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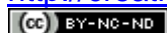*sharing our research*

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Contact UKCEH NORA team at
noraceh@ceh.ac.uk

1 **Wide-spread inconsistency in estimation of lake mixed depth impacts interpretation of**

2 **limnological processes**

3 Emma Gray[1,2], Eleanor B. Mackay[1], J. Alex Elliott[1], Andrew M. Folkard[2], Ian D. Jones[3]

4   1. Centre for Ecology and Hydrology, Lancaster, Lancaster Environment Centre, Library

5   Avenue, Bailrigg, Lancaster, LA1 4AP, UK

6   2. Lancaster Environment Centre, Library Avenue, Lancaster University, Lancaster LA1

7   4YQ

8   3. Biological and Environmental Sciences, University of Stirling, Stirling, FK9 4LA,

9   UK

10 Correspondence: Emma Gray, tel: +44 1524 595952, e-mail: emma.gray9229@gmail.com

11   **Abstract**

12   The mixed layer, or epilimnion, is a physical concept referring to an isothermal layer at the

13   surface of a water body. This concept is ubiquitous within limnology, is fundamental to our

14   understanding of chemical and ecological processes, and is an important metric for water

15   body monitoring, assessment and management. Despite its importance as a metric, many

16   different approaches to approximating mixed depth currently exist. Using data from field

17   campaigns in a small meso-eutrophic lake in the UK in 2016 and 2017 we tested whether

18   different definitions of mixed depth resulted in comparable estimates and whether variables

19   other than temperature could be assumed to be mixed within the layer. Different methods

20   resulted in very different estimates for the mixed depth and ecologically important variables

21   were not necessarily homogenously spread through the epilimnion. Furthermore, calculation

22   of simple ecologically relevant metrics based on mixed depth showed that these metrics were

23   highly dependent on the definition of mixed depth used. The results demonstrate that an

24   idealised concept of a well-defined fully mixed layer is not necessarily appropriate. The

25   widespread use of multiple definitions for mixed depth impairs the comparability of different

26   studies while associated uncertainty over the most appropriate definition limits the

27   confirmability of studies utilising the mixed depths.

28   Keywords: mixed depth, lake, phytoplankton, oxygen, euphotic depth

1.   Introduction

The "mixed layer" of a lake is a physical concept referring to a layer at the surface of a lake within which temperature is uniform (Robertson and Imberger, 1994; Sverdrup, 1953) (Fig.1a). The depth of the mixed layer, or epilimnion, depends on the balance between stratifying and mixing forces, with deepening being driven by wind mixing and convective cooling and shallowing being driven by warming (Wüest and Lorke, 2003). In stratified lakes, this layer typically overlies water in which the mixing rates are significantly smaller, enabling vertical gradients to develop in variables of interest, including temperature, particulate matter and dissolved gasses. This concept is used extensively and underpins our understanding of limnological processes. It is therefore fundamental for monitoring and assessment purposes (Jaša et al., 2019; Peter et al., 2009; Schauser et al., 2003) and studies on the restoration of lakes (Hoyer et al., 2015; Hupfer et al., 2016; Stroom and Kardinaal, 2016) as well as the limnology of lakes (Brainerd and Gregg, 1995; Diehl, 2002; Wüest and Lorke, 2003).

There are, though, many practical problems generated by the concept of an idealised mixed depth. The layer is mixed by turbulence, but turbulence itself is not commonly measured directly. Furthermore, where turbulence has been directly measured it has shown the actively mixing layer can be substantially shallower than the isothermal layer (MacIntyre, 1993; Tedford et al., 2014). These measurements have indicated that temperature differences as little as 0.02 ℃ can delineate regions with different mixing rates (MacIntyre, 1993). The "mixed layer" can therefore be sub-divided into two regions; an actively mixed upper layer and a region below whose depth is determined by recent mixing, and characterised as "mixed" by its homogeneity in terms of one or more variables, most commonly temperature or density (Brainerd and Gregg, 1995). As temperatures are frequently only measured to an accuracy of 0.1 or 0.2 ℃, and at only 0.5 m or 1 m vertical resolution or less, the most

commonly collected limnological temperature profiles cannot identify this actively mixing

layer. It is even questionable whether this depth of recent mixing can be accurately

determined using relatively coarse resolution measurements, as sharp changes in gradient can

become smeared, blurring the boundary between epilimnion and metalimnion. Furthermore,

temperature profiles can be complicated by the presence of secondary thermoclines

developing during the daytime, enhancing the potential for confounding results arising from

different mixed depth definitions. Such diurnal thermoclines can affect gas fluxes (MacIntyre

et al., 2002) and the vertical distribution of nutrients and phytoplankton (MacIntyre and

Melack, 1995). These secondary thermoclines can complicate the estimation of a

systematically defined mixed depth. Each ecological variable is also subject to different

source and sink terms operating at different timescales. Thus, physical mixing within the

epilimnion might be sufficient for homogenising a variable with slow rates of production or

loss, but the same mixing may be insufficient for homogenising a variable with faster

production and loss.

The necessity to infer the mixed depth without direct turbulence measurements has led to a

vast array of methods being developed for defining the depth of the mixed layer, typically

exploiting the notion of a vertical limnological profile being generated by rapid vertical

mixing in the surface waters of a lake and much diminished mixing beneath. A Web of

Science search using terms 'lake' AND 'mix* depth' AND 'layer' followed by removal of

non-lake references or those referring to sediment mixed depths or chemoclines identified at

least 313 research papers explicitly referring to a mixed layer. Often references to the mixed

depth were descriptive (24 %) or theoretical (16 %) rather than quantitative and in 10 % of

papers the mixed depth was arbitrarily or visually defined. The remaining studies determined

the mixed depth using a variety of methods which included being calculated within lake

models (11 %), fixed within mesocosm or laboratory experiments (8 %), directly measured

79   through turbulence (8 %) or calculated using a secondary variable (23 %). The latter method

80   could be categorised into temperature (Coloso et al., 2008) or density gradients (Staehr et al.,

81   2012), temperature (Wilhelm and Adrian, 2007), or density differences (Winder et al., 2009)

82   and isotopic (Imboden et al., 1983) or chemical tracers (Maiss et al., 1994). Temperature

83   gradients were most commonly used to define the mixed depth, followed by density

84   gradients, temperature thresholds and density thresholds. There are, however, at least 20

85   different thresholds and gradients of temperature or density currently being applied to

86   estimate the mixed depth (Table 1).

87   Implicitly, the common usage of such a wide variety of methods suggests that each one is

88   assumed to define approximately the same depth of mixed layer. If the vertical profiles of a

89   lake match the idealised concept, then this should be true, but any discrepancies from an

90   idealised profile could lead to different methods producing different estimates for the mixed

91   depth. This would make a cross comparison of mixed layer depths between different studies

92   meaningless and poses difficulties for the understanding and quantification of linkages to

93   biological or chemical processes.

94   These methodological caveats are of particular concern when using the mixed depth as an

95   explanatory or predictive variable in chemical and ecological studies. For example, the mixed

96   depth can control the vertical distribution of phytoplankton and therefore the light climate to

97   which they are exposed (Diehl et al., 2002). The ability for a phytoplankton community to

98   grow and maintain biomass depends on the ratio of the mixed depth to the euphotic depth

99   (Huisman, van Oostveen, & Weissing, 1999) in addition to the loss of cells due to sinking

100   and the motility and light affinity of the species in the community (Diehl et al., 2002;

101   Huisman et al., 2002; Jäger et al., 2008). Mixing that encroaches into the hypolimnion during

102   stratification can also incorporate nutrients into the mixed layer increasing their availability

103   for phytoplankton near the surface (Kunz and Diehl, 2003) and mix oxygen into the

104 hypolimnion potentially reducing future internal loading (Mackay et al., 2014). Having a

105 robust estimate of mixing is therefore required to understand the vertical positioning and

106 composition of phytoplankton taxa within a lake, along with the mechanisms of bloom

107 formation (Cyr, 2017) and the associated water quality impacts (Dokulil and Teubner, 2000;

108 Jaša et al., 2019).

109 Similarly, the vertical pattern of productivity in the water column is influenced by the mixed

110 depth and water clarity (Obrador et al., 2014); therefore lake metabolism studies require a

111 robust mixed depth estimation. The depth of surface mixing determines how much of the

112 water column has regular contact with the atmosphere, influencing the depth of oxygen

113 penetration. This is particularly important in stratified, productive systems where incomplete

114 mixing can result in anoxia in the hypolimnion due to the oxidisation of organic matter by

115 bacteria (Nürnberg, 1995). The direction of the flux of oxygen into and out of the mixed layer

116 will also vary depending on the vertical distribution of primary producers in the water column

117 relative to the mixed depth (Obrador et al., 2014; Peeters et al., 2016; Staehr et al., 2012,

118 2010).

119 Despite the widespread use of the mixed depth concept and the large number of methods used

120 to estimate mixed depth, there is a lack of research evaluating the consistency among

121 methods of mixed depth estimation and the implications of using different estimates when

122 interpreting ecological and chemical data. This study therefore aims to: (1) determine if

123 different methods of calculating the mixed depth produce comparable estimates; (2) evaluate

124 the extent to which ecological and chemical parameters are homogenously distributed

125 throughout the mixed depth; (3) evaluate how the choice of mixed depth definition may

126 influence the calculation of simple example metrics relevant to studies of phytoplankton

127 dynamics and metabolism. Analysis of vertical profiles of physical, chemical and ecological

128   parameters collected from a small meso-eutrophic lake in the UK were used to address these

129   aims.

130 **2.**   **Materials and methods**

131 *2.1.   Site description*

132 Blelham Tarn is a small (surface area 0.1 km^2), moderate depth lake (mean depth 6.8 m,

133 maximum depth 14.5 m) (Ramsbottom, 1976), which stratifies typically for seven to eight

134 months each year between spring and autumn. It is located in north-west England, UK

135 (54°24'N, 2°58'W) and lies on the meso-eutrophic boundary (mean total phosphorus 24.5

136 mg m^{-3}) (Maberly et al., 2016).

137 *2.2.   Field methods and data collection*

138 Vertical profiles of oxygen, chlorophyll *a* (measured via fluorescence as a proxy for

139 phytoplankton biomass), temperature, specific conductivity and pH were measured using a

140 YSI EXO2 multi-parameter sonde. Given the limitations of chlorophyll *a* fluorescence

141 profiles (Gregor and Maršálek, 2004), water samples for chemical determination of

142 chlorophyll *a* were taken at metre intervals in the water column (1-10 m) using standard

143 methods (Mackereth et al., 1979). Vertical profiles of chlorophyll *a* obtained using both

144 methods were compared visually and statistically using linear regression (R^2=0.53, p<0.001).

145 The probes were calibrated every six weeks according to manufacturer specifications.

146 Profiles were measured weekly between 9:30 am and 11 am during the stratified period (46

147 sample days), defined here as when the density difference from the surface to the bottom was

148 greater than 0.1 kg m^{-3}, at 0.5 m intervals in the water column from 1 m to 13 m (2016) and

149 0.5 m to 13 m (2017).

150 A LI-COR underwater quantum cos-corrected sensor was also used to measure

151 photosynthetically active radiation (PAR); measurements were taken just below the surface

152 and then at one-metre intervals from 1 m to 9 m. The natural logarithm of the PAR

153 measurements were regressed with depth and the slope of the equation was used to estimate

154 the extinction coefficient (k) for each sample day. The euphotic depth (z_{eu}) was then defined

155 as the depth where only 1 % of the surface measurement of PAR remained:

156 $$z_{eu} = \ln(100)\,/k \qquad\qquad (1)$$

157

158 *2.3. Methods for estimating mixed depth, z_{mix}*

159 Four methods of mixed depth estimation were tested for consistency, the first two methods

160 used threshold changes in density (Method 1a) and temperature (Method 1b) from surface

161 values to determine the depth of the mixed layer whereas Methods 2 and 3 determined the

162 depth of the mixed layer statistically.

163 *2.3.1. Method 1a: Density threshold*

164 The baseline mixed depth for this study was calculated as the depth at which the density first

165 became 0.1 kg m^{-3} greater than the density at the surface (e.g. Andersen et al., 2017) (Fig.1b).

166 Water density was calculated using water temperature and salinity from equations within

167 Lake Analyzer (Read et al., 2011). Salinity was calculated from conductivity using the

168 GibbsSeaWater (GSW) Oceanographic Toolbox (McDougall and Barker, 2011).

169 *2.3.2. Method 1b: Temperature threshold*

170 Temperature is frequently used instead of density to define the mixed layer, therefore a 1 $^{\circ}$C

171 difference in temperature from the surface was used, roughly equating to a 0.1 kg m^{-3} density

172 difference at moderate water temperatures. Below these temperatures the density difference

173 will be smaller and vice versa for higher temperatures (Fig.1b).

174 Equivalent and directly comparable threshold methods cannot be applied to chemical and

175 ecological variables due to their different units of measure. Therefore, two statistical methods

176 were used which avoid the use of an arbitrary threshold or gradient and could therefore be

applied to profiles of chlorophyll *a* fluorescence, oxygen, pH and specific conductivity, as well as density profiles. If the idealised concept of the stereotypical shape of the vertical density profile holds true then both these statistical methods should provide estimates of mixed depth which are reasonably consistent with each other and with the mixed depth estimated by a density threshold (Fig. 1). Similarly, if the epilimnion is truly mixed then applying these methods to other limnological variables should also estimate a comparable depth for the bottom of the mixed layer.

2.3.3. Method 2: Intersection of the plane of maximum gradient with the plane of the profile minimum (or maximum)

A Generalised Additive Model (GAM) with a gamma error distribution and logarithm link function was fitted to every profile for each variable collected (46 sample days, 6 variables = 276 profiles in total) using the mgcv package (version 1.8-26) (Wood, 2011) within the R programming language (R Core Team, 2018). The number of knots used in the GAM were optimized and fixed for each variable and the fitted values were predicted at 0.5 m depth intervals. Using the fitted predictions, the first derivative was calculated using forward differences to find the depth of the maximum gradient. At the depth of the maximum gradient the plane was extrapolated to all depths using the intercept and slope. Vertical lines were then drawn corresponding to the mean of three maximum and minimum values from each profile. The depth where the vertical lines intersected the extended maximum gradient line marked the top and bottom of the thermocline, or equivalent for other variables, that is, the mixed layer depth and the top of the hypolimnion, respectively (Fig. 1c).

2.3.4. Method 3: Depth of statistically significant deviation

Using the confidence intervals from the first derivative of the fitted GAM, the sections of the profile where changes in the gradient were significantly different from zero were calculated

201 (Simpson, 2018). The section of the profile that contained the depth of the maximum

202 gradient was identified, with the upper and lower values of this section being the mixed depth

203 and the top of the hypolimnion, respectively (Fig.1d).

2.4. Comparison of mixed depth method estimations

205 To compare the differences in mixed depth estimates, the mean difference (including the

206 directional sign of the difference i.e. shallower or deeper), mean absolute difference (not

207 including the directional sign), root mean square error and the range were calculated for the

208 different estimates of mixed depth for each sample day. The relative shift in the mixed depth

209 (shallowing, deepening or no change) was calculated between sample days as well as the

210 percentage of instances in which the methods were consistent. Initial comparisons were made

211 between temperature and density thresholds (Methods 1a and 1b), followed by comparing

212 Method 1a with the two statistical methods (Methods 2 and 3).

213 Statistical models were then used to determine if the depth of the mixed layer calculated from

214 density using Method 2 was a good predictor for the depth of the mixed layer calculated by

215 Method 2 from the other variables. A similar assessment was carried out using Method 3.

216 This was initially assessed by linear regression of the density-derived mixed depth against the

217 depth of the mixed layer derived from chlorophyll *a*, oxygen, pH and specific conductivity

218 profiles. The residuals from each regression were visually inspected for normality,

219 homoscedasticity, autocorrelation, and the influence of outliers with no issues found. Non-

220 linearity was initially assessed visually and then each model was fitted with a quadratic

221 density-derived mixed depth term to optimise the model fit. The density-derived mixed depth

222 as a predictor of the mixed depth calculated from oxygen and specific conductivity profiles

223 was better described using a quadratic model whereas the equivalent for chlorophyll *a* and pH

224 were best described using a linear model based on the *F*-test.

225 *2.5. Determining the homogeneity of ecological and chemical parameters within the mixed*

226 *depth*

227 The coefficient of variation (expressed as a percentage) and the range of values for

228 temperature, chlorophyll *a*, oxygen, specific conductivity and pH within the mixed layer were

229 calculated for each method of mixed depth estimation and compared to the equivalent

230 variation for the whole water column.

231 *2.6. Calculation of example metrics using different mixed depth estimates*

232 The following metrics were calculated for each sample day using mixed depth estimates for

233 Method 1a, Method 2 and Method 3: (a) the percentage of oxygen and chlorophyll *a* within

234 the mixed layer and whether more than 50% of chlorophyll *a* and oxygen were contained

235 within the mixed layer, (b) the directional flux of oxygen, that is, the sign of the difference in

236 the mean concentration of oxygen in the mixed layer compared to the concentration 0.5 m

237 below and, (c) the ratio between the mixed depth and euphotic depth.

238 **3.   Results**

239 *3.1. Comparing mixed depth estimates*

240 *3.1.1. Methods 1a and 1b*

241 Mixed depth estimates calculated using temperature were on average 0.7 m deeper than

242 estimates calculated from the density baseline, equivalent to an increase of 70 %. The RMSE

243 was 1.1 m. The differences differed temporally (Fig.2) with the maximum daily range in

244 values being 5.5 m.

245 *3.1.2. Methods 1a, 2 and 3*

246 There were large differences between the density-derived estimates of mixed depth calculated

247 using the three different methods (Fig. 3). Method 2 estimates were shallower than Method

248 1a by 0.8 m on average, whereas Method 3 estimates were deeper by 0.6 m (Table 2). The

249   daily differences in the estimates had no consistent systematic pattern (Fig. 3), with the

250   largest daily range in values (5 m) occurring between Method 1a and Method 2. The methods

251   were also inconsistent on whether there was shallowing, deepening or no change in the mixed

252   depth between sample days with methods only being directionally consistent for 51 % of

253   sample days (one method disagreed for 42 % of sample days and three different answers

254   occurred for 7 % of sample days).

255   *3.2. Using the density-derived estimate as a predictor for ecological and chemical derived*

256   *estimates of mixed depth*

257   Mixed depths calculated using ecological and chemical parameters were varied and dissimilar

258   from the estimates calculated from density (Fig. 4). The density-derived estimate was found

259   to be a poor predictor for the estimates using chlorophyll *a,* pH and specific conductivity

260   profiles, with low *F*-statistic values and weak or insignificant r^2 and *p*-values (Table 3). A

261   significant relationship was found between the depth of the oxygen derived mixed depth and

262   the density derived mixed depth using a quadratic model. Further statistical testing, however,

263   demonstrated that at depths shallower than 4.5 m the density derived mixed depth was a poor

264   predictor for the equivalent oxygen derived mixed depth.

265   Mixed depth estimates were also a poor predictor of the chlorophyll *a* maxima for 2016 and

266   2017 and a good predictor for the depth of the oxygen maxima during 2016 using Method 3

267   but not during 2017 when no significance was found (Table 3).

268   *3.4. Determining the homogeneity of limnological variables within the mixed layer*

269   As expected, temperature had a small coefficient of variation and range of values within the

270   mixed layer compared to the whole water column suggesting a homogenous distribution of

271   heat within the mixed layer (Fig. 5; Table 4). The coefficient of variation and range of values

272   in the mixed layer for specific conductivity were also small relative to the whole water

273   column suggesting homogeneity (Fig. 5; Table 4). Though the coefficient of variation was

274   relatively low for oxygen in the mixed layer, values could differ by up to 2.4 mg/L at times

275   suggesting that oxygen concentrations were not always homogenous (Fig. 5; Table 4).

276   Chlorophyll *a* and the concentration of hydrogen ions demonstrated the largest coefficients of

277   variation and range of values in the mixed layer relative to the water column (Table 4) and

278   therefore had a heterogeneous distribution in the mixed layer for much of the stratified period

279   (Fig.5).

280   *3.5. The impact of using different mixed depth estimates when calculating example metrics*

281   *3.5.1. The percentage of chlorophyll* a *and oxygen within the mixed layer*

282   The mean percentage of chlorophyll *a* in the mixed layer during the stratified period differed

283   between methods. Even the proportion of days when the majority (>50 %) of chlorophyll *a*

284   was contained within the mixed layer varied greatly depending upon the mixed layer

285   estimation method (Fig 6). For 2016 the proportion of days when the majority of chlorophyll

286   a was contained within the mixed layer was 35 %, 74 % and 39 % for Methods 1a, 2 and 3

287   respectively, whereas for 2017 the values were 48 %, 65 % and 30 %. The methods only all

288   agreed for 50 % of sampling days on whether the majority of chlorophyll *a* was contained

289   within the mixed layer (Fig.6).

290   The mean percentage of oxygen in the mixed layer for the whole of the stratified period also

291   differed depending on the definition used for mixed depth (Fig. 6). The proportion of days

292   when the percentage of oxygen in the mixed layer was greater than 50 % varied between

293   methods (Fig. 6). For 2016 the proportion of days when the majority of oxygen was

294   contained within the mixed layer was 43 %, 83 %, and 43 % for Methods 1a, 2 and 3

295   respectively whereas for 2017 the values were 61 %, 74 % and 35 %. The methods all agreed

14

296   on whether the majority of oxygen in the water column was in the mixed layer for less than

297   half (46 %) of the sampling days (Fig.6).

298   *3.5.2. The directional flux of oxygen*

299   The direction of the flux of oxygen between the mixed layer and the layer below, as

300   determined by whether concentration was greater within or beneath the mixed layer, was not

301   always consistent between methods with contradictory results occurring 24 % of the time

302   (Fig.7). Even when the direction of the oxygen flux was consistent between methods the size

303   of the gradient between the mixed layer and the water directly underneath was markedly

304   different (Fig.7). Thus, both the direction and magnitude of the flux of oxygen between the

305   mixed layer and the thermocline were highly dependent on how the mixed layer depth was

306   defined.

307   *3.5.3. Mixed layer to euphotic layer depth ratio*

308   The ratio of mixed depth to euphotic depth was very different depending on which method

309   was used to calculate mixed depth (Fig. 8). The mean ratio calculated using Method 2 (0.9)

310   was typically greater than that using Method 1a (0.7), which was itself greater than that using

311   Method 3 (0.6). As well as the systematic differences there was also a lot of temporal

312   variation between the consistency of the estimates (Fig.8). The mean difference between the

313   mixed depth to euphotic depth ratio between Method 1a and Method 2 was 0.32 and between

314   Method 1a and Method 3 was 0.90, with methods being contradictory as to whether the

315   euphotic or the mixed depth was deeper for 20 % of sample days (Fig. 8).

316

4. Discussion

The results demonstrate that different approaches to mixed depth estimation are not
necessarily comparable, even when those methods are underpinned by the same conceptual
description of a mixed depth. This is the case when the same method is used with different
variables (Fig. 4) or when different methods are used with the same variable (Fig. 3). It is
particularly worth noting that, estimations of mixed depth from temperature profiles differ
from estimations of mixed depth derived from density profiles (Fig. 2). This is partly due to
the non-linear relationship between temperature and density and partly due to the deviation of
observed density profiles from an idealised profile, such as when both diel and seasonal
pycnoclines are present. The functional role density gradients have in influencing mixing
rates suggests that density be preferred to temperature as a variable for defining mixing
length scales, despite the frequency with which temperature is still used (Table 1). The
number of methods and variables examined here for estimating mixed depth is a relatively
small sample compared with the vast array of mixed depth definitions in the literature (Table
1). Nevertheless, they indicate that even the direction of change in mixed depth over time can
be dependent on the method chosen for its calculation. To some extent the development of
automated tools for calculating mixed depth such as Lake Analyzer (Read et al., 2011), offers
a means to reduce the proliferation of definitions.

 It is not necessarily the case though, that, a single definition of mixed depth estimation is
always appropriate, as different definitions might be better suited to different conditions or
different ecological questions. An example is the variety of mixed layer definitions used in a
study comparing depth-related oxygen metabolism across disparate lakes (Giling et al.,
2017), where it was considered that no one definition was suitable for all the lakes. It may
also be sometimes appropriate, depending on the purpose of the study, to adopt a definition
using a different variable than density or temperature, as the occurrence of a homogenous

16

342   surface layer in one property does not guarantee that it will be homogenous in another

343   property (Table 4, Fig. 5). Studies interested in identifying homogenous distributions of

344   phytoplankton, for example, for which gradients of light and nutrients as well as turbulence

345   are controlling their distribution (Huisman et al., 1999; Kunz and Diehl, 2003), could be

346   inaccurate if a density definition of mixed layer was used. That the depth of the mixed layer

347   is highly dependent on the definition, and that not all properties will be evenly distributed

348   within it, necessitates caution when analysing vertically resolved limnological data. Even the

349   analysis of simple metrics relating to the distribution of chlorophyll a and oxygen

350   demonstrates that the choice of mixed depth definition could influence the interpretation of

351   results (Fig. 6-8). Thus, where phytoplankton samples are integrated over the epilimnion for

352   assessing water quality (Noges et al., 2010) the assessment could be influenced by the

353   definition of mixed layer adopted. Similarly, whether phytoplankton maxima are within or

354   beneath the mixed layer will depend on the definition chosen. The oxygen flux into and out

355   of the mixed layer is important for metabolism studies (Obrador et al., 2014), but the

356   magnitude of the oxygen gradient between layers, and therefore the magnitude and direction

357   of the oxygen flux, is highly dependent on the definition of mixed depth (Fig. 7). Nutrient

358   fluxes will be similarly dependent on definition, which may have consequences for water

359   quality determination and restoration responses (Hupfer et al., 2016; Read et al., 2014;

360   Schauser et al., 2003). In general, the accuracy of flux estimated will be limited without

361   turbulence measurements. The widely used ratio of the mixed depth to euphotic depth was

362   also dependent on the definition of mixed depth used (Fig. 9). This is consequential, when

363   explaining the formation of sub-surface phytoplankton maxima, which are thought to occur in

364   eutrophic systems when the euphotic depth is deeper than the mixed depth (Hamilton et al.,

365   2010; Leach et al., 2018; Mellard et al., 2011).

366   The interrogation and interpretation of vertical profiles is a fundamental and burgeoning area

367   of limnological study (Brentrup et al., 2016; Hamilton et al., 2010; Leach et al., 2018;

368   Obrador et al., 2014) and will require careful consideration of how best to use mixed depth as

369   a predictive or explanatory variable or as a determinant of water quality monitoring. One

370   approach is to assess the impact of using different mixed depth estimates when analysing

371   results. For example, the Giling et al., (2017) study on metabolism found that halving or

372   doubling the threshold density gradient used to estimate the mixed depth changed the

373   estimated thickness of the metalimnetic depth zone by 22 %. For the study, this inconsistency

374   was deemed relatively insignificant to the findings, however the authors highlighted that this

375   would become problematic when aggregating metabolic rates to the metalimnion and

376   hypolimnion (Giling et al., 2017). Another approach is to examine systematically which

377   method or methods are more consistently useful than others for approximating a mixed depth.

378   **5. Conclusions**

379   By testing three methods of mixed depth and using them to calculate simple ecological and

380   chemical metrics this study has demonstrated that methods of mixed depth estimation are

381   inconsistent and influence the interpretation of chemical and ecological results. Based on

382   these findings we recommend that future studies should:

383   - Favour density over temperature for estimating the mixed depth

384   - Not assume homogeneity of other variables within the mixed layer

385   - Assess the sensitivity of the findings of the study to mixed depth definition or

386   - Examine several methods to choose the most consistent and useful method for the

387   study

388   Ultimately, any method adopted for estimating mixed depth from standard limnological data

389   should be used cautiously and with awareness of the potential deviation of observed profiles

390   from idealised ones.

391

Acknowledgements

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Tables

602 Table 1. Examples of temperature and density thresholds and gradients used in existing

603 literature to calculate the mixed layer depth.

Reference	Method
Temperature thresholds	
(Augusto-Silva and MacIntyre, 2019)	0.02 °C from the surface
(Yang et al., 2018)	0.2 °C from the surface
(Zhao et al., 2018)	0.8 °C from the surface
(Mackay et al., 2011)	1 °C from the surface
(Vidal et al., 2010)	0.04 °C from the surface
Temperature gradients	
(Kasprzak et al., 2017)	1 °C m^{-1}
(Coloso et al., 2008)	1 °C /0.5 m.
(Xie et al., 2017)	0.01 °C m^{-1}
(Yankova et al., 2016)	0.5 °C m^{-1}
(Özkundakci et al., 2011)	0.25 °C m^{-1}
(Hamilton et al., 2010)	0.225°C m^{-1}
(McCullough et al., 2007)	0.05 °C m^{-1}
(Whittington et al., 2007)	0.02 °C m^{-1}
(Wilhelm and Adrian, 2007)	Depth of the maximum temperature gradient
Density thresholds	
(Andersen et al., 2017)	0.1 kg m^{-3} from the surface
Density gradients	

(Staehr et al., 2012)	0.07 kg m^{-3} m^{-1}
(Giling et al., 2017)	0.03 kg m^{-3} m^{-1} - 0.18 kg m^{-3} m^{-1}
(Tonetta et al., 2016)	0.03 kg m^{-3} m^{-1}
(Zwart et al., 2016)	0.1 kg m^{-3} m^{-1}
(Lamont and Laval, 2004.)	0.5 kg m^{-3} m^{-1}

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605

606   Table 2. The mean difference, root mean square error (RMSE) and range in mixed depth

607   estimates as calculated using Methods 1a, 1b, 2 & 3. Negative values indicate that the latter

608   mixed depth estimates are deeper.

	M1a-M1b	M1a-M2	M1a-M3
Mean difference (m)	0.7	0.8	-0.6
Mean absolute difference (m)	0.7	1.2	1.3
Mean percentage difference (%)	70	108	77
RMSE (m)	1.1	1.7	1.6
Range (m)	5.5	5	4.5

609

610

611 Table 3. Statistical model coefficients and adjusted R² values for the depth of the density-derived mixed depth compared with the mixed depth

612 calculated from chlorophyll-*a*, oxygen, specific conductivity and pH as well as the depth of the chlorophyll *a* and oxygen maxima for Method 2

613 and Method 3. The significance level is denoted as ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$, ·$p < 0.1$, ns- not significant. Quadratic models were

614 used for oxygen and specific conductivity whereas linear models were used for chlorophyll *a,* chlorophyll *a* maxima, oxygen maxima and pH,

615 2016 n=23; 2017 n=23.

	2016								2017							
	Residual SE		*F*-statistic		Adjusted R^2		*p*-value		Residual SE		*F*-statistic		Adjusted R^2		*p*-value	
	M2	M3	M2	M3	M2	M3	M2	M3	M2	M3	M2	M3	M2	M3	M2	M3
Chlorophyll *a*	1.57	1.58	1.50	4.64	0.02	0.14	ns	*	0.88	1.07	20.74	1.00	0.47	<0.01	***	ns
Oxygen	0.93	0.99	31.57	23.33	0.74	0.67	***	***	1.24	1.57	11.61	6.16	0.49	0.38	***	***
pH	1.25	1.45	1.84	7.29	0.04	0.26	ns	ns	0.75	1.27	18.18	4.67	0.44	0.14	***	ns
Specific Conductivity	2.07	2.23	1.46	1.17	0.04	0.02	ns	ns	2.51	2.47	2.2	1.07	0.1	<0.01	ns	ns
Chlorophyll *a* maxima	1.71	1.23	0.20	0.92	-0.04	<0.01	ns	ns	2.02	0.96	0.02	1.04	-0.05	<0.01	ns	ns
Oxygen maxima	1.59	1.46	3.77	15.87	0.11	0.40	.	***	2.02	1.22	0.03	0.28	-0.05	-0.03	ns	ns

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618 Table 4. The coefficient of variation (COV) and the range of temperature, oxygen, chlorophyll *a*, concentration of hydrogen ions (exponential of

619 pH) and specific conductivity values in the water column (WC) and the mixed layer for Method 1a (M1a), Method 2 (M2) and Method 3 (M3),

620 percentage values in brackets depict the percentage variation in the mixed layer relative to the whole water column variation.

	Mean coefficient of variation (COV) (%)				*Mean Range*			
Variable	WC	M1a	M2	M3	WC	M1a	M2	M3
Temperature (°C)	24.7	1.7 (7 %)	2.1 (9 %)	0.6 (2 %)	7.1	0.7 (10 %)	0.9 (13 %)	0.2 (3 %)
Oxygen (mg L^{-1})	94.7	9.0 (10 %)	9.4 (10 %)	5.3 (6 %)	8.8	2.3 (26 %)	2.4 (27 %)	1.3 (15 %)
Chlorophyll *a* (mg m^{-3})	74	17.1 (23 %)	24.5 (33 %)	11.6 (16 %)	19.7	8.2 (42 %)	11.4 (58%)	5.3 (27 %)
pH	48.7	16.2 (33 %)	20.2 (42 %)	11.8 (24 %)	1778.2	950.3 (53 %)	1073.6 (60 %)	641.0 (36 %)
Specific Conductivity	8.7	1.1 (13 %)	0.9 (10 %)	0.4 (5 %)	28.1	3.3 (12 %)	2.5 (9 %)	1.2 (4 %)

621
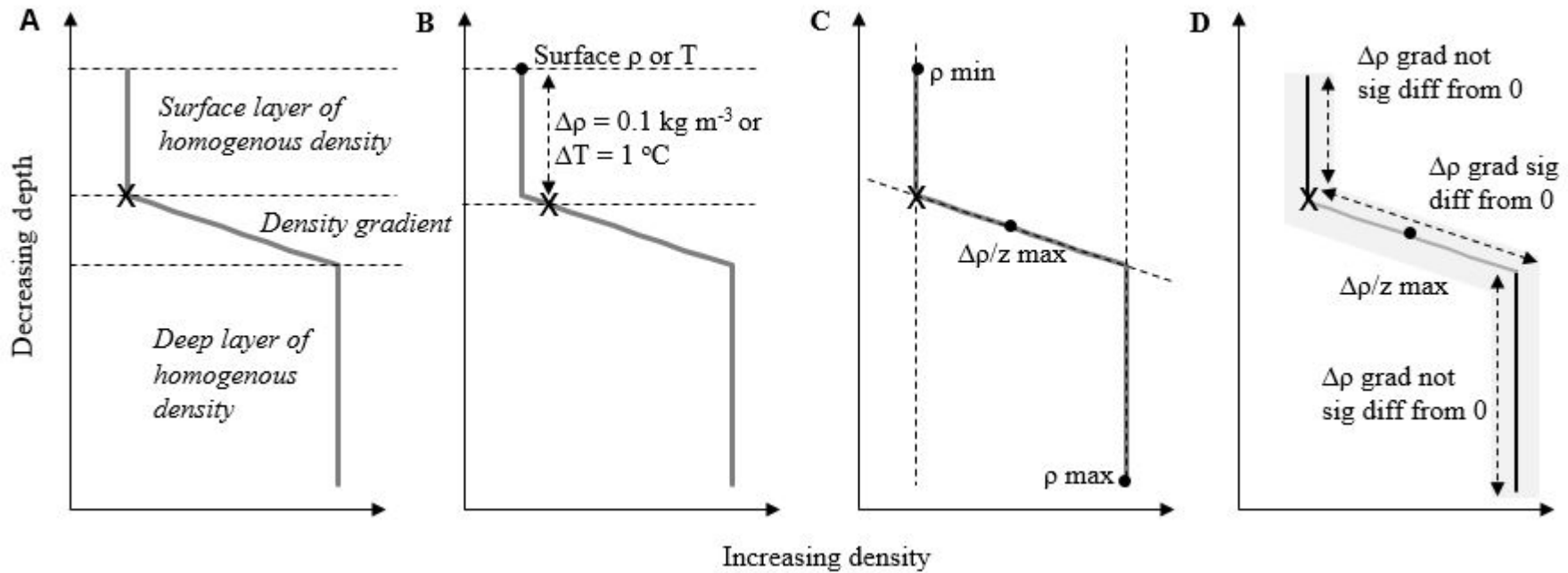
Figure 1. Diagram of density profiles marking the mixed depth (X) for (a) a theoretical mixed depth; (b) estimating the mixed depth using a 0.1 kg m^{-3} or 1 °C difference from the surface (Surface ρ or T) (Methods 1a and b); (c) estimating the mixed depth using Method 2 where lines are extended from the depth of the maximum gradient ($\Delta\rho/\Delta z$ max), the density minimum (ρ min) and the density maximum (ρ max) with the upper intersection of the lines marking the top of the pycnocline or base of the mixed depth and (d) estimating the mixed depth using Method 3 were the upper and lower values of the section of the profile containing the depth of the maximum gradient ($\Delta\rho/\Delta z$ max) and a change in the density gradient ($\Delta\rho$ grad) significantly different from zero marking the mixed depth and the top of the hypolimnion, respectively, the grey shading marks the profile confidence intervals.
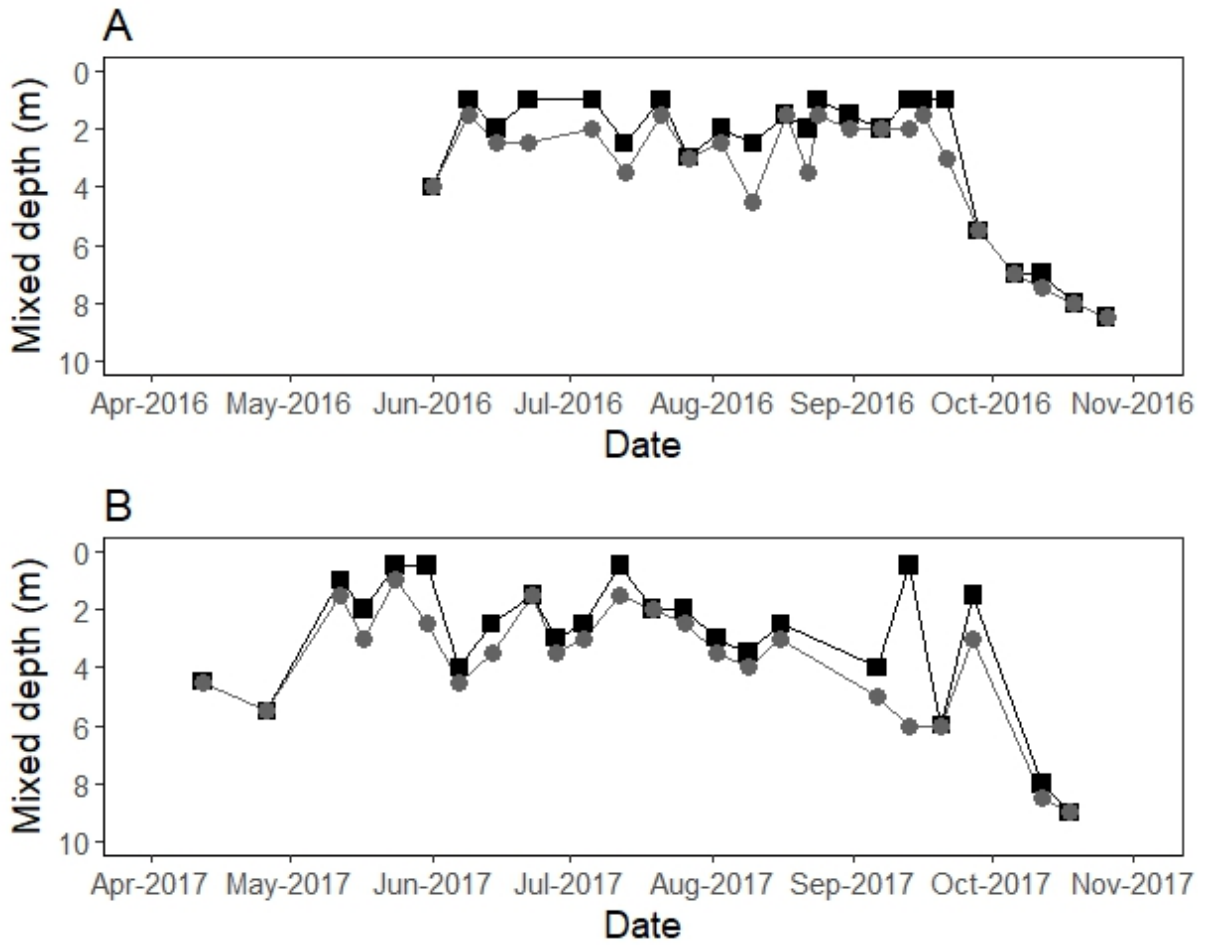
Figure 2. Mixed depth estimates using Method 1a (density threshold; black square) and Method 1b (temperature threshold; grey diamond) in (a) 2016 and (b) 2017.
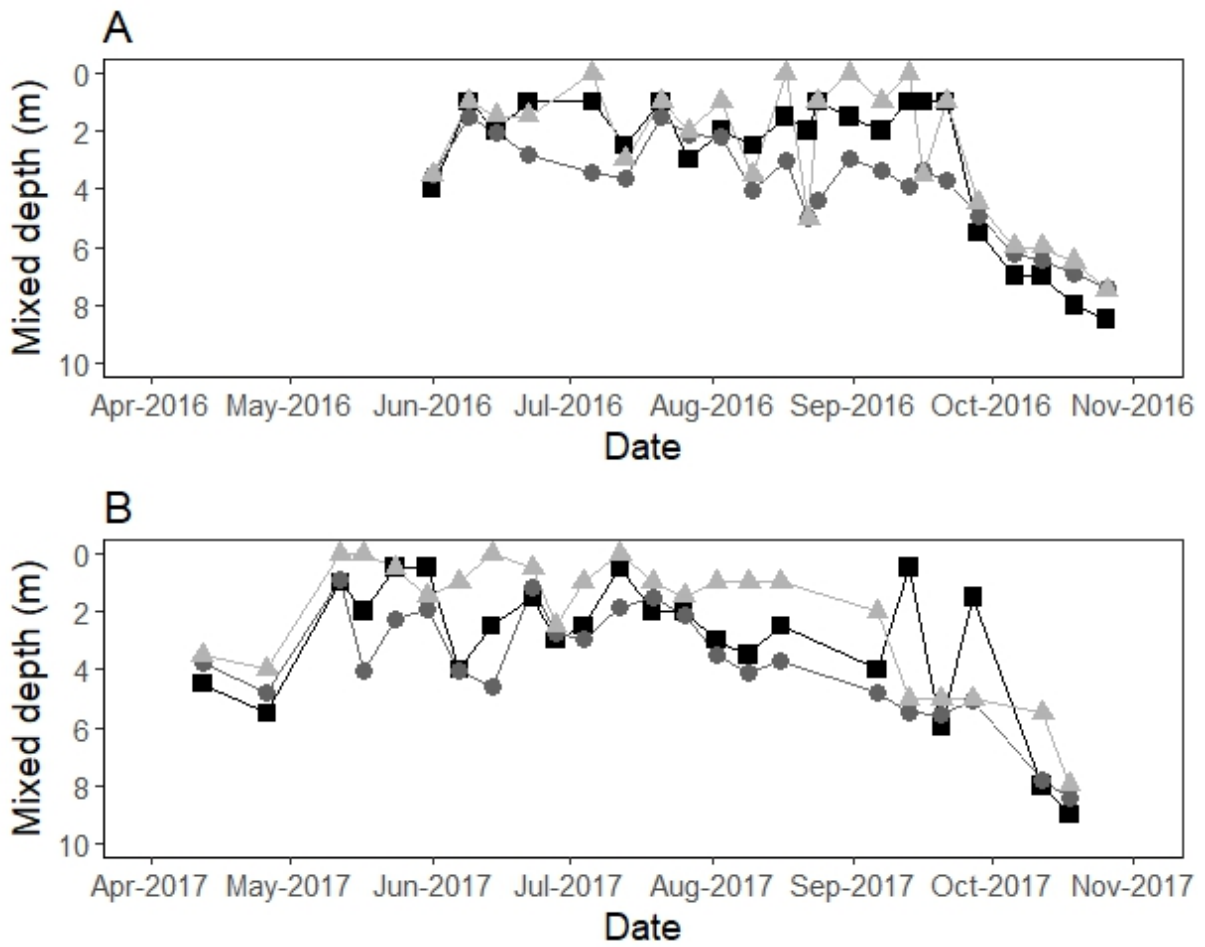
Figure 3. Density-derived mixed depth estimates using Method 1a (black square), Method 2 (grey circle) and Method 3 (light grey triangle) (a) 2016 and (b) 2017.
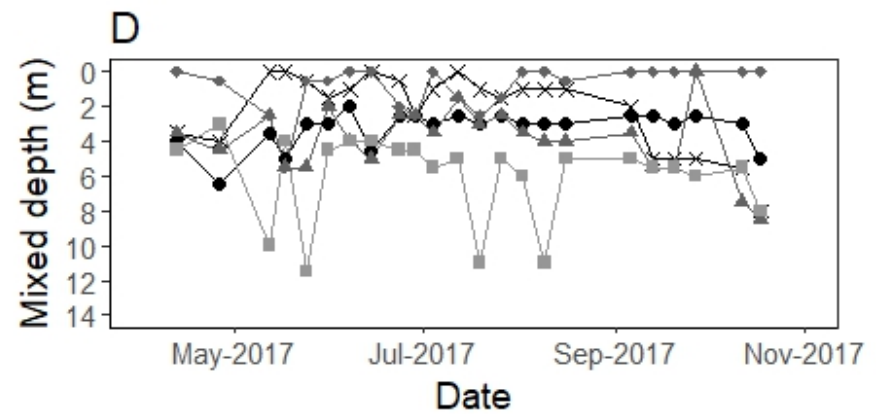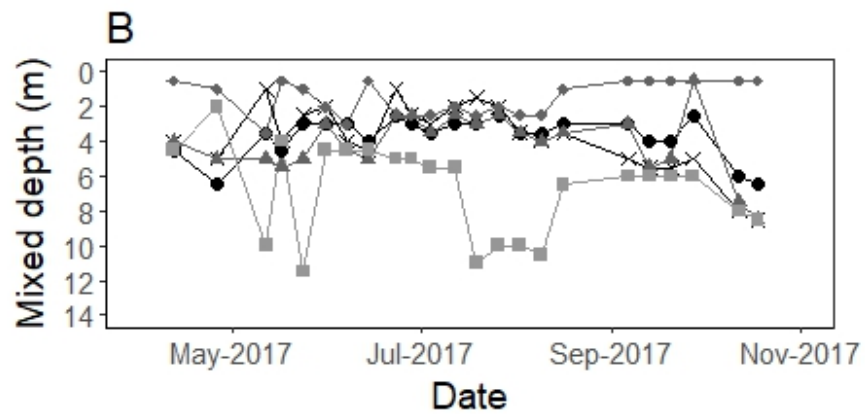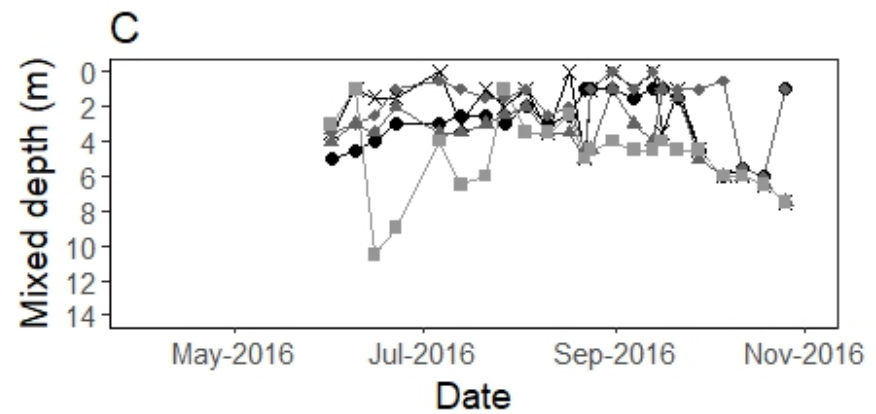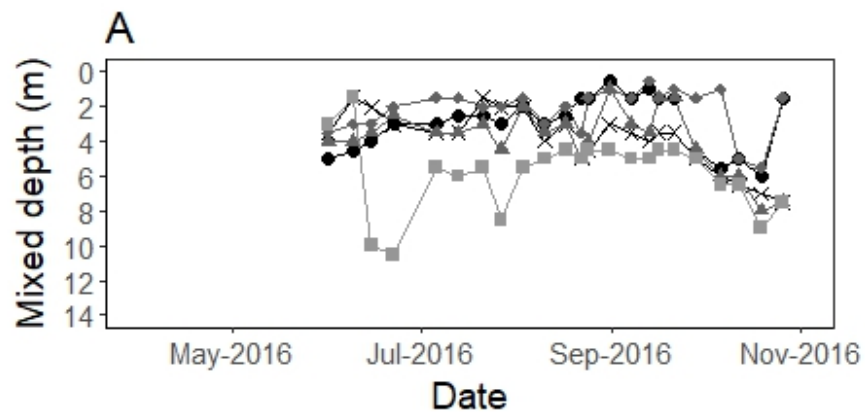
Figure 4. Depth of the mixed layer calculated from density (✳), chlorophyll-*a* (●), oxygen (▲), pH (◆) and specific conductivity (■) for (a) 2016 using Method 2, (b) 2016 Method 3 (c) 2017 Method 2 and (d) 2017 Method 3.
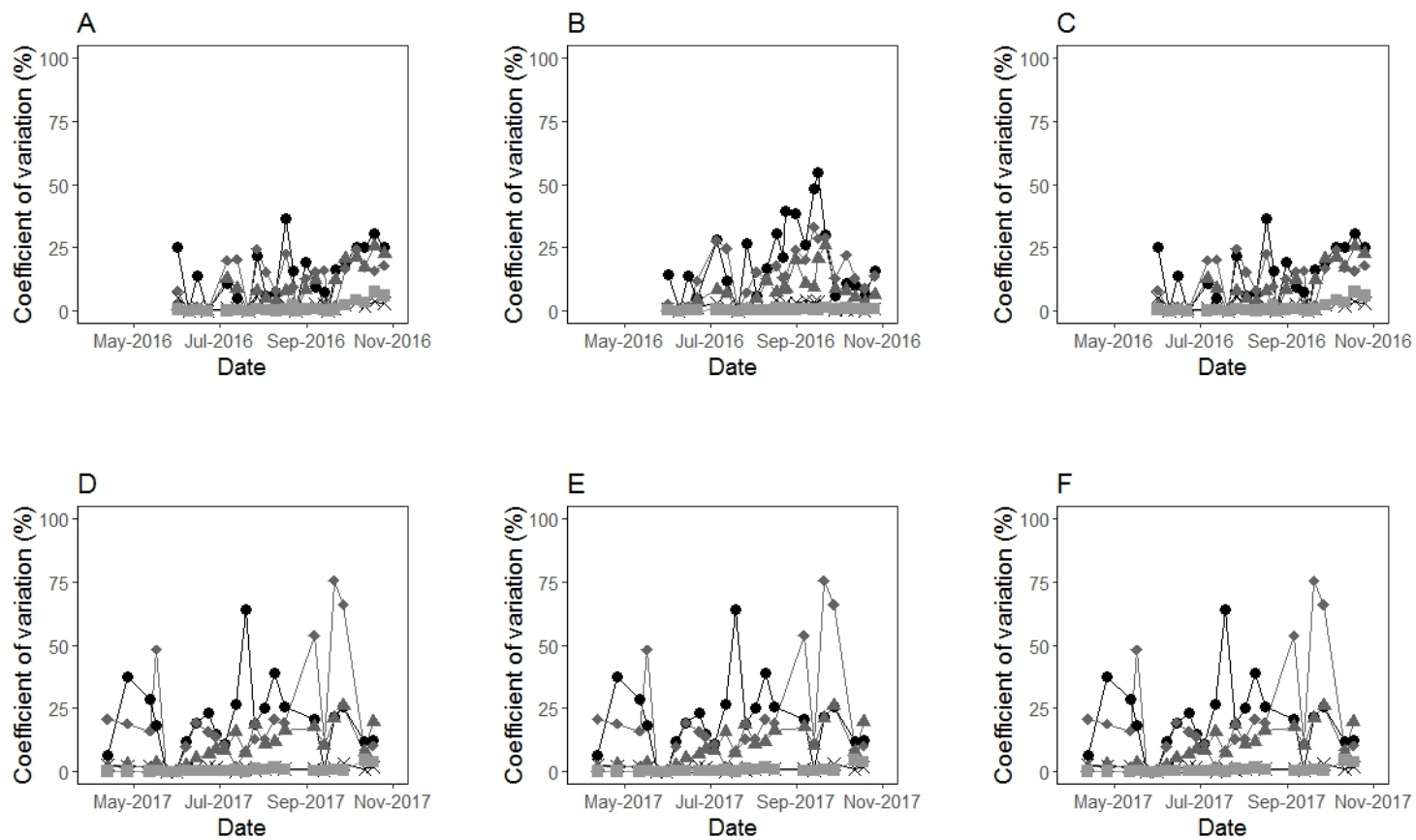
Figure 5. The coefficient of variation in the mixed layer for temperature (✳), chlorophyll *a* (●), oxygen (▲), concentration of hydrogen ions (pH) (◆) and specific conductivity (■) for (a) 2016 Method 1a, (b) 2016 Method 2 , (c) 2016 Method 3, (d) 2017 Method 1a, (e) 2017 Method 2 and (f) 2017 Method 3.
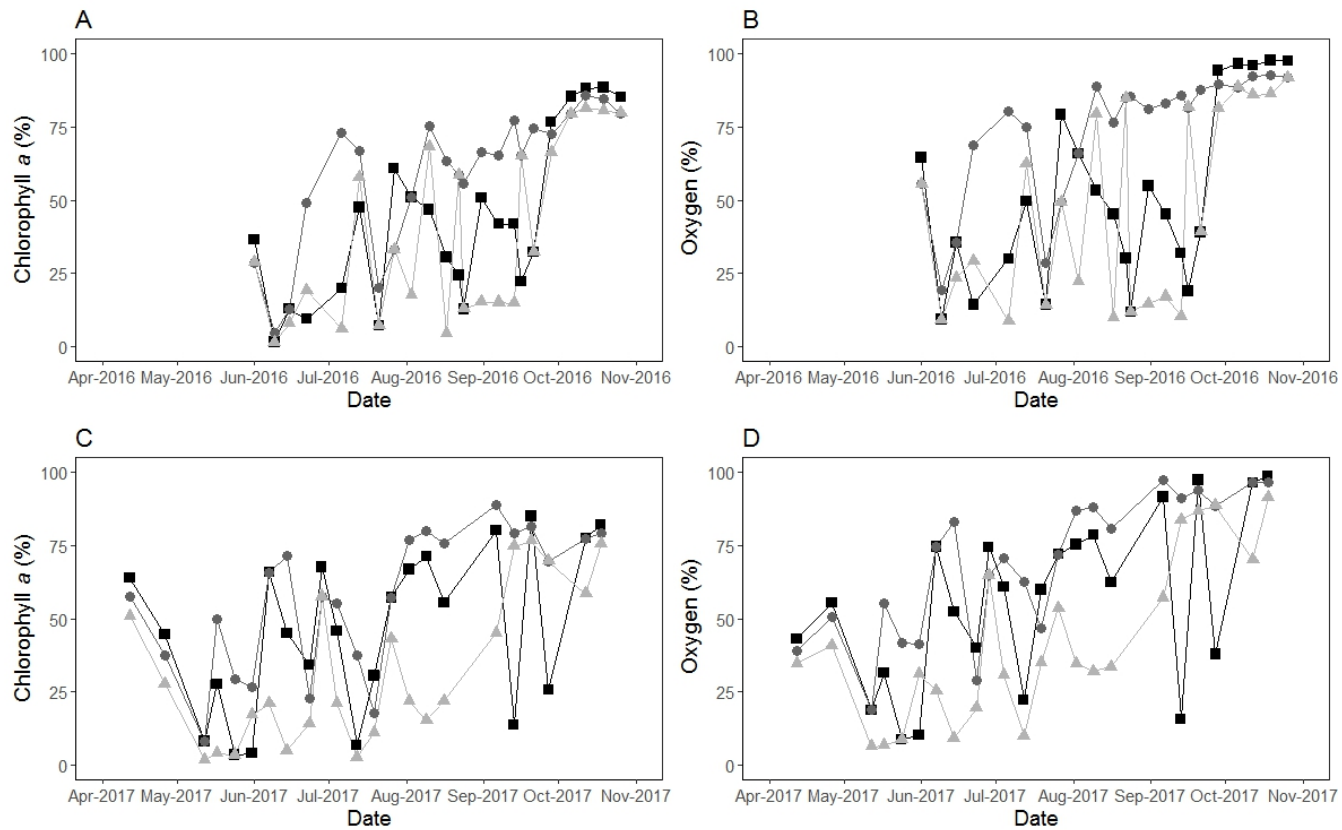
Figure 6. The percentage of chlorophyll *a* and oxygen within the mixed layer using mixed depth estimates calculated using Method 1a (black square), Method 2 (grey circle) and Method 3 (light grey triangle) for (a) chlorophyll *a* in 2016, (b) oxygen in 2016, (c) chlorophyll *a* in 2017 and (d) oxygen in 2017
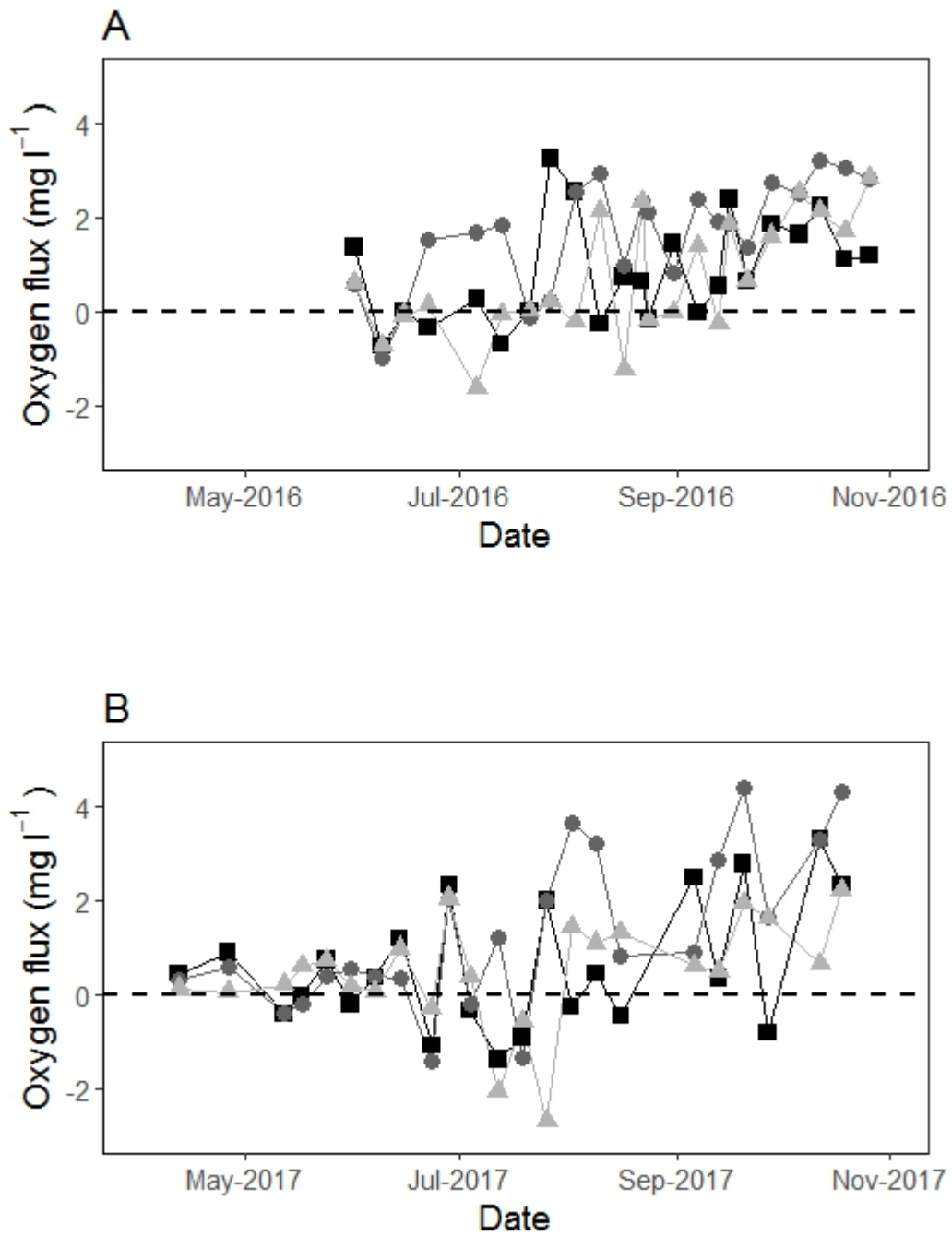
Figure 7. The difference in the concentration of oxygen within the mixed layer compared to the concentration in the layer 0.5 m below using mixed depth estimates calculated from Method 1a (black square), Method 2 (grey circle) and Method 3 (light grey triangle) for (a) 2016 and (b) 2017 .
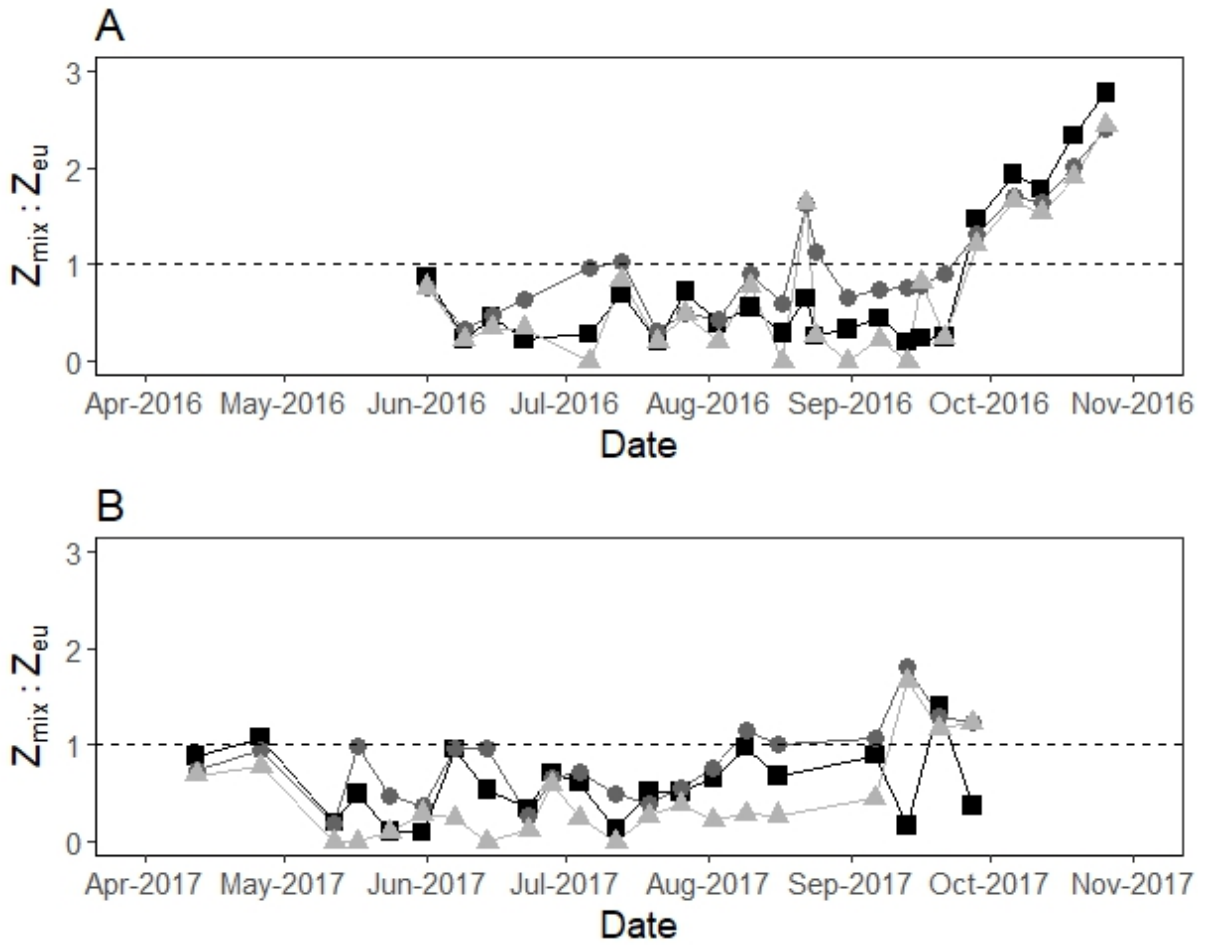
Figure 8. The z_{mix}:z_{eu} ratio calculated using density derived mixed depth estimated using Method 1a (black square), Method 2 (grey circle) and Method 3 (light grey triangle) for (a) 2016 and (b) 2017. Values below the horizontal y intercept line at 1 z_{mix}:z_{eu} mark when mixed depths are shallower than the euphotic depth and vice versa for values above.