 etc. Cyanobacteria-induced turbidity may also interfere with foraging (Keshavanath et al.,
356 1994), predator avoidance (Lehtiniemi et al., 2005), and refuge use of fish (Maes et al., 1998).
357 Recently, Vejřìk et al. (2016) found that a high abundance of YOY perch was associated with
358 low oxygen concentrations, suggesting that juvenile perch are using the hypoxic layer as a
359 refuge from large predators. We do not know if this hypoxia was associated with *Microcystis*
360 bloom, which often occurs in the studied lake. Nevertheless, juvenile perch may survive in
361 oxygen concentrations of ca. 1 mgL^{-1} (Suthers and Gee, 1986), while for example adult perch,
362 one of the main predators of juvenile perch, avoid oxygen concentrations below 6.7 mgL^{-1}
363 (Alabaster and Robertson, 1961). Therefore, the use of the hypoxic pelagic zone as a refuge
364 by YOY fish is highly probable. Engström-Őst et al. (2006) have shown in a laboratory
365 experiment that cyanobacteria bloom may function as an important refuge for small fish
366 during predation pressure in pelagic algal blooms. According to our acoustic results, average
367 size of fish within a bloom was statistically significantly smaller than that outside of the
368 bloom, which supports the above literature findings. However, the gillnet catches have
369 brought about the opposite pattern resulting with mean TL = 24.9 cm and 21.2 cm in high and

low cyanobacteria densities, respectively (Fig. 7). One of the possible explanations is that the gillnet catches can give biased results, especially when low fish number is caught (CEN, 2005; Šmejkal et al., 2015). Lower number of fish caught in the area with higher cyanobacteria density gives lower reliability in statistical sense. The gillnets were situated only at two positions in the reservoir and they can reflect characteristics of the site and not the presence or absence of the bloom. So, due to the low fishing effort, the gillnet data may not be treated as representative for the whole reservoir and their value is limited to the species discrimination. Additionally, one must be aware that acoustic sizing of fish when beaming horizontally is also problematic. We do not know the orientation of the fish relative to the transducer, so normally it is assumed that fish are oriented randomly, which means that each aspect is equally probable. To account for this the deconvolution method is used (Kubečka et al., 1996). Our measurements were performed only in the lacustrine part of the reservoir, where such assumption seems to be reasonable (Tušer et al., 2009). However, if this assumption is not fulfilled, and majority of fish are swimming along the main axis of the reservoir, while hydroacoustic transects are perpendicular to this axis, than the fish will be mainly seen from their head or tail aspect, and they will look smaller than they really are. Another possibility for bias in the acoustically obtained fish size distribution is the processing of the data. Tracking targets with a method like the CFD, where the threshold adaptively follows the cyanobacteria layer, do raise the question whether some detections regarded as fish, simply contained cyanobacteria echoes. A large number of such detections could shift the average fish size down. There are, however, three arguments against this hypothesis. Firstly, larger fish echoes contribute essentially more to the average target size than smaller targets, due to the logarithmic character of the data. One would need an essential number of false detections to shift the average size noticeably down. Secondly, since the CFD only apply phase stable single echo detections with strict criteria for echo length and positions, the

chance of falsely included cyanobacteria echoes is low. Thirdly and finally, visual studies of the fish echogram set did not reveal tracks looking as if they originate from blobs in the cyanobacteria. Even if a few erroneous tracks could have slipped our attention, it is unlikely that the number of false detections of plankton needed to shift the average target strength down, would have been easily spotted.

Apart from lowered visibility, other factors, such as diurnal instability of oxygen concentrations or pH values following the bloom physiological activity (Gągała et al., 2013; Rashidan and Bird, 2001), could also affect fish behavior. In areas of dense bloom, night time oxygen depletion and low pH can be expected. In our case, however, the values of oxygen and pH did not exceed the values that could fall outside the tolerance range for cyprinids (Alabaster and Lloyd, 1982). Unfortunately we do not know how these values changed through the night, as each station was sampled only once. We can only judge from the 3 hours difference between the first and the last station, that these changes were not noticeable. According to Tom (1998), the oxygen requirements of roach and bream are 1.51 and 1.41, respectively, comparing to the oxygen requirement of common carp *Cyprinus carpio*, which can perform well at oxygen levels of 3–5 mgL^{-1} (critical value 2 mgL^{-1}) (Bryliński, 2000). During our studies, oxygen dropped below 4 mgL^{-1} only at 4 stations and only at the narrow layer near the bottom; at all other stations and depths it was above 6 mgL^{-1}, which is sufficient even for large fish, especially for cyprinid dominants, i.e. common bream and roach. The pH values were fairly homogenous across the study area and did not noticeably differ between the stations situated within and outside the bloom (mean pH = 8.56; SD = 0.18). So, in our case environmental parameters were unlikely to be responsible for fish avoidance. The threshold in cyanobacterial biomass causing fish avoidance in the Sulejów Reservoir was estimated in this study at 7 mgL^{-1}. This was higher than the threshold (3 mgL^{-1}) estimated in the Sulejów Reservoir in July 2013 (Godlewska et al., 2016). The threshold might depend on the toxicity

420 of cyanobacteria. The number of intracellular microcystins recorded in 2013 was much higher
421 than that in 2015 (Kaczkowski et al., 2017), which may suggest that toxicity is one of the
422 factors responsible for fish avoidance.

423 Cyanobacteria distribution obtained with phycocyanin fluorescence and hydroacoustic
424 methods differed only slightly (Fig. 4) and without statistical significance. There are several
425 reasons, which could cause such discrepancy. Firstly, fluorescence measurements were
426 collected at a depth of 0.5 m, i.e. practically only in the surface layer, while hydroacoustics
427 surveyed the total water column. Secondly, one must be aware that cyanobacterial biomass
428 estimated acoustically may incidentally include biomass of small fish, whose TS is so small
429 that they are buried in signals from cyanobacteria, as well as zooplankton. Zooplankton
430 without gas bubbles is a much weaker scatterer than objects possessing gas bubbles in their
431 bodies. Usually it is below the threshold, but when it occurs in high densities, it can be
432 recorded by a high frequency echosounder (Guillard et al., 2014). These additional scatterers
433 could cause acoustic estimates of cyanobacteria biomass to become higher. However, it is
434 unlikely that this was the case in our studies. Fig.5d shows that we observed even smaller fish
435 within the bloom than outside the bloom, which indicates that filter did effectively separate
436 cyanobacteria and small fish. Also zooplankton biomass during our studies in 2015, but also
437 in 2013 (Kaczkowski et al., 2017), was higher outside the bloom than within the bloom. Since
438 with our threshold of -45 dB we did not see any zooplankton in areas free of bloom, we
439 assume it was not present within the bloom either.

440 Cyanobacterial and fish biomass showed opposite trends in their spatial distribution, with the
441 most dramatic slopes at the edge of the bloom. It is particularly evident from small scale
442 analyses (100 m) that both fish and cyanobacteria maximum concentrations do not overlap in
443 space, but they are rather situated next to each other. Therefore, the reason why Sotton et al.
444 (2011) concluded that *P. rubescens* distribution did not seem to affect the vertical or

445 horizontal distribution of whitefish could be the too low spatial resolution of their
446 measurements. They estimated fish biomass at intervals of 250 m (ESDU), while
447 cyanobacteria were measured at 3 single points about 1,000 m apart, so the comparison was
448 based on the assumption that the concentrations of *P. rubescens* were constant within this
449 range, which might not have been true. While their conclusion was well supported by the
450 vertical data, we wonder if the ESDU and spatial resolution of cyanobacteria measurements
451 were small enough to account for the heterogeneous distributions of fish and *P. rubescens* in
452 the horizontal dimension.

453 Fish avoidance behavior was also confirmed by gillnet catches, with over twice less fish
454 caught in the net situated on the periphery of the bloom than in the net situated outside of the
455 bloom. Clearly lower fish numbers and bream dominance in gillnets placed in the bloom area
456 were observed in the Sulejów Reservoir in 2013 (Godlewska et al., 2016; Kaczkowski et al.,
457 2017). In 2015, the dominant fish in the bloom area was roach (over 60% of all caught fish
458 specimens), but there were only large specimens of this species (over 27 cm in total length).
459 There are at least two possible explanations for our observation. At the size of 27 cm or more,
460 roach prey mostly on zebra mussel *Dreissena polymorpha*, which becomes an available food
461 source for fish longer than 16 cm (Kobak et al., 2010; Nagelkerke and Sibbing, 1996; Prejs et
462 al., 1990). Utilization of this food source might prevail over any disadvantage related to
463 bloom presence. Alternatively, fish utilizing almost entirely this benthic food source are partly
464 avoiding bloom by occupying the deepest layers of the water, where autotrophic algae density
465 is lower. In comparison, breams are able to utilize zebra mussels when they reach lengths over
466 37 cm and even then they are only able to consume much smaller mussels (mainly 6–8 mm
467 long and a maximum of 13 mm) than roach (mainly 6–18 mm long and a maximum 19 mm),
468 and thus are not able to compete effectively with the latter species. Bream prefer other food
469 sources such as zooplankton and chironomids, similar to smaller roach specimens, of average

length 18 cm, present in gillnets situated outside of the bloom (Kakareko, 2001; Lammens, 1989; Martyniak et al., 1987; Nagelkerke and Sibbing, 1996; Persson and Hansson, 1999; Prus, 2009; van den Berg, 1993).

Applied quantitative genetic analyzes confirmed the presence of cyanobacteria of the genus *Microcystis* (based on 16S rRNA gene analysis) and their toxigenic genotypes (based on *mcy*A gene analysis) at all 14 stations. The fact that MCs were not found in the fish tissue may indicate that the avoidance occurred fairly fast, preventing accumulation of microcystins by fish, or that they were present in fish bodies only in small concentrations, making impossible their identification by chromatographic methods using diode array detection. Moreover, the absence of microcystins in the water could explain lack of presence of identified toxins in the organs of fishes, although they are known to be well accumulated by fish (Papadimitriou et al., 2012; Xie et al., 2004). Thus, our results confirm the observations of Kamjunke et al. (2002) that microcystins are not dangerous for fish as long as they are closed in the *Microcystis* cells, because they are not digested when passing through fish guts.

5. Conclusions

Fish avoidance has been clearly documented, indicating that fish can actively react to their exposure to microcystin-producing cyanobacteria and minimize adverse effects, regardless of the nature of such interaction, for example intoxication (Sotton et al., 2012a, 2012b), visual deprivation (Gray et al., 2014) or food shortages (Reichwaldt et al., 2013). Both, hydroacoustic measurements and gillnet catches confirmed that maxima of fish and cyanobacteria biomass mismatch in space. In spite of toxic bloom occurrence, no microcystins were detected in fish bodies, suggesting that in the Sulejów Reservoir fish avoidance was effective. The paper also demonstrates that a high frequency echosounder provides an effective tool for monitoring bloom development and propagation and its results

are highly correlated with measurements of cyanobacteria biomass based on fluorescence of phycocyanin.

Further investigations are needed to fully understand the mechanisms of the effect of *Microcystis* on fish, and to determine the densities of cyanobacteria which trigger fish avoidance behavior for different densities and species of fish and cyanobacteria. An interesting question is whether avoidance behavior depends on the density of cyanobacteria alone or also on their toxicity.

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Figure captions

Fig. 1. Localization of hydroacoustic transects (A), point water sampling stations and gillnet location (B) in the Sulejów Reservoir.

Fig. 2. An example of a horizontal beam echogram with a *Microcystis* bloom border crossed during the hydroacoustic survey in the Sulejów Reservoir on September 2, 2015.

826

827 Fig. 3. Fish and cyanobacterial biomass expressed as an area sound backscattering coefficient
828 Sa total (m^2ha^{-1}) for the horizontal beam echogram of transect 7, at which the bloom edge
829 (segment 8 and partly 9) presented in Fig.2 occurred.

830

831 Fig. 4. Spatial distribution of fish abundance estimated acoustically (A), cyanobacterial
832 biomass estimated acoustically (B) and cyanobacterial biomass estimated from fluorescence
833 of phycocyanin (C) based on 100 m intervals along 10 transects in the Sulejów Reservoir on
834 September 2, 2015, plotted in Surfer software. Tr2 and Tr9 correspond to the position of the
835 gillnets.

836

837 Fig. 5. Fish abundance (A), fish biomass (B), number of single echo detections (C) and mean
838 fish size TS in dB (D) along cyanobacterial biomass gradient for 100 m segments of a survey
839 track in the Sulejów Reservoir on September 2, 2015.

840

841 Fig. 6. Diagram of the frequency (number of fish) of length classes of fish caught in multi-
842 mesh gillnets near transects 2 and 9 at the Sulejów Reservoir.

843

844 Fig.7. The dynamics of the genus *Microcystis* (16S rRNA) and its toxigenic genotypes
845 (*mcy*A) characterized in molecular analyses in the Sulejów Reservoir at 14 stations on
846 September 3, 2015.

847

848 Fig.8. Distribution of intracellular microcystins determined by HPLC in water samples
849 collected from the Sulejów Reservoir on September 3, 2015.

850

851 **Tables**

852 Table 1. Results of statistical comparisons (t-test for unequal sample sizes) of the acoustic
853 parameters characterizing fish abundance, biomass and fish sizes below and above the
854 threshold of cyanobacterial biomass estimated as 7 mgL^{-1} in the Sulejów Reservoir on 2
855 September 2015.

856

857 Table 2. Abundance of fish species (D, %) and fish sizes (total length–TL) in the gillnet catch
858 with respect to cyanobacterial chlorophyll *a* concentration in the Sulejów Reservoir on
859 September 2, 2015. The dominants are in bold.

860

861 Table 3. Water temperature and dissolved oxygen concentration values at 14 stations in the
862 Sulejów Reservoir, measured from the surface till depth at 1 m intervals on September 2,
863 2015 simultaneously with hydroacoustic measurements.

864 Tab.1

			Cyanobacterial biomass						
			< 7 mgL^{-1}		> 7 mgL^{-1}				
			mean	SD	mean	SD	t-value	df	p-value
Fish abundance	$fishha^{-1}$	198.5	145.9		54.6	24.4	8.6	101	0.001
Sa	$m^2\ ha^{-1}$		12.33	13.26	2.89	2.70	6.06	103	0.001
SED			76.21	86.17	5.00	2.73	7.69	87	0.001
TS	dB		-29.09	2.87	-33.73	5.37	3.36	17	0.01

865

866

867

868

869 Table 2

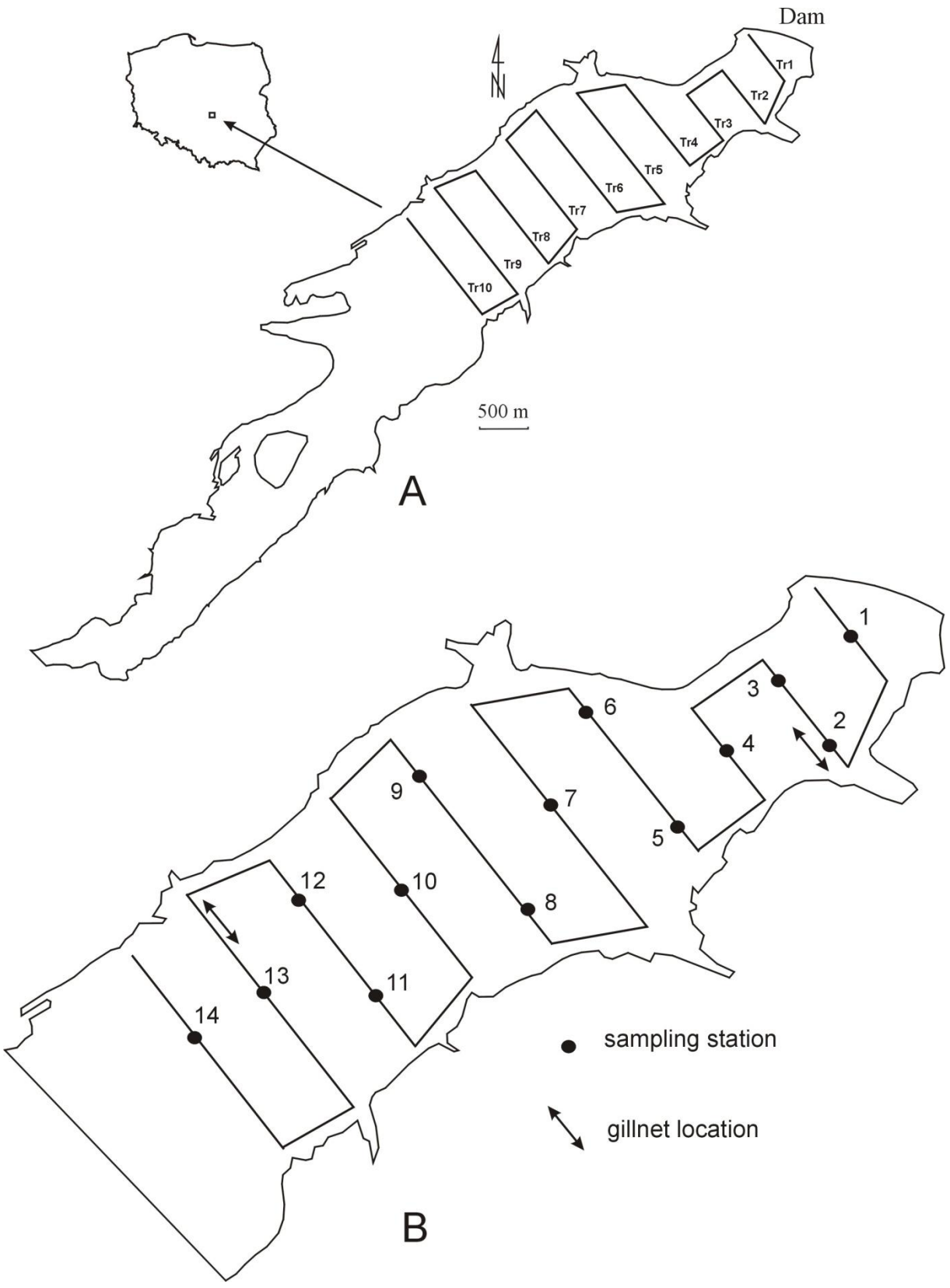
Transect number	2	9
Cyanobacterial chlorophyll*a* [µg L^{-1}]	5.93	27.92

Bleak *Alburnus alburnus*	0.91	10.9		
Common bream *Abramis brama*	6.36	40.4 ± 2.9		
White bream *Blicca bjoerkna*	**63.64**	21.2 ± 5.4	**20.00**	18.9 ± 6.7
Roach *Rutillus rutillus*	**20.00**	18.0 ± 5.7	**64.44**	27.2 ± 5.4
Eurasian perch *Perca fluviatilis*	0.91	8.7	6.67	22.9 ± 8.1
Pikeperch *Sander lucioperca*	1.82	44.2 ± 3.2		
Ruffe *Gymnocephalus cernua*	6.36	9.3 ± 0.3	6.67	9.4 ± 3.0
Wels catfish *Silurus glanis*			2.22	65.2
n tot.	**110**		**45**	

870

	depth. m	0	0	1	1	2	2	3	3	4	4	5	5	6	6	7	7
Time (h:min)	station	Temp. Deg.	Oxyg. mg/L	Temp. Deg..	Oxyg. mg/L	Temp. Deg.	Oxyg. mg/L	Temp. Deg.	Oxyg. mg/L	Temp. Deg.	Oxyg. mg/L	Temp. Deg.	Oxyg. mg/L	Temp. Deg.	Oxyg. mg/L	Temp. Deg.	Oxyg. mg/L
20:51	1	22.5	11.44	22.5	11.34	22.4	10.1	21.4	6.17	21.1	5.45	21,00	5.14	20.9	4.24	20.7	4.24
21:07	2	22.2	9.39	22.1	7.24	21.7	5.19	21.6	5.89								
21:18	3	22.6	11.48	22.6	11.55	22.7	11.55	21.8	6.94	21.2	5.42	20.9	4.76	20.8	4.3	20.5	1.66
21:33	4	22.1	10.51	22.1	10.49	22.1	10.34	21.9	9.59	21.3	7.23						
21:44	5	21.9	10.11	21.9	10,00	21.9	9.89										
21:57	6	22.7	11.64	22.8	11.65	22.9	11.48	22.7	10.4	22,00	6.95	21.4	5.19				
22:12	7	21.8	10.67	22,00	10.61	21.9	10.16	21.7	9.88	21.6	8.34	21.5	7.24				
22:26	8	21.2	9.11	21.3	9.09	21.3	9.05	21.3	9.01	21.3	8.98	21.2	7.12	21.1	6.44		
22:42	9	22.7	12.86	22.9	12.89	22.9	12.9										
22:51	10	21.5	9.29	21.6	9.27	21.6	9.25	21.6	9.23	21.6	9.22	21.6	8.92	20.7	2.9		
23:06	11	22.4	12.23	22.5	12.32	22.5	12.34										
23:21	12	21.4	10.21	21.5	10.15	21.5	9.75	21.2	8.09	21.2	7.66	21.1	6.88				
23:32	13	21.9	11.51	22,00	11.52	22.1	11.4	22.1	11.26	22,00	9.91	21.6	10.28				
23:45	14	22.4	12.4	22.4	12.4	22.4	12.41	22.4	12.42	22.4	12.22	20.9	3.54				

Tab.3


Dam
Tr1
Tr2
Tr3
Tr4
Tr5
Tr6
Tr7
Tr8
Tr9
Tr10
500 m
A
1
2
3
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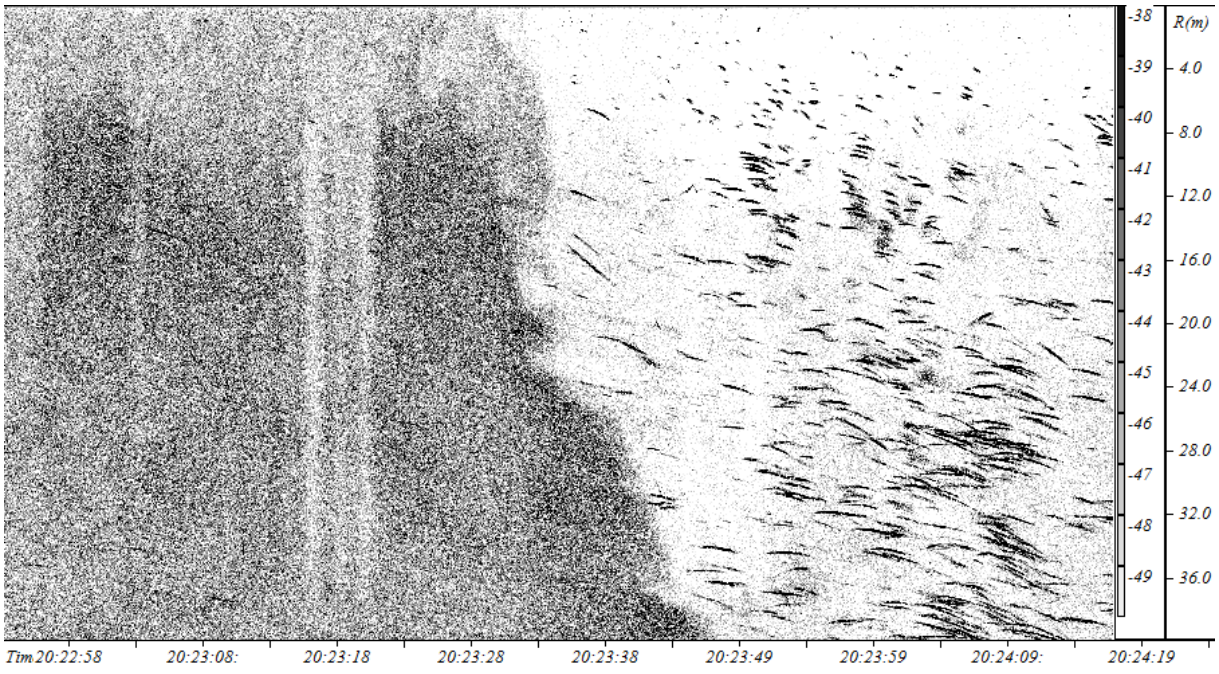
9
10
11
12
13
14
sampling station
gillnet location
B

875

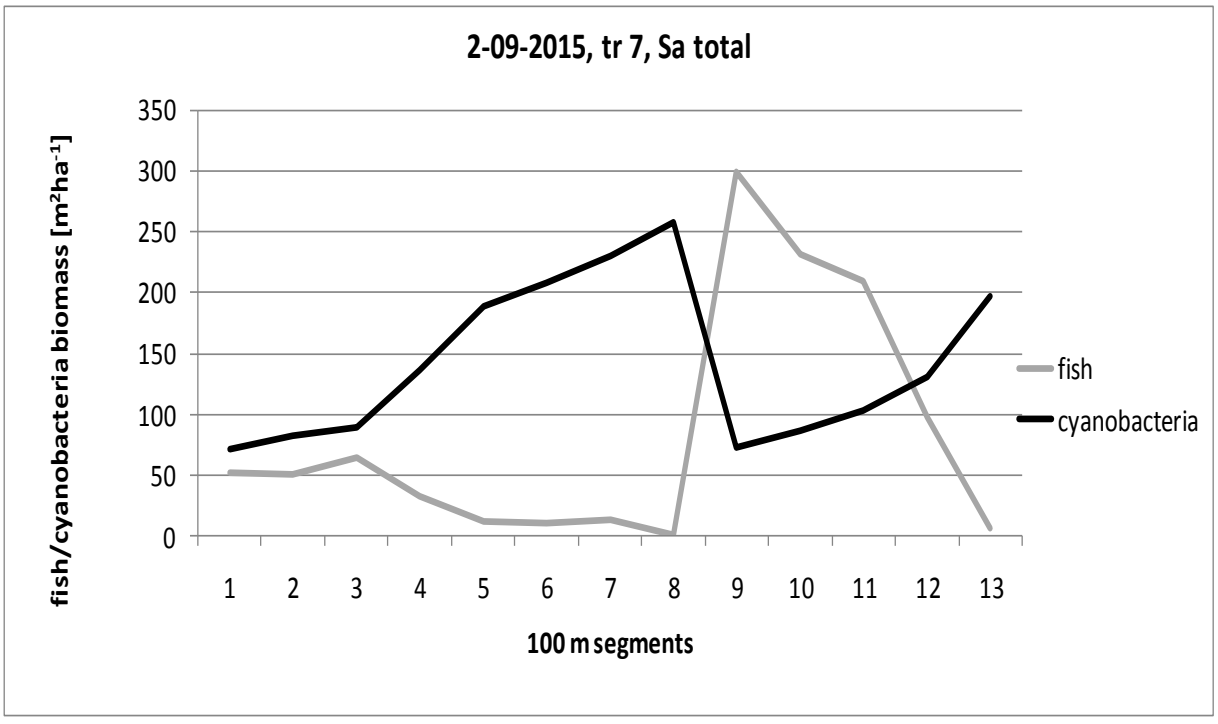
876 Fig. 1



877

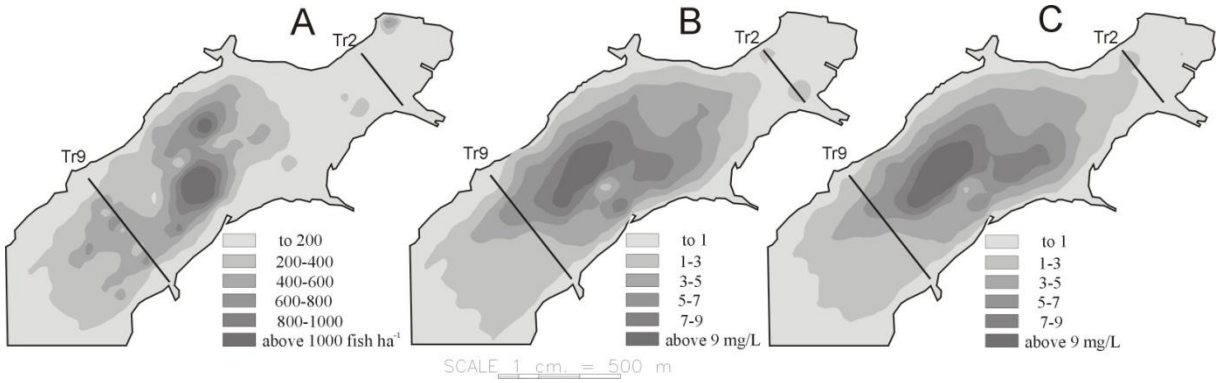
878 Fig. 2

879



880
881

882 Fig. 3



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884 Fig. 4

885

886

887

888 Fig. 5

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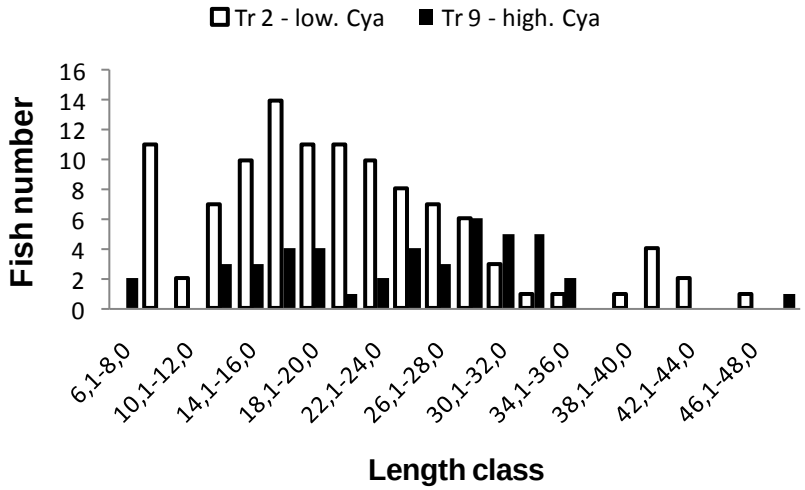

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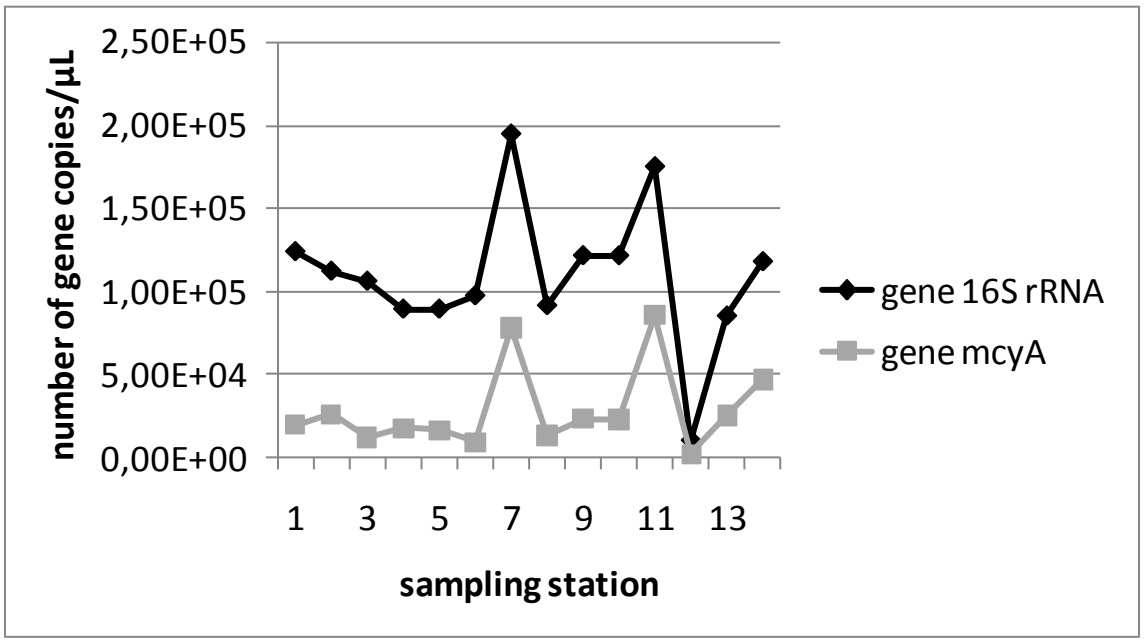
891 Fig. 6



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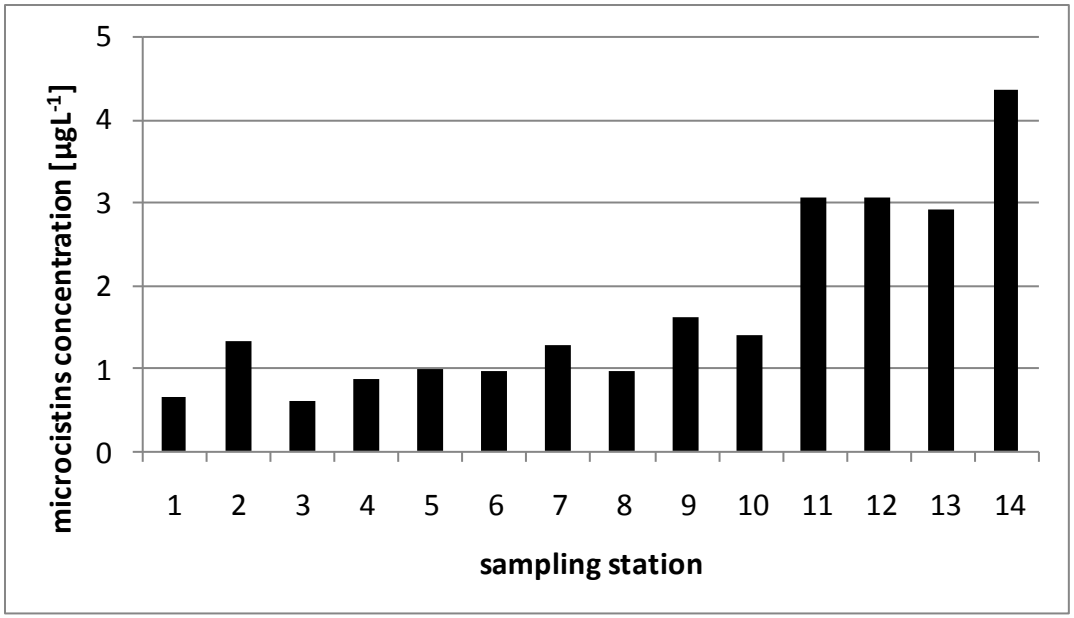

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895 Fig.8

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