

1 **The vertical distribution of maraena whitefish (*Coregonus maraena*) early juveniles**  
2 **in different times of day in a newly created oligotrophic lake.**

3

4

5 **Tomáš Jůza<sup>a\*</sup>, Vladislav Drašík<sup>a</sup>, Martin Čech<sup>a</sup>, Zuzana Sajdlová<sup>a</sup>, Maria Anton-**  
6 **Pardo <sup>a</sup>, Petr Blabolil<sup>a</sup>, Jiří Peterka<sup>a</sup>**

7

8 <sup>a</sup> Biology Centre of the Czech Academy of Sciences, Institute of Hydrobiology, Na

9 Sádkách 7, 37005, České Budějovice

10 [\\*tomas.juza@seznam.cz](mailto:*tomas.juza@seznam.cz)

11

12

13

14

15

16

17

18

19

20

21

22

23

24 **Abstract**

25 Diel vertical distribution of strictly pelagic juvenile (23 - 47 mm total length) maraena  
26 whitefish *Coregonus maraena* (Bloch, 1779) was repeatedly investigated in spring  
27 primarily using hydroacoustics in the artificial post-mining Most Lake in the Czech  
28 Republic. At the same time, an ichthyoplankton trawl was used to identify acoustical  
29 targets. During the day, fish performed extensive shoaling behaviour in depths between 2  
30 and approximately 40 meters and were not accessible for trawling. By evening, with  
31 decreasing light intensity, shoals started to disintegrate and at night fish were relatively  
32 homogeneously distributed in the water column from the surface down to a depth of 40  
33 m. Juvenile maraena whitefish could be caught by trawl as the only fish species at night.  
34 Shoaling behaviour started again approximately 1.5 hour before sunrise. The data showed  
35 steep decreases in fish density between the two surveys in spring which indicates  
36 significant mortality of early juvenile coregonids as a result of poor availability of  
37 zooplankton in a highly oligotrophic post-mining lake.

38

39 Key words: Most Lake; ichthyoplankton trawling; hydroacoustics; shoaling behavior;  
40 vertical migrations

41

42

43

44

45

46

47 **1 Introduction**

48 Fish stocks in water bodies are based on the occurrence of species found in a specific  
49 region, and are formed by the particular characteristics of a given water body (Gassner et  
50 al., 2005; Irz et al., 2006). Reservoirs, created by damming the original river valley,  
51 comprise the majority of standing waters in the Czech Republic, because natural lakes are  
52 relatively scarce in Central Europe. Typical features of these mostly eutrophic water  
53 bodies are strong summer stratification with significant decrease of water temperature  
54 and oxygen concentration below approximately 5 meters (Prchalová et al.; 2009, Jůza et  
55 al., 2009).

56 Since the beginning of 21<sup>st</sup> century new large water bodies have been constructed in  
57 the northern part of the Czech Republic – post-mining lakes created by flooding the  
58 former surface coal mines (Kružíková, 2013; Šusterka et al.; 2012). These artificial lakes  
59 usually have a similar area as reservoirs (few hundreds of hectares), and are also  
60 relatively deep (usually few tens of meters). Due to a relatively oligotrophic character,  
61 none or only metalimnetic oxygen depletions occur in deeper layers (Peterka, 2014).  
62 Dominant fish species in these lakes are cyprinids - usually roach and rudd *Scardinius*  
63 *erythrophthalmus* (Linnaeus, 1758) and also one percid species -perch *Perca fluviatilis*  
64 Linnaeus, 1758 (Peterka at al., 2013). In addition to these species, inhabiting especially  
65 warmer littoral habitats and to some extent pelagic habitats above thermocline, oxygen  
66 throughout the entire water column enables the presence of coldwater coregonids that  
67 usually are found in deep, well oxygenated lakes at similar latitudes. Coregonids are  
68 common in pelagic areas of deep stratified lakes across northern parts of Europe, Asia  
69 and North America (Rudstam and Magnuson, 1985, Marjomäki and Huolila, 1995; Busch

70 and Mehner, 2009; Jurvelius et al., 2011) and natural lakes with similar characteristics as  
71 the post-mining lakes in Northern Bohemia, with coregonids being the important  
72 component of the fish stock, are situated also in neighbouring Germany and Poland  
73 (Mehner et al., 2003; Godlewska et al., 2014). As mentioned above deep natural lakes,  
74 representing suitable water bodies for coregonids are missing in the Czech Republic but  
75 the natural reproduction of maraena whitefish in newly created post-mining lakes was  
76 recognized shortly after stocking (Peterka, 2014) indicating the suitability of these  
77 artificial waterbodies for this species.

78         Significant and in comparison with other coregonid localities a unique characteristic  
79 of the post-mining lake investigated in this study is its age. Newly created lakes are  
80 usually extremely oligotrophic with high water transparency and low phosphorus  
81 concentration (below 10  $\mu\text{g l}^{-1}$ ). The availability of food may be a limiting factor for  
82 growth and survival of larval fish if low concentration of nutrients limits primary  
83 production (Hardy et al., 2008). Coregonids are predominantly zooplankton consumers  
84 and their mortality is significantly influenced by zooplankton density (Rellstab et al.,  
85 2004). A newly filled lake therefore provides the unique possibility to investigate density,  
86 mortality, growth, depth distribution and shoaling behavior in an extremely oligotrophic  
87 system with low primary production and high water transparency.

88         Diel vertical migrations (DVM) and shoaling behaviour were described as common  
89 strategies in coregonid populations (Hamrin, 1986; Ventling-Schwank and Meng, 1995;  
90 Gjelland et al., 2009) but the significance of this behaviour can differ during ontogeny. It  
91 is generally understood that the DVM is the result of a compromise between conflicting  
92 demands, since food availability, light, temperature and predation change with depth

93 (Ventling-Schwank and Meng, 1995) and their relative importance should be dependent  
94 on the size and environmental factors (Hamrin, 1986). Shoaling is recognized as an  
95 important antipredator behaviour and represents an alternative or supplementary defence  
96 strategy to DVM for pelagic fish (Gjelland et al., 2009). Distribution of larval, older  
97 juvenile and adult native coregonid populations in lakes has been studied many times  
98 (Lahnsteiner and Wanzenböck, 2004; Hamrin, 1986; Sandlund et al., 1992; Gjelland et  
99 al., 2009) but knowledge of the spatio-temporal distribution of coregonid juveniles in  
100 their earliest stages (May-June, *total length* (TL) <5 cm) is mostly lacking and this paper  
101 aims to elaborate on their early life history.

102 The main aim of this study is to investigate pelagic diurnal spatio-temporal  
103 distribution of maraena whitefish early juveniles and a newly created oligotrophic lake  
104 with a confirmed naturally reproducing coregonid population was used for this purpose.  
105 Repeated sampling during one season provides primary information about mortality and  
106 growth rates of coregonid juveniles in a lake with low productivity. This study attempts  
107 to address why these behavioural patterns are important during the early juvenile stage,  
108 and their differences and similarities with other ontogenetic stages of other coregonid  
109 species described in literature are discussed.

110

## 111 **2 Material and methods**

112

### 113 **1.1 Study area**

114 The study was carried out in the post-mining Most Lake situated near the town of  
115 Most (Northern Bohemia, 50.5379136N, 13.6456339E, Fig. 1a). The mining activity

116 ended in the summer of 1999 and filling of the opencast mine started in autumn 2008  
117 (Fultner and Valvoda, 2013). The lake was completely filled in 2012, and it has a surface  
118 area of 309.4 ha, a volume of  $70.5 \times 10^6 \text{ m}^3$ , a maximum depth of 75 m, and a mean depth  
119 of 22 m. In spring, the mean depth of the thermocline is about 7 m, the secchi depth is  
120 around 5 m and the concentration of dissolved oxygen is about  $10 \text{ mg l}^{-1}$  throughout  
121 almost the whole water column (Fig. 2). The trophic status of the lake is characterized as  
122 oligotrophic with total phosphorus concentrations lower than  $10 \text{ } \mu\text{g l}^{-1}$  in the whole water  
123 column during the whole season. The springtime pH value is about 8. The dominant fish  
124 species are perch, roach, ruffe *Gymnocephalus cernua* (Linnaeus, 1758) and rudd  
125 (Peterka, 2014). Two predatory species, pike *Esox lucius* (Linnaeus, 1758) and wels,  
126 *Silurus glanis* (Linnaeus, 1758) were stocked in the lake. Maraena whitefish have been  
127 regularly stocked in this lake since 2011 (46,000 inds. 70 - 155 mm standard length in  
128 2011, 46,000 inds. 95 - 170 mm standard length in 2012, 46,000 inds. 83 - 163 mm  
129 standard length in 2013).

130

## 131 **1.2 Data collection and analysis**

132

### 133 **1.2.1 Hydroacoustic survey**

134 A vertically oriented echosounder, fixed on a trawling vessel was the main method  
135 used to monitor the spatio-temporal distribution and abundance of maraena whitefish  
136 early juveniles. Transects for mobile surveys and trawling were positioned in the central  
137 part of lake from depths of 25 to 75 m. Mobile surveys were carried out simultaneously  
138 with the trawling activities in the day and at night on May 20 and June 3, 2015.

139 Continuous stationary recording was conducted on June 10 and 11 between 6 pm and 7  
140 am in the central part of the lake, deeper than 31 m, which allowed safe anchoring (Fig.  
141 1a).

142 The Simrad EK 60 hydroacoustic system connected to an ES 120-7C composite  
143 transducer with a nominal angle of 7 degrees operating at a frequency of 120 kHz was  
144 employed for both mobile and stationary recordings. During the surveys, the transducer  
145 was mounted 0.5 m below the water surface. The system was properly calibrated  
146 following the manufacturer's recommendations using the calibration procedure as per  
147 Foote et al. (1987) before the surveys. The pulse repetition rate was set to 2.5 pings per  
148 second, using a pulse duration of 0.128 ms.

149 Recorded data were analyzed using Sonar5-Pro post-processing software, version  
150 6.0.2 (Balk & Lindem, 2014). All recorded echograms were manually cleaned of noise  
151 (bubbles) and non-fish echoes. The bottom was automatically detected using the  
152 software's algorithm with a margin of 0.25 m, manually scrutinized and corrected when  
153 necessary to avoid the inclusion of bottom echoes in the analysis. The upper limit of the  
154 echogram area was set 2 m below the transducer face and the lower limit was set at the  
155 detected bottom line. Single echo detection (SED) criteria were set during the conversion  
156 of data files to Sonar5-Pro and were the same for the mobile and stationary surveys. The  
157 echo length was set from 0.6 to 1.8 relative to the length of the transmitted pulse, the  
158 maximum gain compensation was set to 3 (one way) and the maximum standard  
159 deviation of axis angles was set to 0.6. Automatic tracking was performed to produce fish  
160 tracks. All fish targets had to meet the following criteria to be recognized as a track:  
161 minimum track length (MTL), maximum ping gap (MPG) and vertical range gating.

162 MTL was set in depth dependent steps; for the upper part of the echogram (2 – 5 m) MTL  
163 was set to 1 echo, for the mid-ranges (5.1 – 7 m) MTL was set to 2 echoes, and for the  
164 lower part of the echogram (7.1 m to the bottom) MTL was set to 3 echoes. MPG was set  
165 to 1 and the vertical range gating was set to 0.15 m for the whole water column. The echo  
166 integration method was used for estimating fish density (CEN, 2014). Volume  
167 backscattering strength ( $S_v$ ) was scaled by average target strength (TS) of in situ tracks as  
168 a source of density estimates. A regression for vendace (*Coregonus albula* Linnaeus,  
169 1758) was used (Mehner, 2006b) to convert TS (dB) to fish total length (mm). The  
170 threshold for the SED echogram was set to -64 dB (1.8 cm) and threshold for the Amp  
171 echogram (40 logR) was set to -70 dB. Both thresholds were based on the minimum  
172 length of maraena whitefish juveniles caught in the trawl. For the mobile survey,  
173 transects were 10 minutes long and corresponded to the duration of trawling. Density  
174 estimation was conducted in 5 m depth layers down to 50 m depth, or the bottom, with  
175 the exception of the first layer, which was set from 2 m to 5 m. Each file of the stationary  
176 survey was approximately 23 minutes long. Density analysis for the stationary survey  
177 was done for whole water column from 2 m to 31 m (bottom). Maraena whitefish  
178 juveniles were clearly acoustically distinguishable from the other potential fish targets  
179 because they were unique in their size, and were also the only catch in the trawl (see  
180 results chapter).

181

### 182 **1.2.2 Trawling**

183 Fifteen trawl tows on May 20, 2015 (9 daytime tows, 6 night time tows), and 24 tows  
184 on June 3, 2015 (10 daytime tows, 14 night time tows) at different depth layers between 0

185 and 23 m were performed simultaneously with hydroacoustics. Only the pelagic area was  
186 taken into account in this study because fry beach seining around the lake confirmed the  
187 complete absence of coregonids in the littoral during both day and night (10 seine hauls  
188 in each term; the only catch were cyprinid larvae and a few perch juveniles). The main  
189 aim of trawling in this study was to identify acoustical targets, calibrate acoustical fish  
190 sizing, and sample the surface water layer (0-2 m; blind zone of echosounder). A pelagic,  
191 fixed frame ichthyoplankton trawl (mouth opening 2x2 m, mesh size 1 mm x 1.35 mm)  
192 with the collecting bucket at the end (Fig. 1b; Jůza et al., 2010) was used for fish  
193 sampling. The trawl had a funnel to prevent fish from escaping (Jůza and Kubečka,  
194 2007). A floater attached to the upper part of the frame regulated the sampling depth of  
195 the trawl. The length of the rope between the frame and the floater kept the trawl at the  
196 required depth. The lower part of the trawl frame was equipped with two weights to keep  
197 the trawl vertically in required depth. The volume of water sampled was calculated for  
198 each haul. The research vessel Ota Oliva (64 HP diesel engine) towed the trawl  
199 approximately 100 m behind it at velocities of approximately 1 m/s.

200 Juvenile maraena whitefish captured by each trawl tow were immediately  
201 anesthetized and preserved in 4% formaldehyde solution. In the laboratory, each fish was  
202 measured for TL to the nearest mm. The catch was expressed in terms of density (number  
203 of fish per 100 m<sup>3</sup> of water sampled).

204

### 205 **1.2.3 Zooplankton sampling and vertical profiling**

206 In each session (May 20, June 3, June 10) zooplankton was collected during the day  
207 in the central part of the lake (Fig. 1a) concurrently as fish were monitored. A closing

208 zooplankton net with a mouth diameter of 25 cm and mesh size of 200  $\mu\text{m}$  was used for  
209 zooplankton sampling. In each sampling session the mixed contents of three vertical  
210 hauls in surface water layer (0-2 m) and also in depth around thermocline (6-8 m) was  
211 taken. The hypolimnetic layer (11-55 m) was sampled only during the third sampling  
212 campaign (June 10). The zooplankton material was immediately preserved in 4%  
213 formaldehyde solution. Zooplankton samples were counted in the laboratory under a  
214 microscope using a Sedgewick-Rafter chamber. Cladocerans were determined to species  
215 level, copepods were determined as cyclopoid or calanoid and their larval stage  
216 (nauplius) was distinguished. The density was calculated as number of individuals per 1  
217 litre in each depth layer.

218 Water temperature and oxygen concentration were simultaneously measured during  
219 the day in 1 m steps in the central part of the lake using an ISY ProODO probe during  
220 each round of zooplankton sampling. Water transparency was also measured using a  
221 Secchi disk.

222

#### 223 **1.2.4 Statistical analysis**

224 To compare the density and size of maraena whitefish juveniles between dates (May  
225 20 and June 3) night data were used separately for hydroacoustics and trawling and a  
226 paired t-test was used ( $\alpha=0.05$ ). For comparison of trawling data only tows from layers  
227 sampled in both dates were analyzed (6 observations in each date). In order to stabilize  
228 the variance of the differences in the t-test, a logarithmic transformation was performed  
229 for density data.

230 Percentage differences in density between surveys performed on May 20 and June 3  
231 represented a 14-day mortality value ( $\pm$  standard deviation).

232 Statistica software (Statsoft Inc., 2011) was used to perform the statistical analysis.

233

## 234 **2 Results**

235

### 236 **2.1 Depth distribution of maraena whitefish juveniles**

237 During both sampling sessions 187 juvenile maraena whitefish were captured at  
238 night by trawling and this species was the only one caught. This proved that the targets  
239 observed by hydroacoustics were maraena whitefish. Relatively similar patterns in depth  
240 distribution of juvenile maraena whitefish were observed hydroacoustically during day  
241 and night in both sessions. During the day the highest hydroacoustical density was  
242 observed in the depth layer between 5 and 10 meters (4 ind./100 m<sup>3</sup> in May and 1  
243 ind./100 m<sup>3</sup> in June; Fig. 3a) and maraena whitefish juveniles were present from the  
244 surface down to 35 m in May and 40 m in June (Fig. 3a). Night hydroacoustical data  
245 revealed that the majority of maraena whitefish juveniles occupied the uppermost layer,  
246 which could be monitored by vertical hydroacoustics (2-5 m; Fig. 3b, 15 ind/100 m<sup>3</sup> in  
247 May and 3 ind./100 m<sup>3</sup> in June). Maraena whitefish juveniles were regularly captured in  
248 the surface water layer (0-2 m) at night. Night occurrence of maraena whitefish juveniles  
249 was found from the surface down to 45 m in May and 35 m in June using hydroacoustics  
250 (Fig. 3b).

251

252 **2.2 Comparison of overall density and size of maraena whitefish juveniles between**  
253 **two sampling points**

254 Both sampling methods found that the overall density of maraena whitefish juveniles  
255 decreased significantly between May 20 and June 3 (Fig. 4; trawl:  $p < 0.001$ ,  $t = 6.1$ ,  $Df =$   
256  $10$ ; hydroacoustics:  $p < 0.001$ ,  $t = 3.6$ ,  $Df = 13$ ). Mortality during 14 days was  $89 \pm 24 \%$   
257 and  $64 \pm 14.3 \%$  based on trawling and hydroacoustical data respectively.

258 In trawl catches, size increased only slightly between the sessions (mean TL in May  
259  $34.3$  mm, mean TL in June  $35.4$  mm) and the difference was not statistically significant  
260 ( $p = 0.16$ ). Hydroacoustics revealed a slight decrease in TL (mean TL in May  $29.7$  mm,  
261 mean TL in June  $28$  mm), which was found significant ( $p < 0.001$ ).

262

263 **2.3 Vertical distribution of maraena whitefish juveniles during day and night using**  
264 **stationary hydroacoustics.**

265 In the daytime the echogram was practically empty during stationary monitoring  
266 (Fig. 5a) with the occasional occurrence of shoals (in fact shoals were much better  
267 detected during the mobile survey, when a larger volume was monitored). In the  
268 evening, with decreasing light intensity, the shoals started to disintegrate (Fig. 5b) and  
269 during the night fish were distributed homogeneously in water column (Fig. 5c,d). In  
270 early morning but still before sunrise the homogeneous distribution started to disappear  
271 and shoaling behavior was observed again (Fig. 5e). In the morning during sunrise the  
272 echogram was again usually empty during stationary recording with an occasional  
273 occurrence of shoals (Fig. 5f). Fish density started to increase shortly after sunset and

274 peaked around midnight. At 03:30 a.m. (1.5 hour before sunrise), the water column was  
275 again almost empty and showed the daytime characteristics (Fig. 6).

276

## 277 **2.4 Zooplankton density**

278 The zooplankton community showed a similar vertical distribution pattern during the  
279 surveys. Higher density was observed in the metalimnion (6-8 m) rather than the surface  
280 layer or hypolimnetic layer (11-55 m, in the case of June 10), all and all the zooplankton  
281 density was very low in all sessions (Tab. 1). In the surface water layer, less than 1 ind./l  
282 was always observed in May and the zooplankton density was lower in later occasions. In  
283 the metalimnion zooplankton density was around 2 ind/l with the highest density in the  
284 first sampling session in May (2.54 ind./l, Tab.1). Also on June 10 in hypolimnetic layer,  
285 zooplankton density was approximately one tenth of that in metalimnion.

286 Nauplii of cyclopoid and calanoid copepods dominated the samples (Tab. 1). The  
287 “others” group consisting of minor components of samples was represented by *Bosmina*  
288 *longirostris* (Müller, 1776), *Chydorus sphaericus* (Müller, 1776), *Diaphanosoma*  
289 *brachyurum* (Liévin, 1848), *Alonella nana* (Baird, 1850), adult calanoid copepods and  
290 chironomids.

291

## 292 **3 Discussion**

293 This study presents the first attempt to describe basic ecological characteristics of  
294 early juveniles of an introduced population of coregonids in an extremely young post-  
295 mining oligotrophic lake with high water transparency. In the spring during the day  
296 juvenile maraena whitefish gathered in shoals practically throughout the whole water

297 column up to 40 m whereas at night they were relatively homogeneously distributed up to  
298 the depth of 45 m with a peak between 2 and 5 m. Both hydroacoustics and trawling  
299 revealed a significant decrease of maraena whitefish density between sampling sessions,  
300 which points to high mortality in spring of the first year of life. Any significant increase  
301 of size between sessions with either method was used, which is a sign of very slow  
302 growth in a zooplankton poor lake with low productivity.

303 Mehner et al. (2011) stated the hydroacoustical density of juvenile coregonids in  
304 Lake Stechlin in June to be 6.1 ind./100 m<sup>3</sup> and 0.3 ind./100 m<sup>3</sup> in 2002 and 2010  
305 respectively. Thus, the spring juvenile maraena whitefish density observed in Most Lake,  
306 is comparable with the density of coregonids in the natural lake in Germany. Our data  
307 however revealed extremely high mortality of maraena whitefish juveniles in spring.  
308 Urpanen et al. (2005) mentions the three weeks mortality of another coregonid, vendace  
309 *Coregonus albula* (Linnaeus, 1758), to be 64%-95% directly after hatching. During the  
310 larval stage mortality rates are usually the highest because of yolk sac reduction and the  
311 necessity to switch to exogenous feeding, and this stage in general represents the most  
312 sensitive stage in the development of the cohort (Lahnsteiner and Wanzenböck, 2004).  
313 The intensity of mortality in the juvenile stage is much lower - approximately 0.5-1.5%  
314 per day (Bradford and Cabana, 1997). In Most Lake, mortality of maraena whitefish early  
315 juveniles was several times higher (15 and 7 % per day in trawl and hydroacoustics  
316 respectively), rather comparable to mortality during the larval stage in vendace.

317 Because food concentration is the key factor to the survival and growth of young fish  
318 (Rellstab et al., 2004; Müller et al., 2007) and coregonids are predominantly zooplankton  
319 consumers (Eckmann, 1985; Hanazato et al., 1990), zooplankton density is probably the

320 most important factor influencing mortality and growth. Rellstab et al. (2004) showed a  
321 clear relationship between food concentration and mortality of coregonid larvae. During  
322 the first 34 days of life, an elevated mortality of 40% or more resulted from a food  
323 concentration of 20 zooplankton organisms per liter or fewer (Rellstab et al., 2004). Also  
324 the growth rate of maraena whitefish juveniles was extremely low in Most Lake. Based  
325 on the trawling data the average growth rate was 0.08 mm per day and according to  
326 hydroacoustical recordings, the growth rate was even negative. Due to the large number  
327 of observations, a 1.7 mm difference between both occasions observed by hydroacoustics  
328 was statistically significant. It is evident that the approximately 1 mm difference revealed  
329 in trawl catches between sampling sessions is out of the resolving power of precise sizing  
330 by hydroacoustics means. Pelczarski (2004) noted that the mean growth rate of stocked  
331 whitefish juveniles in the southern Baltic area was 0.82 mm per day, approximately 10  
332 times faster than in this study. The most probable reason for high mortality and slow  
333 growth was the extremely low zooplankton density in Most Lake (maximum of 2.5  
334 ind./l), which is significantly below the threshold value of 20 ind./l noticed by Rellstab et  
335 al. (2004). Especially during the June survey, maraena whitefish juveniles were skinny  
336 and clearly not in good condition. Negatively density dependent growth is the same  
337 reason for slow growth in the coregonids populations when high fish densities cause slow  
338 growth (Marjomäki and Kirjasniemi, 1995; Viljanen et al., 2004; Urpanen et al., 2005).  
339 The density of juvenile maraena whitefish observed in Most Lake was comparable to the  
340 density of coregonids in the natural lake in Germany (see above in discussion) but  
341 extremely low zooplankton density per individual was probably the main reason for the  
342 slow growth and high mortality.

343 Another important aspect of high mortality can be predation. Maraena whitefish  
344 juveniles were predated only by larger perch in the lake. Seventeen percent of all  
345 piscivorous perch had maraena whitefish juveniles in their digestive tracts during the  
346 gillnet survey in 2015 and no maraena whitefish were found in the digestive tracts of  
347 catfish and pike (Peterka, pers. comm.). In such an oligotrophic lake, with limited  
348 densities of juvenile fish of other species, juvenile maraena whitefish obviously represent  
349 an important food source, especially for piscivorous perch. Our results show that the low  
350 productivity of the newly created lake leads to a limited amount of zooplankton, which  
351 became insufficient for the usual densities of maraena whitefish juveniles. This leads to  
352 slow growth during the first year of life and also enhanced mortality, because small  
353 maraena juveniles are longer under predation pressure of perch. Perch predation should  
354 be also taken into account when considering maraena whitefish mortality in a young  
355 oligotrophic lake.

356 Diel vertical distribution of coregonids has been investigated especially for the  
357 earliest (larval) stages (Urpanen et al., 2009; Ylönen et al., 2005; Ventling-Schwank and  
358 Meng, 2005), late summer juveniles (Hamrin, 1986; Sandlund et al.; 1992, Jůza et al.,  
359 2012,) and adults (Jensen et al., 2006; Gjelland et al., 2009; Mehner, 2006a). Diel  
360 distribution of early juveniles in spring is scarce in literature. Coregonid larvae prefer the  
361 warmer upper layer that enables them to grow faster during early spring (Eckmann,  
362 1989). Urpanen et al. (2009) found that vendace and whitefish larvae were aggregated  
363 near the water surface mostly in the top 30 cm during the day in the first weeks after the  
364 ice melted, while Ventling-Schwank and Meng (1995) also found most of whitefish  
365 larvae 12-14 mm in size close to the water surface during both day and night. During this

366 time period schooling was observed for the first time by these authors. It is evident that  
367 DVM of significant extent are not the case of coregonid larvae, however short vertical  
368 migrations caused by avoidance of UV radiation during the day were described (Ylönen  
369 et al., 2005). Clear DVM were described for juvenile vendace in July in lakes in southern  
370 Sweden (Hamrin, 1986). These migrations, depending on temperature, might include  
371 movement to the epilimnion at night, whereas during the day metalimnion around  
372 thermocline is utilized (Hamrin, 1986). In lakes with a strong temperature gradient and  
373 warm epilimnion, juvenile vendace also stayed below thermocline during the night (Jüza  
374 et al., 2012). The DVM are also common in adult coregonids and the trend is similar to  
375 that observed for juvenile coregonids in late summer. The only difference is that juvenile  
376 fish often utilize warmer water with higher light intensities during the day, whereas adults  
377 remain in deeper cold water (Hamrin, 1986). In coregonids diel vertical migrations can be  
378 also partial, when part of population does not migrate and exhibits a resident strategy  
379 (Mehner and Kasprzak, 2011). Spatio-temporal distribution of maraena whitefish in the  
380 early juvenile period with TL of about 35 mm was investigated in this study. According  
381 to Ventling-Schwank and Meng (1995) coregonids in the last larval stage (size 28-29  
382 mm) congregated between 1.5 m depth and the water surface during the night and in the  
383 morning the fish swam in schools as far down as 4 m depth or stayed in loose shoals  
384 between this depth stratum and the surface. Our results indicate that the spatiotemporal  
385 distribution of early juveniles is unique, different from the distribution of larvae and late  
386 summer juveniles. We did not found significant DVM, because the whole epilimnion,  
387 metalimnion and a significant part of the hypolimnion were utilized during both day and  
388 night. During the night fish were homogenously distributed in water column and they

389 were catchable for trawl, whereas during the day obvious shoaling behaviour was  
390 observed. Catching the shoal during the day by ichthyoplankton trawling is improbable  
391 because of their patchy distribution. Light intensities are also important, because in the  
392 absence of vision, fish are unable to react in the ordered manner to an approaching net  
393 (Glass and Wardle, 1989). During the day the catchability of the trawl is therefore  
394 reduced significantly.

395 Shoaling reduces the probability of being preyed on, and the rapid, coordinated  
396 movement by shoals serves to protect individual members (Pitcher and Parrish, 1993).  
397 Our data suggests that the aggregation to shoals during the day is a better strategy for  
398 early juveniles than extensive depth shifts, because the peak of fish density was only  
399 slightly shallower at night than during the day. Changes in activity patterns and vertical  
400 use of habitat typically occur during crepuscular periods (Pitcher and Parrish, 1993).  
401 Disintegration of shoals was obviously connected with decreasing light intensity. During  
402 the night fish were relatively homogeneously distributed in open water and the re-  
403 creation of shoals started again approximately 1.5 hour before sunrise, still in period with  
404 low light intensity. In comparison with shoal disintegration in the evening, shoaling  
405 behaviour was not directly driven by light. Pitcher et al. (1976) found for saithe  
406 *Pollachius virens* (Linnaeus, 1758) that vision is not required in order for the fish to  
407 school and that the lateral line organ could play an important role during schooling. Also  
408 our observation shows that the shoaling behaviour of early juveniles of maraena whitefish  
409 is not driven by light and shoals can be formed during low light intensities at night. The  
410 eye of coregonids is very sensitive to light. when at a light level of 0.05 lx they are able  
411 to prey on zooplankton (Ohlberger et al., 2008) or observe partners during a spawning

412 (Karjalainen and Marjomäki, 2018). Residual light in clear water of an oligotrophic lake  
413 close to the water surface, where the majority of coregonids spend the night, can be  
414 sufficient for starting of shoaling behaviour. More than one hour later, when the light  
415 intensity increases quickly, coregonids juveniles are safe in formed shoals.

416 This study is the first attempt to describe basic ecological parameters such as  
417 mortality, growth and spatiotemporal distribution of early juvenile maraena whitefish in a  
418 post-mining lake shortly after its filling. It shows that in a zooplankton-poor, oligotrophic  
419 lake the early juveniles are affected by extremely high mortality and slow growth.

420 Vertical-temporal distribution was different from the distribution patterns of larvae and  
421 late summer juveniles that are well described in literature. A similar pattern with larvae  
422 was that the majority of early juveniles utilized the epipelagic area but also partly  
423 occurred in deeper layers below thermocline, which is typical for late summer juveniles.  
424 Homogeneous distribution of larvae at night changed to aggregated during the day  
425 (Ventling-Schwank and Meng, 1995). Extensive diurnal depth changes described for late  
426 summer juveniles of coregonids (Hamrin, 1986) were not the case in early juvenile  
427 stages, because the peak of occurrence shifted only slightly between day and night.  
428 However, vertical distribution of vendace was investigated in the study of Hemrin (1986)  
429 so the comparison with maraena whitefish is not straightforward.

430 While maraena whitefish are able to reproduce naturally in a post mining lake,  
431 scarcity of food limits their growth and as such their consequent survival into more stable  
432 second year of life is therefore also limited. Shoaling behaviour during the day, as an  
433 efficient antipredator behaviour, supports the survival of earliest stages but scarcity of  
434 food seems to be the main bottleneck. Future more detailed studies will be necessary to

435 investigate the population dynamics of artificially stocked maraena whitefish in a newly  
436 created post-mining lake.

437

#### 438 **4 Acknowledgements**

439 The study was supported by the Norwegian Financial Mechanism 2009-2014 under  
440 contract number MSMT-28477/2014 (project number 7F14316). The work has also  
441 received funding from the European Union's Horizon 2020 research and innovation  
442 programme under grant agreement No. 677039" project CLIMEFISH. This publication  
443 reflects the views only of the authors, and the Commission cannot be held responsible for  
444 any use that may be made of the information contained therein.

445 We thank the FishEcu team ([www.fishecu.cz](http://www.fishecu.cz)) for the critical reading of the manuscript,  
446 Leslie Tse for English correction and to an anonymous referee for significant improving  
447 of the previous version of the manuscript.

448

449 Authors Contribution Statement:

450 TJ, VD, MČ, ZS, PB, MA, JP performed field work, VD analyzed acoustical data, TJ  
451 wrote the manuscript and processed trawl catches, PB performed statistical analysis, MA  
452 processed zooplankton samples, VD, MČ, ZS, MA, PB, JP did the final editing of the  
453 manuscript and JP provided financial support.

454

455 Conflicts of interest:

456 The authors have declared that no competing interest exist.

457

458 Ethics statement:

459 Fish sampling and treatment was conducted in compliance with guidelines from the  
460 Experimental Animal Welfare Commission under the Ministry of Agriculture of the  
461 Czech Republic.

462

## 463 **5 References**

464 Balk, H., Lindem, T., 2014. Sonar4 and Sonar5-Pro post processing systems.

465 Operator manual version 6.0.3, Lindem Data Acquisition A/S: 464 pp.

466 Bradford, M.J., Cabana, G., 1997. Interannual variability in stage-specific survival  
467 rates and the causes of recruitment variation. In: Chambers, R.C. and Trippel, E.A.  
468 ed. Early life history and recruitment in fish population. Chapman and Hall, London,  
469 469-492.

470 Busch, S., Mehner, T., 2009. Hydroacoustics estimates of fish population depths and  
471 densities at increasing longer time scales. Int. Rev. Hydrobiol. 94, 91-102. doi:  
472 10.1002/iroh.200811092

473 CEN (European Committee for Standardization), 2014. EN 15910, Water quality  
474 guidance on estimation of fish abundance with mobile hydroacoustic methods.  
475 Brussels: 41 pp.

476 Eckmann, R., 1989. The distribution of coregonid larvae (*Coregonus lavaretus* and *C.*  
477 *fera*) from Lake Constance in a vertical temperature gradient. Pol. Arch. Hydrobiol.  
478 36, 485-494.

479 Foote, K.G., Knudsen, H.P., Vestnes, G., MacLennan, D.N., Simmonds, E.J., 1987.  
480 Calibration of acoustic instruments for fish-density estimation: a practical guide.

481 ICES Cooper. Res. Rep. 144, 1-69.

482 Fultner, J., Valvoda, P., 2013. Jezero Most – zhodnocení stabilních poměrů konečných  
483 svahů v okolí zbytkové jámy bývalého lomu Most-Ležáky. In: Sborník příspěvků  
484 konference: Jezera a mokřady ve zbytkových jámách po těžbě nerostů, 44-48.

485 Gassner, H., Wanzenböck, J., Zick, D., Tischler, G., Pamminer-Lahnsteiner, B., 2005.  
486 Development of a fish based lake typology for natural Austrian Lakes >50 ha based  
487 on the reconstructed historical fish communities. *Int. Rev. Hydrobiol.* 90, 422-432.  
488 doi: 10.1002/iroh.200510798

489 Gjelland, K.Ø., Bøhn, T., Horne, J.K., Jensvoll, I., Knudsen, F.R., Amundsen, P., 2009.  
490 Planktivore vertical migration and shoaling under a subarctic light regime. *Can. J. of*  
491 *Fish. Aquat. Sci.* 66, 525-539. doi: 10.1139/F09-014

492 Glass, C.W., Wardle, C.S., 1989. Comparison of the reactions of fish to a trawl gear, at  
493 high and low light intensities. *Fish. Res.* 7, 249–266. doi: 10.1016/0165-  
494 7836(89)90059-3

495 Godlewska, M., Doroszczyk, L., Dlugoszewski, B., Kanigowska, E., Pyka, J., 2014.  
496 Long-term decrease of the vendace population in Lake Pluszne (Poland) – result of global  
497 warming, eutrophication or both? *Ecohydrology and Hydrobiology* 14, 89-95. doi:  
498 10.1016/j.ecohyd.2014.01.004

499 Hamrin, S.F., 1986. Vertical distribution and habitat partitioning between different size  
500 classes of vendace, *Coregonus albula*, in thermally stratified lakes. *Can. J. Fish.*  
501 *Aquat. Sci.* 43, 1617-1625. doi: 10.1139/f86-200

502 Hardy, R., Paragamian, V.L., Neufeld, M.D., 2008. Zooplankton communities and burbot  
503 relative abundance of some oligotrophic lakes of Idaho, USA and British Columbia,

504 Canada. American Fisheries Society Symposium 59, 79-89.

505 Hanazato, T., Iwakuma, T., Hayashi, H., 1990. Impact of whitefish on an enclosure  
506 ecosystem in a shallow eutrophic lake: selective feeding of fish and predation effect  
507 on the zooplankton communities. *Hydrobiologia* 200/201, 129-140.

508 Irz, P., Odion, M., Argillier, C., Pont, D., 2006. Comparison between the fish  
509 communities of lakes, reservoirs and rivers: can natural systems help define the  
510 ecological potential of reservoirs? *Aquat. Sci.* 68, 109-116. doi: 10.1007/s00027-005-  
511 0812-3

512 Jensen, O.P., Hrabik, T.R., Martell, S.J.D., Walters, C.J., Kitchell, J.F., 2006. Diel  
513 vertical migration in the Lake Superior pelagic community. II. Modeling trade-offs at  
514 an intermediate trophic level. *Can. J. Fish. Aquat. Sci.* 63, 2296-2307. doi:  
515 10.1139/f06-125

516 Jurvelius, J., Kolari, I., Leskelä, A., 2011. Quality and status of fish stock in lakes:  
517 gillnetting, seining, trawling and hydroacoustics as sampling methods. *Hydrobiologia*  
518 660, 29-36. doi: 10.1007/s10750-010-0385-6

519 Jůza, T., Kubečka, J., 2007. The efficiency of three fry trawls for sampling the freshwater  
520 pelagic fry community. *Fish. Res.* 85, 285-290. doi: 10.1016/j.fishres.2007.03.001

521 Jůza, T., Vašek, M., Kubečka, J., Sed'a, J., Matěna, J., Prchalová, M., Peterka, J., Říha,  
522 M., Jarolím, O., Tušer, M., Kratochvíl, M., Čech, M., Draštík, V., Frouzová, J.,  
523 Hohausová, E., Žaloudík, J., 2009. Pelagic underyearling communities in a canyon-  
524 shaped reservoir in late summer. *J. Limnol.* 68, 304-314. doi:  
525 10.4081/jlimnol.2009.304

526 Jůza, T., Čech, M., Kubečka, J., Vašek, M., Peterka, J., Matěna, J., 2010. The influence

527 of the trawl mouth opening size and net colour on catch efficiency during sampling of  
528 early fish stages. Fish. Res. 105, 125-133. doi: 10.1016/j.fishres.2010.03.010

529 Jůza, T., Frouzová, J., Brämick, U., Draštík, V., Mrkvička, T., Kubečka, J., 2012. The  
530 vertical distribution of fish in the open water area of a deep temperate mesotrophic  
531 lake assessed by hydroacoustics and midwater trawling. Int. Rev. Hydrobiol. 97, 509-  
532 525. doi: 10.1002/iroh.201101440

533 Karjalainen, J., Marjomäki, T.J., 2018. Communal pair spawning behavior of vendace  
534 (*Coregonus albula*) in the dark. Ecol. Freshw. Fish 27: 542-548.  
535 doi:10.1111/eff.12368

536 Kružíková, L., 2013. Vývoj napouštění jezera Most. In: Sborník příspěvků konference:  
537 Jezera a mokřady ve zbytkových jámách po těžbě nerostů, 89-93.

538 Lahnsteiner, B., Wanzenböck, J., 2004. Variability in the spatio-temporal distribution of  
539 larval European whitefish (*Coregonus lavaretus* (L.)) in two Austrian lakes. Ann.  
540 Zool. Fennici 41, 75-83.

541 Marjomäki, T.J., Huolila, M., 1995. Monitoring the density of Lake Puulavesi vendace  
542 (*Coregonus albula* (L.)) by hydroacoustics, catch per unit effort, virtual population  
543 and catch per swept area. Arch. Hydrobiol. Spec. Issues Advanc. Limnol. 46, 267-  
544 276.

545 Marjomäki, T.J., Kirjasniemi, J., 1995. Density dependent growth of vendace (*Coregonus*  
546 *albula* (L.)) in Lake Puulavesi: A modelling analysis. Arch. Hydrobiol. Spec. Issues  
547 Advanc. Limnol. 46: 89-96.

548 Mehner, T., 2006a. Individual variability of diel vertical migrations in European vendace  
549 (*Coregonus albula*) explored by stationary vertical hydroacoustics. Ecol. Freshw.

550 Fish 15, 146-153. doi: 10.1111/j.1600-0633.2006.00137.x

551 Mehner, T., 2006b. Prediction of hydroacoustic target strength of vendace (*Coregonus*  
552 *albula*) from concurrent trawl catches. Fish. Res. 79, 162-169. doi:  
553 10.1016/j.fishres.2006.01.014

554 Mehner, T., Gassner, H., Schulz, M., Wanzenböck, J., 2003. Comparative fish stock  
555 estimates in Lake Stechlin by parallel split-beam echosounding with 120 kHz. Arch  
556 Hydrobiol. Spec. Issues Advanc. Limnol. 58, 227-236.

557 Mehner, T., Emmrich, M., Kasprzak, P., 2011. Discrete thermal windows cause opposite  
558 response of sympatric cold-water fish species to annual temperature variability.  
559 Ecosphere 2, Article 104. doi: 10.1890/ES11-00109.1

560 Mehner, T., Kasprzak, P., 2011. Partial diel vertical migrations in pelagic fish. J. Animal  
561 Ecol. 80, 761-770. doi: 10.1111/j.1365-2656.2011.01823.x

562 Müller, R., Breitenstein, M., Bia, M.M., Rellstab, C., Kirchhofer, A., 2007. Bottom-up  
563 control of whitefish populations in ultra-oligotrophic Lake Brienz. Aquat. Sci. 69,  
564 271-288. doi: 10.1007/s00027-007-0874-5

565 Ohlberger, J., Mehner, T., Staaks, G., Hölker, F., 2008. Is ecological segregation in a  
566 pair of sympatric coregonines supported by divergent feeding efficiencies? Can. J.  
567 Fish. Aquat. Sci. 65, 2105–2113. doi: 10.1139/F08-120

568 Pelczarski, W., 2004. Mass rearing of juvenile whitefish in brackish water using live  
569 zooplankton. Ann. Zool. Fennici, 41, 165-170.

570 Peterka, J., Čech, M., Draštík, V., Frouzová, J., Jůza, T., Blabolil, P., Vejřík, L., Richta,  
571 J., Kubečka, J., 2013. Vývoj rybích společenstev důlních jezer Milada, Most a  
572 Medard. In: Sborník příspěvků konference: Jezera a mokřady ve zbytkových jámách

573 po těžbě nerostů: 113-116.

574 Peterka, J. 2014. Výsledky průzkumu rybí obsádky jezera Most v roce 2014. Zpráva  
575 Biologického centra AVČR, v.v.i., Hydrobiologického ústavu: 3 pp.

576 Pitcher, T.J., Partridge, L.B., Wardle, C.S., 1976. A blind fish can school. *Science* 194,  
577 963-965. doi: 10.1126/science.982056

578 Pitcher, T.J., Parrish, J.K., 1993. Functions of shoaling in teleost fishes. *In: Behaviour of*  
579 *teleost fishes*. Ed: Pitcher, T.J. Chapman & Hall, London, UK, 363-439.

580 Prchalová, M., Kubečka, J., Čech, M., Frouzová, J., Draštík, V., Hohausová, E., Jůza, T.,  
581 Kratochvíl, M., Matěna, J., Peterka, J., Říha, M., Tušer, M., Vašek, M. 2009., The  
582 effect of depth, distance from dam and habitat on spatial distribution of fish in an  
583 artificial reservoir. *Ecol. Freshwat. Fish* 18, 247-260. doi: 10.1111/j.1600-  
584 0633.2008.00342.x

585 Rellstab, C., Bürgi, H.R., Müller, R., 2004. Population regulation in coregonids: the  
586 significance of zooplankton concentration for larval mortality. *Ann. Zool. Fennici* 41,  
587 281-290.

588 Rudstam, L., Magnuson, J.J., 1985. Predicting the vertical distribution of fish  
589 populations: Analysis of cisco, *Coregonus artedii*, and yellow perch, *Perca*  
590 *flavescens*. *Can. J. Fish Aquat. Sci.* 42, 1178-1188. doi: 10.1139/f85-146

591 Říha, M., Jůza, T., Prchalová, M., Mrkvička, T., Čech, M., Draštík, V., Muška, M.,  
592 Kratochvíl, M., Peterka, J., Tušer, M., Vašek, M., Kubečka, J., 2012. The size  
593 selectivity of the main body of a sampling pelagic pair trawl in freshwater reservoirs  
594 during the night. *Fish. Res.* 127-128: 56-60. doi: 10.1016/j.fishres.2012.04.012

595 Sandlund, O.T., Naesje, T.F., Jonsson, B., 1992. Ontogenetic changes in habitat use by

596 whitefish, *Coregonus lavaretus*. Environ. Biol. Fish. 33, 341-349.

597 Šutera, V., Lenc, P., Kroupa, F., Bělohoubek, J., Kuncová, J., Vrba, F., Egrtová, Z.,  
598 Skála, E., Kříž, M., Beran, L., Holec, M., Vysoký, V., Černý, J., Benda, P., Peterka,  
599 J., Majer, P., Vondráček, J., 2012. Příroda nádrže Milada. Území po zatopení lomu  
600 Chabařovice, 208 pp.

601 Urpanen, O., Huuskonen, H., Marjomäki, T.J., Karjalainen, J., 2005. Growth and size-  
602 selective mortality of vendace (*Coregonus albula* (L.)) larvae. Boreal Environ. Res.  
603 10, 225-238.

604 Urpanen, O., Marjomäki, T.J., Viljanen, M., Huuskonen, H., Karjalainen, J., 2009.  
605 Population size estimation of larval coregonids in large lakes: Stratified sampling  
606 design with a simple prediction model for vertical distribution. Fish. Res. 96, 109-  
607 117. doi: 10.1016/j.fishres.2008.09.004

608 Ventling-Schwank, A.R., Meng, H.J., 1995. Vertical migration of coregonid larvae in the  
609 first two months of development. Aquat. Sci. 57/1, 1-13.

610 Viljanen, M., Turunen, T., Väisänen, P., 2004. Fluctuations in year-class strength and  
611 growth of the vendace (*Coregonus albula* (L.)) in the small, mesohumic, oligotrophic  
612 Suomunjärvi, a lake in eastern Finland. Ann. Zool. Fennici 41, 241-248.

613 Ylönen, O., Huuskonen, H., Karjalainen, J., 2005. Effect of UV radiation on the vertical  
614 distribution of vendace [*Coregonus albula* (L.)] larvae in Finnish lakes. Ecol.  
615 Freshwat. Fish. 14, 161-167. doi: 10.1111/j.1600-0633.2005.00085.x

616

617

618 **Figure captions**

619

620 **Fig. 1:** (a) Map of the Most Lake and its location within the Czech Republic – lines in the  
621 lake map represent 10 m isobaths. Triangles sign the locations of stationary echosounding  
622 (black) and zooplankton sampling (white). (b) Diagram showing the sampling operation.

623

624 **Fig. 2:** Thermal and oxygen stratification in the Most Lake on May 20 (a), June 3 (b) and  
625 June 10 (c). Secchi depth (SecD) is also displayed. Only depth up to 25 m is shown.

626 Below this depth temperature and oxygen concentration was constant.

627

628 **Fig. 3:** Depth distribution from hydroacoustic monitoring of juvenile maraena whitefish  
629 during day (a) and night (b) on May 20 (black column) and June 3 (white column) 2015.

630 Error bars represent standard errors of mean.

631

632 **Fig. 4:** Density of juvenile maraena whitefish in the Most Lake on May 20 (black  
633 column) and June 3 (white column) in trawl catches and hydroacoustical observations.

634 Error bars represent standard errors of mean.

635

636 **Fig. 5:** Echograms showing typical distribution of juvenile maraena whitefish during day  
637 18:30 (a), growing dark 19:40 (b), night 22:30; 1:00 (c,d), morning before sunrise 2:30

638 (e) and morning after sunrise 6:00 (f) on June 10-11. Twenty-three minutes of monitoring  
639 are shown in the echograms.

640

641 **Fig. 6:** Temporal changes in density of maraena whitefish juveniles during stationary  
 642 hydroacoustic monitoring on June 10-11 above the depth of 31 m. Sunset and sunrise are  
 643 displayed by arrows.

644

645

646

647 Table 1: Density and species composition of zooplankton during three sampling sessions  
 648 in two (three 10 June) depth layers in the Most Lake in 2015. For groups included in  
 649 category “Others” see Results section.

650

651

Date	Depth (m)	Zoopl.dens. (ind./l)	Cyclopoid nauplii	Cyclopoid copepodites	Species composition %			
					Calanoid nauplii	Calanoid copepodites	Daphnia longispina	Others
20.5.	0-2	0.84	39	29	24	4	1	3
	6-8	2.54	62	12	9	15		2
3.6.	0-2	0.09	11	22	29	4	15	19
	6-8	1.03	72	4	15	5	3	1
10.6.	0-2	0.02		29	43		14	14
	6-8	2	63	4	15	12	2	4
	11-55	0.22	1	5	63	18	9	4

652

653

654

655

656

657

658

659

660

661

662

663

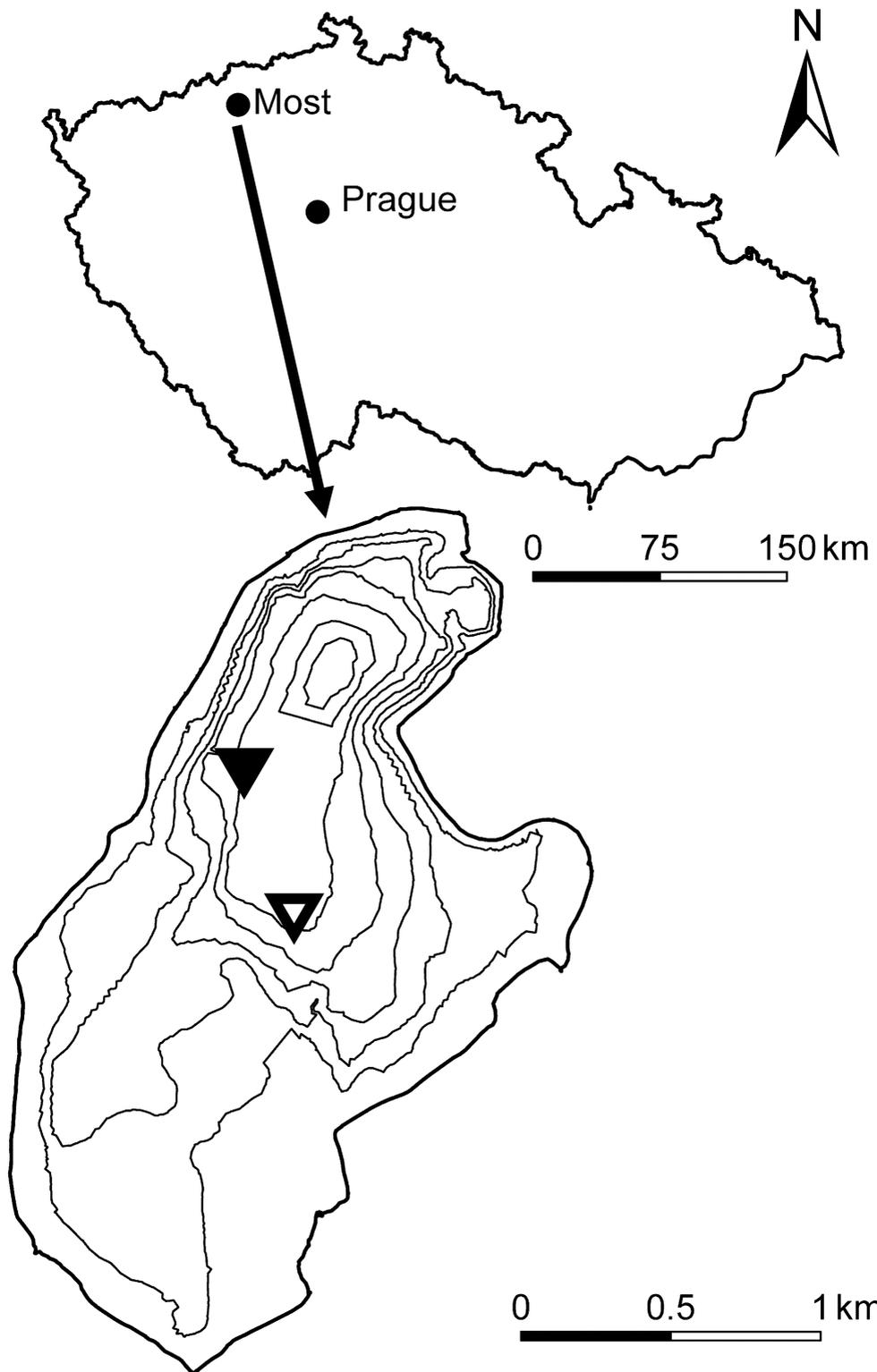
664

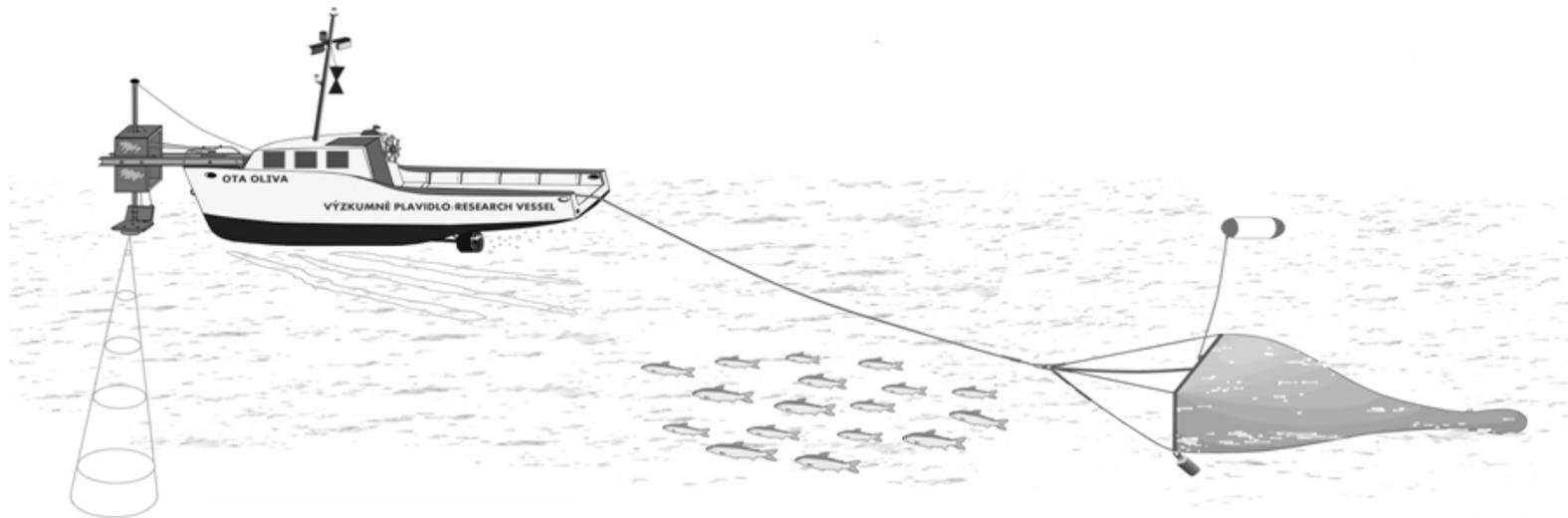
665

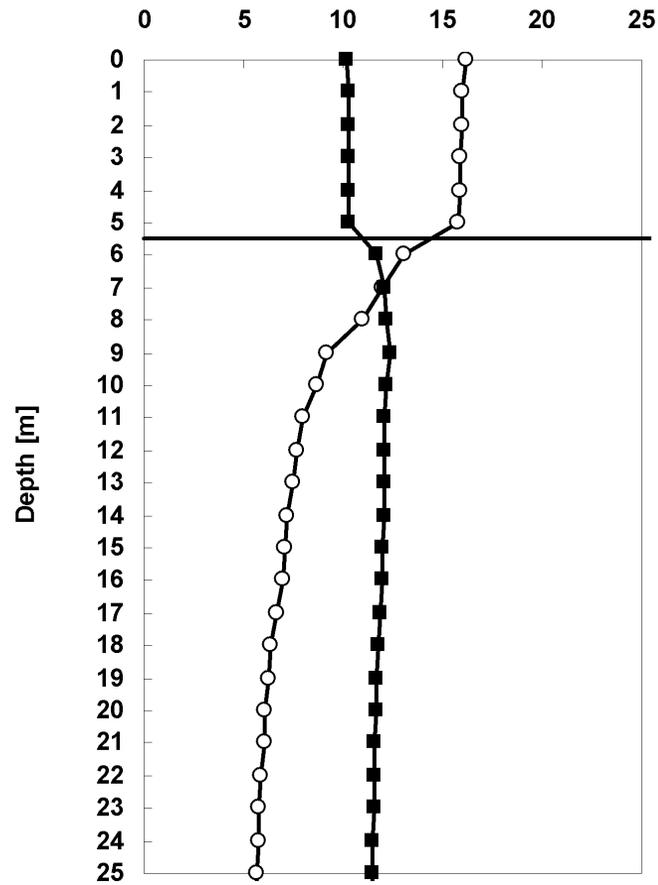
666

667

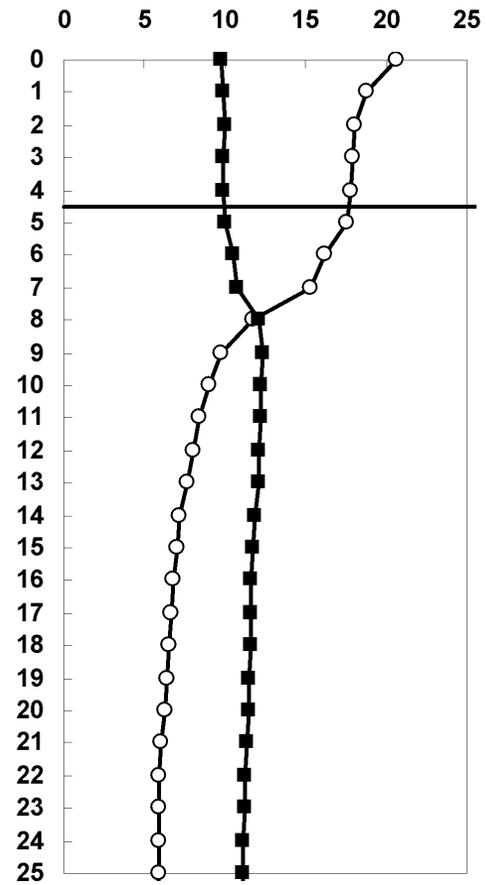
668  
669  
670  
671



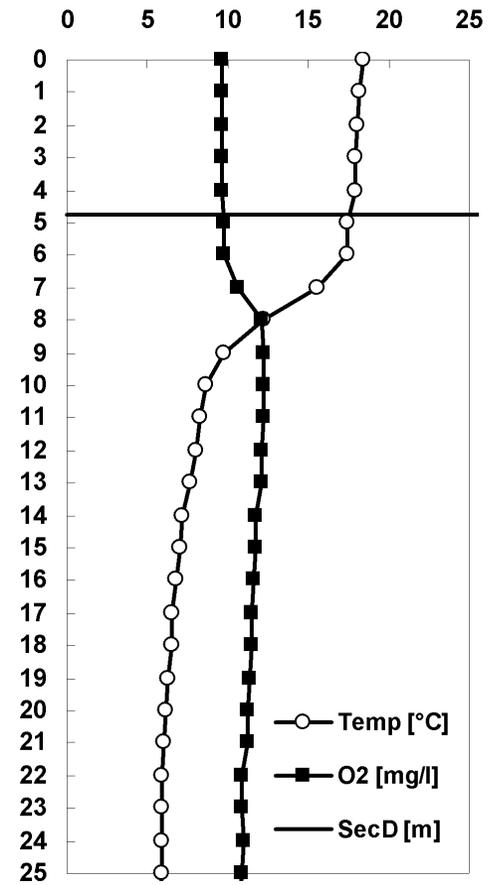




a)



b)



c)

