

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

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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 homogeneously 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

29 1. Introduction

30 The “mixed layer” of a lake is a physical concept referring to a layer at the surface of a lake
31 within which temperature is uniform (Robertson and Imberger, 1994; Sverdrup, 1953)
32 (Fig.1a). The depth of the mixed layer, or epilimnion, depends on the balance between
33 stratifying and mixing forces, with deepening being driven by wind mixing and convective
34 cooling and shallowing being driven by warming (Wüest and Lorke, 2003). In stratified
35 lakes, this layer typically overlies water in which the mixing rates are significantly smaller,
36 enabling vertical gradients to develop in variables of interest, including temperature,
37 particulate matter and dissolved gasses. This concept is used extensively and underpins our
38 understanding of limnological processes. It is therefore fundamental for monitoring and
39 assessment purposes (Jaša et al., 2019; Peter et al., 2009; Schauser et al., 2003) and studies on
40 the restoration of lakes (Hoyer et al., 2015; Hupfer et al., 2016; Stroom and Kardinaal, 2016)
41 as well as the limnology of lakes (Brainerd and Gregg, 1995; Diehl, 2002; Wüest and Lorke,
42 2003).

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

54 commonly collected limnological temperature profiles cannot identify this actively mixing
55 layer. It is even questionable whether this depth of recent mixing can be accurately
56 determined using relatively coarse resolution measurements, as sharp changes in gradient can
57 become smeared, blurring the boundary between epilimnion and metalimnion. Furthermore,
58 temperature profiles can be complicated by the presence of secondary thermoclines
59 developing during the daytime, enhancing the potential for confounding results arising from
60 different mixed depth definitions. Such diurnal thermoclines can affect gas fluxes (MacIntyre
61 et al., 2002) and the vertical distribution of nutrients and phytoplankton (MacIntyre and
62 Melack, 1995). These secondary thermoclines can complicate the estimation of a
63 systematically defined mixed depth. Each ecological variable is also subject to different
64 source and sink terms operating at different timescales. Thus, physical mixing within the
65 epilimnion might be sufficient for homogenising a variable with slow rates of production or
66 loss, but the same mixing may be insufficient for homogenising a variable with faster
67 production and loss.

68 The necessity to infer the mixed depth without direct turbulence measurements has led to a
69 vast array of methods being developed for defining the depth of the mixed layer, typically
70 exploiting the notion of a vertical limnological profile being generated by rapid vertical
71 mixing in the surface waters of a lake and much diminished mixing beneath. A Web of
72 Science search using terms 'lake' AND 'mix* depth' AND 'layer' followed by removal of
73 non-lake references or those referring to sediment mixed depths or chemoclines identified at
74 least 313 research papers explicitly referring to a mixed layer. Often references to the mixed
75 depth were descriptive (24 %) or theoretical (16 %) rather than quantitative and in 10 % of
76 papers the mixed depth was arbitrarily or visually defined. The remaining studies determined
77 the mixed depth using a variety of methods which included being calculated within lake
78 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 (Staeher 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 homogeneously 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 km²), 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:

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

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 $1 \text{ }^\circ\text{C}$
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

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

184 *2.3.3. Method 2: Intersection of the plane of maximum gradient with the plane of the profile*
185 *minimum (or maximum)*

186 A Generalised Additive Model (GAM) with a gamma error distribution and logarithm link
187 function was fitted to every profile for each variable collected (46 sample days, 6 variables =
188 276 profiles in total) using the mgcv package (version 1.8-26) (Wood, 2011) within the R
189 programming language (R Core Team, 2018). The number of knots used in the GAM were
190 optimized and fixed for each variable and the fitted values were predicted at 0.5 m depth
191 intervals. Using the fitted predictions, the first derivative was calculated using forward
192 differences to find the depth of the maximum gradient. At the depth of the maximum gradient
193 the plane was extrapolated to all depths using the intercept and slope. Vertical lines were then
194 drawn corresponding to the mean of three maximum and minimum values from each profile.
195 The depth where the vertical lines intersected the extended maximum gradient line marked
196 the top and bottom of the thermocline, or equivalent for other variables, that is, the mixed
197 layer depth and the top of the hypolimnion, respectively (Fig. 1c).

198 *2.3.4. Method 3: Depth of statistically significant deviation*

199 Using the confidence intervals from the first derivative of the fitted GAM, the sections of the
200 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).

204 *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

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

317 **4. Discussion**

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

335 It is not necessarily the case though, that, a single definition of mixed depth estimation is
336 always appropriate, as different definitions might be better suited to different conditions or
337 different ecological questions. An example is the variety of mixed layer definitions used in a
338 study comparing depth-related oxygen metabolism across disparate lakes (Giling et al.,
339 2017), where it was considered that no one definition was suitable for all the lakes. It may
340 also be sometimes appropriate, depending on the purpose of the study, to adopt a definition
341 using a different variable than density or temperature, as the occurrence of a homogenous

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

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397 **References**

- 398 Andersen, M.R., Sand-Jensen, K., Iestyn Woolway, R., Jones, I.D., 2017. Profound daily
399 vertical stratification and mixing in a small, shallow, wind-exposed lake with submerged
400 macrophytes. *Aquat. Sci.* 79, 395–406. <https://doi.org/10.1007/s00027-016-0505-0>
- 401 Augusto-Silva, P., MacIntyre, S., 2019. Stratification and mixing in large floodplain lakes
402 along the lower Amazon River. *J. Gt. Lakes.* <https://doi.org/10.1016/j.jglr.2018.11.001>
- 403 Brainerd, K.E., Gregg, M.C., 1995. Surface mixed and mixing layer depths. *Deep Sea Res.*
404 Part I *Oceanogr. Res. Pap.* 42, 1521–1543. [https://doi.org/10.1016/0967-0637\(95\)00068-](https://doi.org/10.1016/0967-0637(95)00068-)
405 H
- 406 Brentrup, J.A., Williamson, C.E., Colom-Montero, W., Eckert, W., de Eyto, E., Grossart, H.-
407 P., Huot, Y., Isles, P.D.F., Knoll, L.B., Leach, T.H., McBride, C.G., Pierson, D., Pomati,
408 F., Read, J.S., Rose, K.C., Samal, N.R., Staehr, P.A., Winslow, L.A., 2016. The
409 potential of high-frequency profiling to assess vertical and seasonal patterns of
410 phytoplankton dynamics in lakes: an extension of the Plankton Ecology Group (PEG)
411 model. *Inl. Waters* 6, 565–580. <https://doi.org/10.5268/IW-6.4.890>
- 412 Coloso, J.J., Cole, J.J., Hanson, P.C., Pace, M.L., 2008. Depth-integrated, continuous
413 estimates of metabolism in a clear-water lake. *Can. J. Fish. Aquat. Sci.* 65, 712–722.
414 <https://doi.org/10.1139/f08-006>
- 415 Cyr, H., 2017. Winds and the distribution of nearshore phytoplankton in a stratified lake.
416 *Water Res.* 122, 114–127. <https://doi.org/10.1016/J.WATRES.2017.05.066>
- 417 Diehl, S., 2002. Phytoplankton, Light, and Nutrients in a Gradient of Mixing Depths: Theory.
418 *Ecology* 83, 386–398. <https://doi.org/10.1890/0012->
419 [9658\(2002\)083\[0386:PLANIA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[0386:PLANIA]2.0.CO;2)

420 Diehl, S., Berger, S., Ptacnik, R., Wild, A., 2002. Phytoplankton, Light, and Nutrients in a
421 Gradient of Mixing Depths: Field Experiments. *Ecology* 83, 399–411.
422 [https://doi.org/10.1890/0012-9658\(2002\)083\[0399:PLANIA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[0399:PLANIA]2.0.CO;2)

423 Dokulil, M., Teubner, K., 2000. Cyanobacterial dominance in lakes. *Hydrobiologia* 431, 1–
424 12. <https://doi.org/10.1023/A:1004155810302>

425 Giling, D.P., Staehr, P.A., Grossart, H.P., Andersen, M.R., Bohrer, B., Escot, C.,
426 Evrendilek, F., Gómez-Gener, L., Honti, M., Jones, I.D., Karakaya, N., Laas, A.,
427 Moreno-Ostos, E., Rinke, K., Scharfenberger, U., Schmidt, S.R., Weber, M., Woolway,
428 R.I., Zwart, J.A., Obrador, B., 2017. Delving deeper: Metabolic processes in the
429 metalimnion of stratified lakes. *Limnol. Oceanogr.* 62, 1288–1306.
430 <https://doi.org/10.1002/lno.10504>

431 Gregor, J., Maršálek, B., 2004. Freshwater phytoplankton quantification by chlorophyll a: A
432 comparative study of in vitro, in vivo and in situ methods. *Water Res.* 38, 517–522.
433 <https://doi.org/10.1016/j.watres.2003.10.033>

434 Hamilton, D.P., O'Brien, K.R., Burford, M.A., Brookes, J.D., McBride, C.G., 2010. Vertical
435 distributions of chlorophyll in deep, warm monomictic lakes. *Aquat. Sci.* 72, 295–307.
436 <https://doi.org/10.1007/s00027-010-0131-1>

437 Hoyer, A.B., Schladow, S.G., Rueda, F.J., 2015. A hydrodynamics-based approach to
438 evaluating the risk of waterborne pathogens entering drinking water intakes in a large,
439 stratified lake. *Water Res.* 83, 227–236. <https://doi.org/10.1016/J.WATRES.2015.06.014>

440 Huisman, J., Arrayás, M., Ebert, U., Sommeijer, B., 2002. How do sinking phytoplankton
441 species manage to persist? *Am. Nat.* 159, 245–54. <https://doi.org/10.1086/338511>

442 Huisman, J., van Oostveen, P., Weissing, 1999. Critical depth and critical turbulence: two

443 different mechanisms for the development of phytoplankton blooms. *Limnol. Oceanogr.*
444 44, 1781–1787. <https://doi.org/10.4319/lo.1999.44.7.1781>

445 Hupfer, M., Kleeberg, A., Lewandowski, J., 2016. Long-term efficiency of lake restoration
446 by chemical phosphorus precipitation: Scenario analysis with a phosphorus balance
447 model. *Water Res.* 97, 153–161. <https://doi.org/10.1016/J.WATRES.2015.06.052>

448 Imboden, D.M., Lemmin, U., Joller, T., Schurter, M., 1983. Mixing processes in lakes:
449 mechanisms and ecological relevance. *Schweizerische Zeitschrift für Hydrol.* 45, 11–44.
450 <https://doi.org/10.1007/BF02538150>

451 Jäger, C., Diehl, S., Schmidt, G., 2008. Influence of water-column depth and mixing on
452 phytoplankton biomass, community composition, and nutrients. *Limnol. Oceanogr.* 53,
453 2361–2373. <https://doi.org/10.4319/lo.2008.53.6.2361>

454 Jaša, L., Sadílek, J., Kohoutek, J., Straková, L., Maršálek, B., Babica, P., 2019. Application
455 of passive sampling for sensitive time-integrative monitoring of cyanobacterial toxins
456 microcystins in drinking water treatment plants. *Water Res.* 153, 108–120.
457 <https://doi.org/10.1016/J.WATRES.2018.12.059>

458 Kasprzak, P., Shatwell, T., Gessner, M.O., Gonsiorczyk, T., Kirillin, G., Selmečzy, G.,
459 Padišák, J., Engelhardt, C., 2017. Extreme Weather Event Triggers Cascade Towards
460 Extreme Turbidity in a Clear-water Lake. *Ecosystems* 20, 1407–1420.
461 <https://doi.org/10.1007/s10021-017-0121-4>

462 Kunz, T.J., Diehl, S., 2003. Phytoplankton, light and nutrients along a gradient of mixing
463 depth: a field test of producer-resource theory. *Freshw. Biol.* 48, 1050–1063.
464 <https://doi.org/10.1046/j.1365-2427.2003.01065.x>

465 Lamont, G., Laval, B., Pawlowicz, R., Pieters, R., Lawrence, G. A., 2004. Physical

466 mechanisms leading to upwelling of anoxic bottom water in Nitinat Lake. 17th ASCE
467 Eng. Mech. Conf. June 13-16, 2004, Univ. Delaware, Newark, Delaware, EEUU.

468 Leach, T.H., Beisner, B.E., Carey, C.C., Pernica, P., Rose, K.C., Huot, Y., Brentrup, J.A.,
469 Domaizon, I., Grossart, H.-P., Ibelings, B.W., Jacquet, S., Kelly, P.T., Rusak, J.A.,
470 Stockwell, J.D., Straile, D., Verburg, P., 2018. Patterns and drivers of deep chlorophyll
471 maxima structure in 100 lakes: The relative importance of light and thermal
472 stratification. *Limnol. Oceanogr.* 63, 628–646. <https://doi.org/10.1002/lno.10656>

473 Maberly, S.C., De Ville, M.M., Thackeray, S.J., Ciar, D., Clarke, M., Fletcher, J.M., James, J.
474 Ben, Keenan, P., Mackay, E.B., Patel, M., Tanna, B., Winfield, I.J., Bell, K., Clark, R.,
475 Jackson, A., Muir, J., Ramsden, P., Thompson, J., Titterington, H., Webb, P., 2016. A
476 survey of the status of the lakes of the English Lake District: the Lakes Tour 2015.

477 MacIntyre, S., 1993. Vertical mixing in a shallow, eutrophic lake: Possible consequences for
478 the light climate of phytoplankton. *Limnol. Oceanogr.* 38, 798–817.
479 <https://doi.org/10.4319/lo.1993.38.4.0798>

480 MacIntyre, S., Melack, J.M., 1995. Vertical and Horizontal Transport in Lakes: Linking
481 Littoral, Benthic, and Pelagic Habitats. *J. North Am. Benthol. Soc.* 14, 599–615.
482 <https://doi.org/10.2307/1467544>

483 MacIntyre, S., Romero, J.R., Kling, G.W., 2002. Spatial-temporal variability in surface layer
484 deepening and lateral advection in an embayment of Lake Victoria, East Africa. *Limnol.*
485 *Oceanogr.* 47, 656–671. <https://doi.org/10.4319/lo.2002.47.3.0656>

486 Mackay, E., Jones, I., Thackeray, S., Folkard, A., 2011. Spatial heterogeneity in a small,
487 temperate lake during archetypal weak forcing conditions. *Fundam. Appl. Limnol.* 179,
488 27–40. <https://doi.org/10.1127/1863-9135/2011/0179-0027>

489 Mackay, E.B., Folkard, A.M., Jones, I.D., 2014. Interannual variations in atmospheric forcing
490 determine trajectories of hypolimnetic soluble reactive phosphorus supply in a eutrophic
491 lake. *Freshw. Biol.* 59, 1646–1658. <https://doi.org/10.1111/fwb.12371>

492 Mackereth, F.J., Heron, J., Talling, J., 1979. *Water Analysis: Some Revised Methods for*
493 *Limnologists*, Freshwater Biological Association Scientific Publication. John Wiley &
494 Sons, Ltd. <https://doi.org/10.1002/iroh.19790640404>

495 Maiss, M., Ilmberger, J., Munnich, K.O., 1994. Vertical mixing in Uberlingersee (Lake
496 Constance) traced by SF₆ and heat. *Aquat. Sci.* 56, 329–347.
497 <https://doi.org/10.1007/BF00877180>

498 McCullough, G., Barber, D., Cooley, P., 2007. The vertical distribution of runoff and its
499 suspended load in Lake Malawi. *J. Gt. Lakes Re.* [https://doi.org/10.3394/0380-](https://doi.org/10.3394/0380-1330(2007)33[449:TVDORA]2.0.CO;2)
500 [1330\(2007\)33\[449:TVDORA\]2.0.CO;2](https://doi.org/10.3394/0380-1330(2007)33[449:TVDORA]2.0.CO;2)

501 McDougall, T., Barker, P., 2011. Getting started with TEOS-10 and the Gibbs Seawater
502 (GSW) Oceanographic Toolbox. SCOR/IAPSO WG12.

503 Mellard, J.P., Yoshiyama, K., Litchman, E., Klausmeier, C.A., 2011. The vertical distribution
504 of phytoplankton in stratified water columns. *J. Theor. Biol.* 269, 16–30.
505 <https://doi.org/10.1016/j.jtbi.2010.09.041>

506 Noges, P., Poikane, S., Kõiv, T., Noges, T., 2010. Effect of chlorophyll sampling design on
507 water quality assessment in thermally stratified lakes. *Hydrobiologia* 649, 157–170.
508 <https://doi.org/10.1007/s10750-010-0237-4>

509 Nürnberg, G.K., 1995. Quantifying anoxia in lakes. *Limnol. Oceanogr.* 40, 1100–1111.
510 <https://doi.org/10.4319/lo.1995.40.6.1100>

511 Obrador, B., Staehr, P.A., Christensen, J.P.C., 2014. Vertical patterns of metabolism in three

512 contrasting stratified lakes. *Limnol. Oceanogr.* 59, 1228–1240.
513 <https://doi.org/10.4319/lo.2014.59.4.1228>

514 Özkundakci, D., Hamilton, D.P., Gibbs, M.M., 2011. Hypolimnetic phosphorus and nitrogen
515 dynamics in a small, eutrophic lake with a seasonally anoxic hypolimnion.
516 *Hydrobiologia* 661, 5–20. <https://doi.org/10.1007/s10750-010-0358-9>

517 Peeters, F., Atamanchuk, D., Tengberg, A., Encinas-Fernández, J., Hofmann, H., 2016. Lake
518 Metabolism: Comparison of Lake Metabolic Rates Estimated from a Diel CO₂- and the
519 Common Diel O₂-Technique. *PLoS One* 11, e0168393.
520 <https://doi.org/10.1371/journal.pone.0168393>

521 Peter, A., Köster, O., Schildknecht, A., von Gunten, U., 2009. Occurrence of dissolved and
522 particle-bound taste and odor compounds in Swiss lake waters. *Water Res.* 43, 2191–
523 2200. <https://doi.org/10.1016/J.WATRES.2009.02.016>

524 Ramsbottom, A., 1976. Depth charts of the Cumbrian lakes, Freshwater Biological
525 Association. Kendal.

526 RCoreTeam, 2018. R: a language and environment for statistical computing. Vienna: R
527 Foundation for Statistical Computing; 2018.

528 Read, E.K., Ivancic, M., Hanson, P., Cade-Menun, B.J., McMahon, K.D., 2014. Phosphorus
529 speciation in a eutrophic lake by ³¹P NMR spectroscopy. *Water Res.* 62, 229–240.
530 <https://doi.org/10.1016/J.WATRES.2014.06.005>

531 Read, J.S., Hamilton, D.P., Jones, I.D., Muraoka, K., Winslow, L.A., Kroiss, R., Wu, C.H.,
532 Gaiser, E., 2011. Derivation of lake mixing and stratification indices from high-
533 resolution lake buoy data. *Environ. Model. Softw.* 26, 1325–1336.
534 <https://doi.org/10.1016/J.ENVSOF.2011.05.006>

535 Robertson, D.M., Imberger, J., 1994. Lake Number, a Quantitative Indicator of Mixing Used
536 to Estimate Changes in Dissolved Oxygen. *Int. Rev. der gesamten Hydrobiol. und*
537 *Hydrogr.* 79, 159–176. <https://doi.org/10.1002/iroh.19940790202>

538 Schauser, I., Lewandowski, J., Hupfer, M., 2003. Decision support for the selection of an
539 appropriate in-lake measure to influence the phosphorus retention in sediments. *Water*
540 *Res.* 37, 801–812. [https://doi.org/10.1016/S0043-1354\(02\)00439-6](https://doi.org/10.1016/S0043-1354(02)00439-6)

541 Simpson, G.L., 2018. Modelling Palaeoecological Time Series Using Generalised Additive
542 Models. *Front. Ecol. Evol.* 6, 149. <https://doi.org/10.3389/fevo.2018.00149>

543 Staehr, P.A., Bade, D., Van de Bogert, M.C., Koch, G.R., Williamson, C., Hanson, P., Cole,
544 J.J., Kratz, T., 2010. Lake metabolism and the diel oxygen technique: State of the
545 science. *Limnol. Oceanogr. Methods* 8, 628–644.
546 <https://doi.org/10.4319/lom.2010.8.0628>

547 Staehr, P.A., Christensen, J.P.A., Batt, R.D., Read, J.S., 2012. Ecosystem metabolism in a
548 stratified lake. *Limnol. Oceanogr.* 57, 1317–1330.
549 <https://doi.org/10.4319/lo.2012.57.5.1317>

550 Stroom, J.M., Kardinaal, W.E.A., 2016. How to combat cyanobacterial blooms: strategy
551 toward preventive lake restoration and reactive control measures. *Aquat. Ecol.* 50, 541–
552 576. <https://doi.org/10.1007/s10452-016-9593-0>

553 Sverdrup, H.U., 1953. On Conditions for the Vernal Blooming of Phytoplankton. *ICES J.*
554 *Mar. Sci.* 18, 287–295. <https://doi.org/10.1093/icesjms/18.3.287>

555 Tedford, E.W., Macintyre, S., Miller, S.D., Czikowsky, M.J., 2014. Similarity scaling of
556 turbulence in a temperate lake during fall cooling. *J. Geophys. Res. Ocean.* 119, 4689–
557 4713. <https://doi.org/10.1002/2014JC010135>

558 Tonetta, D., Staehr, P., Schmitt, R., Petrucio, M., 2016. Physical conditions driving the
559 spatial and temporal variability in aquatic metabolism of a subtropical coastal lake.
560 *Limnologica* 58, 30–40. <https://doi.org/10.1016/j.limno.2016.01.006>

561 Vidal, J., Moreno-Ostos, E., Escot, C., Quesada, R., Rueda, F., 2010. The effects of diel
562 changes in circulation and mixing on the longitudinal distribution of phytoplankton in a
563 canyon-shaped Mediterranean reservoir. *Freshw. Biol.* 55, 1945–1957.
564 <https://doi.org/10.1111/j.1365-2427.2010.02428.x>

565 Whittington, J., Sherman, B., Green, D., Oliver, R., 2007. Growth of *Ceratium hirundinella* in
566 a subtropical Australian reservoir: the role of vertical migration. *J. Plankton Res.*

567 Wilhelm, S., Adrian, R., 2007. Impact of summer warming on the thermal characteristics of a
568 polymictic lake and consequences for oxygen, nutrients and phytoplankton. *Freshw.*
569 *Biol.* 53, 226–237. <https://doi.org/10.1111/j.1365-2427.2007.01887.x>

570 Winder, M., Reuter, J.E., Schladow, S.G., 2009. Lake warming favours small-sized
571 planktonic diatom species. *Proc. R. Soc. B Biol. Sci.* 276, 427–435.
572 <https://doi.org/10.1098/rspb.2008.1200>

573 Wood, S.N., 2011. Fast stable restricted maximum likelihood and marginal likelihood
574 estimation of semiparametric generalized linear models. *J. R. Stat. Soc. Ser. B*
575 *(Statistical Methodol.* 73, 3–36. <https://doi.org/10.1111/j.1467-9868.2010.00749.x>

576 Woolway, R.I., Meinson, P., Nöges, P., Jones, I.D., Laas, A., 2017. Atmospheric stilling
577 leads to prolonged thermal stratification in a large shallow polymictic lake. *Clim.*
578 *Change.* <https://doi.org/10.1007/s10584-017-1909-0>

579 Wüest, A., Lorke, A., 2003. Small-Scale Hydrodynamics in Lakes. *Annu. Rev. Fluid Mech.*
580 35, 373–412. <https://doi.org/10.1146/annurev.fluid.35.101101.161220>

581 Xie, Q., Liu, Z., Fang, X., Chen, Y., Li, C., MacIntyre, S., 2017. Understanding the
582 temperature variations and thermal structure of a subtropical deep river-run reservoir
583 before and after impoundment. *Water* 9. <https://doi.org/doi:10.3390/w9080603>

584 Yang, Y., Wang, Y., Zhang, Z., Wang, W., Ren, X., Gao, Y., Liu, S., Lee, X., 2018. Diurnal
585 and seasonal variations of thermal stratification and vertical mixing in a shallow fresh
586 water lake. *J. Meteorological Res.* 32, 219–232. [https://doi.org/10.1007/s13351-018-](https://doi.org/10.1007/s13351-018-7099-5)
587 [7099-5](https://doi.org/10.1007/s13351-018-7099-5). 1.

588 Yankova, Y., Villiger, J., Pernthaler, J., Schanz, F., Posch, T., 2016. Prolongation, deepening
589 and warming of the metalimnion change habitat conditions of the harmful filamentous
590 cyanobacterium *Planktothrix rubescens* in a prealpine lake. *Hydrobiologia* 776, 125–
591 138. <https://doi.org/10.1007/s10750-016-2745-3>

592 Zhao, Q., Ren, Y., Wang, J.X.L., 2018. Temporal and spatial characteristics of potential
593 energy anomaly in Lake Taihu. *Environ. Sci. Pollut. Res.* 25, 24316–24325.
594 <https://doi.org/10.1007/s11356-018-2204-y>

595 Zwart, J.A., Craig, N., Kelly, P.T., Sebestyen, S.D., Solomon, C.T., Weidel, B.C., Jones,
596 S.E., 2016. Metabolic and physiochemical responses to a whole-lake experimental
597 increase in dissolved organic carbon in a north-temperate lake. *Limnol. Oceanogr.* 61,
598 723–734. <https://doi.org/10.1002/lno.10248>

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601 **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
<i>Temperature thresholds</i>	
(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
<i>Temperature gradients</i>	
(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
<i>Density thresholds</i>	
(Andersen et al., 2017)	0.1 kg m ⁻³ from the surface
<i>Density gradients</i>	

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

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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 R^2 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 *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$, $\cdot p < 0.1$, 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		<i>F</i> -statistic		Adjusted R^2		<i>p</i> -value		Residual SE		<i>F</i> -statistic		Adjusted R^2		<i>p</i> -value	
	M2	M3	M2	M3	M2	M3	M2	M3	M2	M3	M2	M3	M2	M3	M2	M3
Chlorophyll <i>a</i>	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 <i>a</i> 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

616

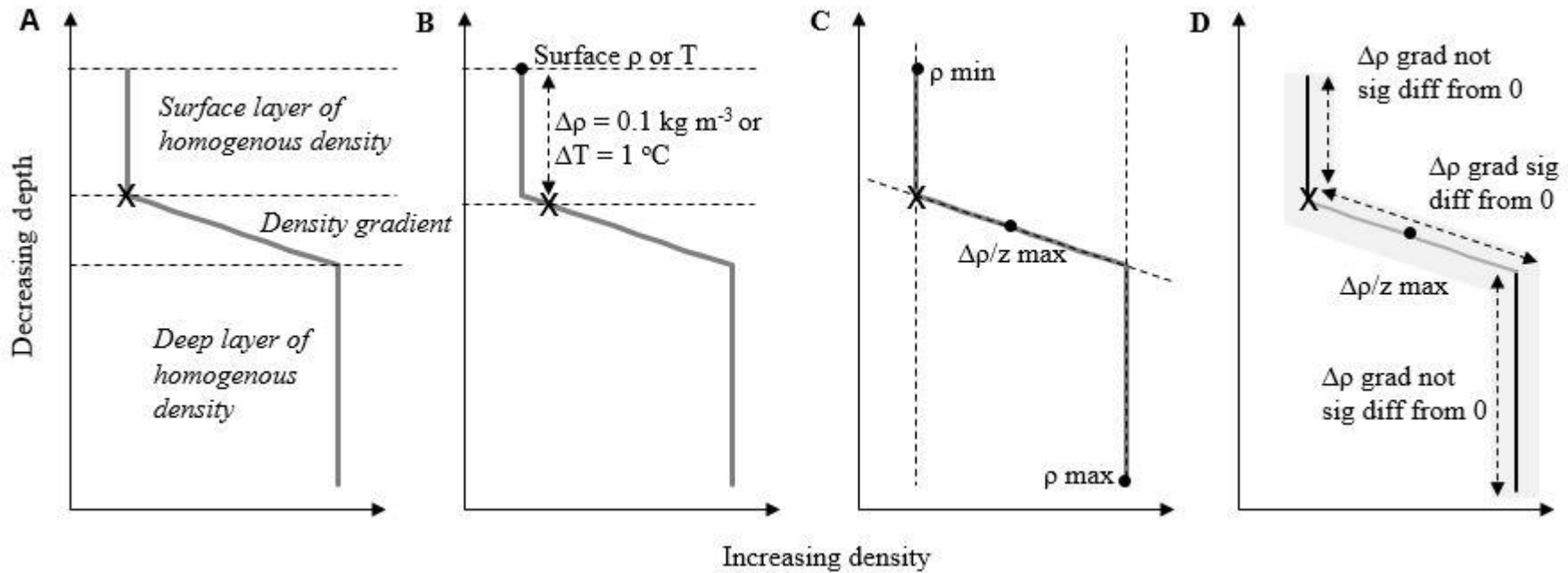
617

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.

<i>Variable</i>	<i>Mean coefficient of variation (COV) (%)</i>				<i>Mean Range</i>			
	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 <i>a</i> (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

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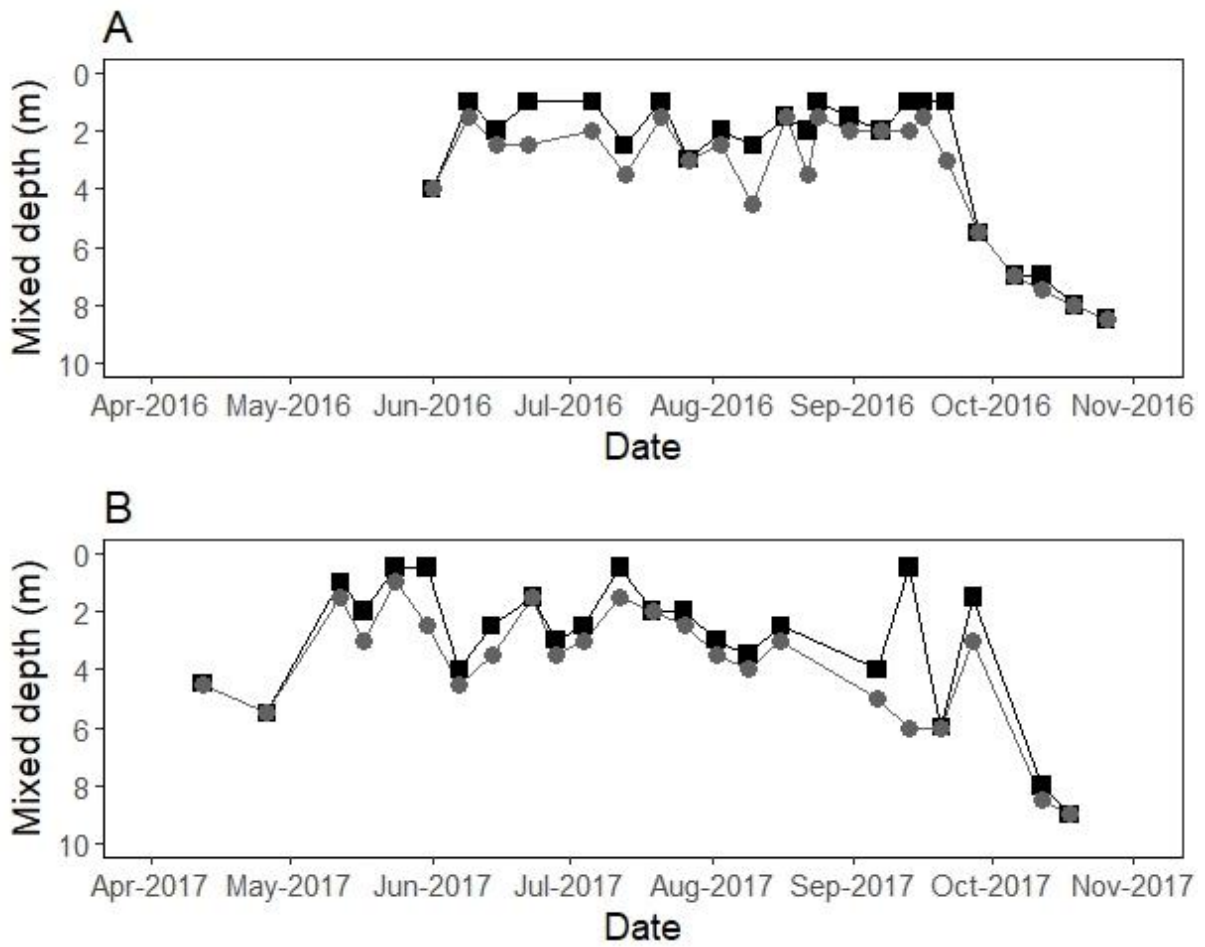


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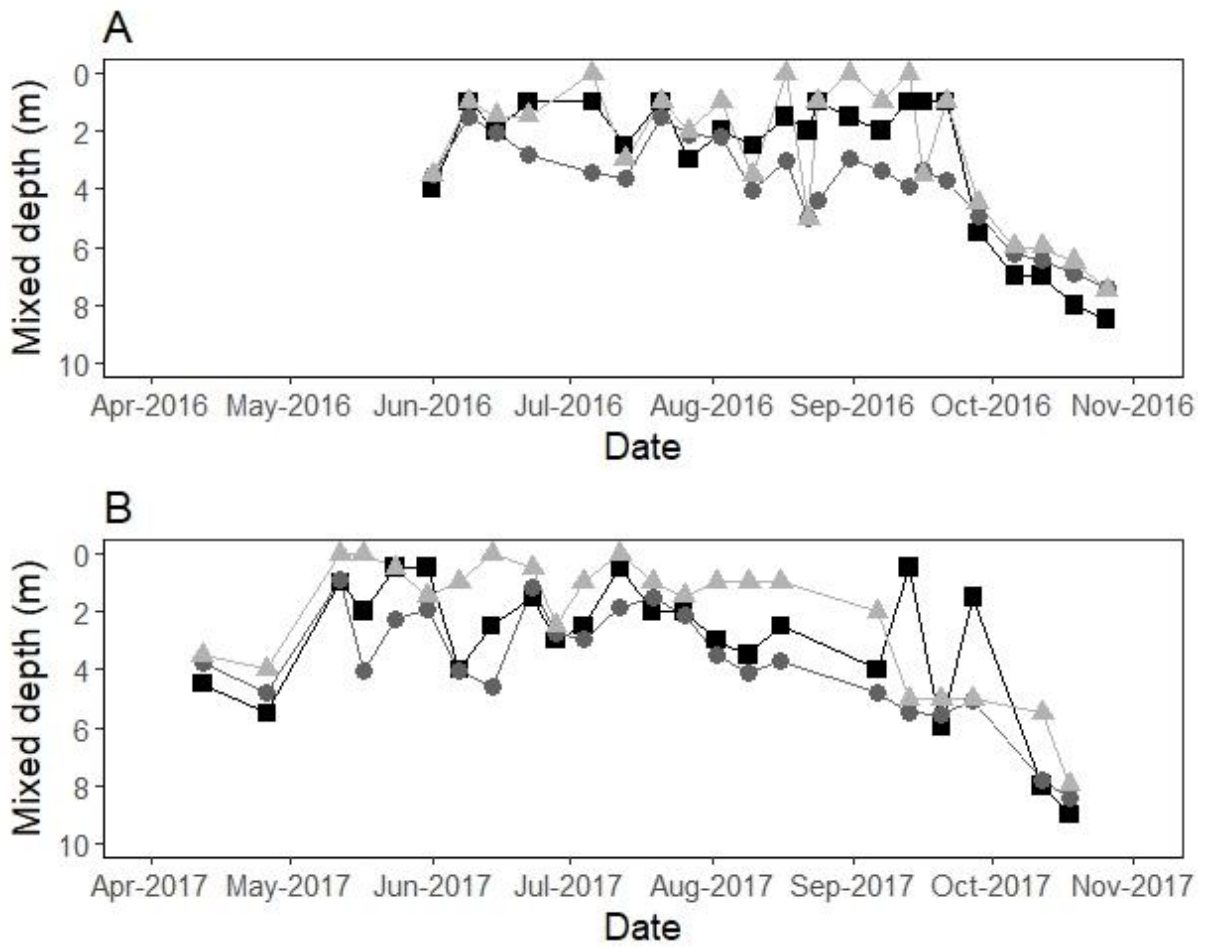
625 Figure 1. Diagram of density profiles marking the mixed depth (X) for (a) a theoretical mixed depth; (b) estimating the mixed depth using a 0.1
 626 kg m^{-3} or $1 \text{ }^\circ\text{C}$ difference from the surface (Surface ρ or T) (Methods 1a and b); (c) estimating the mixed depth using Method 2 where lines are
 627 extended from the depth of the maximum gradient ($\Delta\rho/\Delta z \text{ max}$), the density minimum ($\rho \text{ min}$) and the density maximum ($\rho \text{ max}$) with the upper
 628 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 where
 629 the upper and lower values of the section of the profile containing the depth of the maximum gradient ($\Delta\rho/\Delta z \text{ max}$) and a change in the density
 630 gradient ($\Delta\rho \text{ grad}$) significantly different from zero marking the mixed depth and the top of the hypolimnion, respectively, the grey shading
 631 marks the profile confidence intervals.

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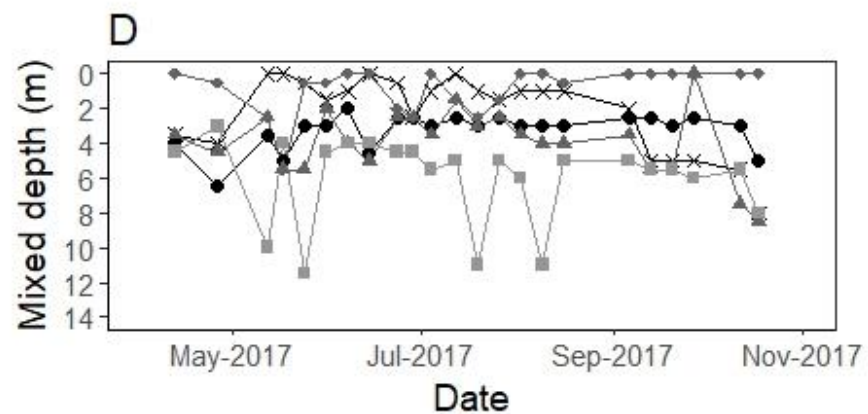
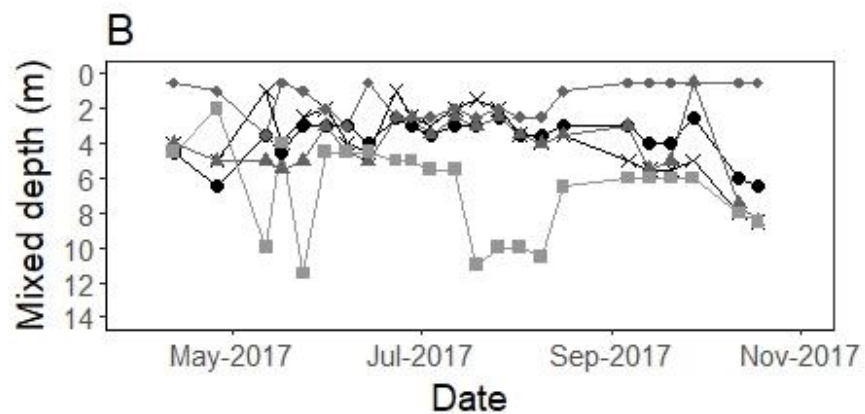
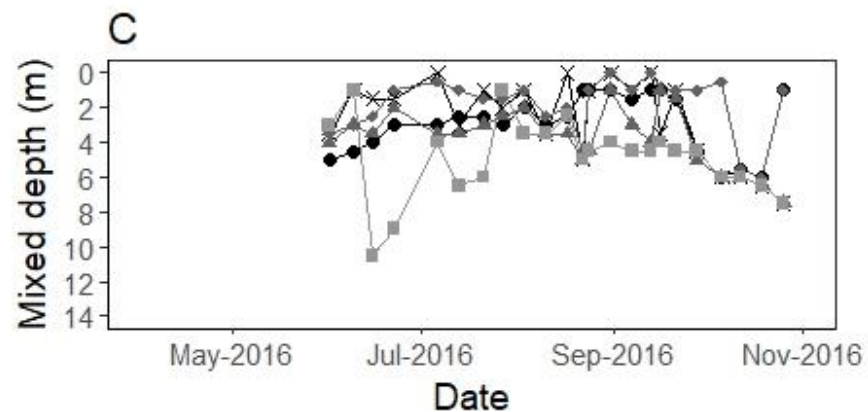
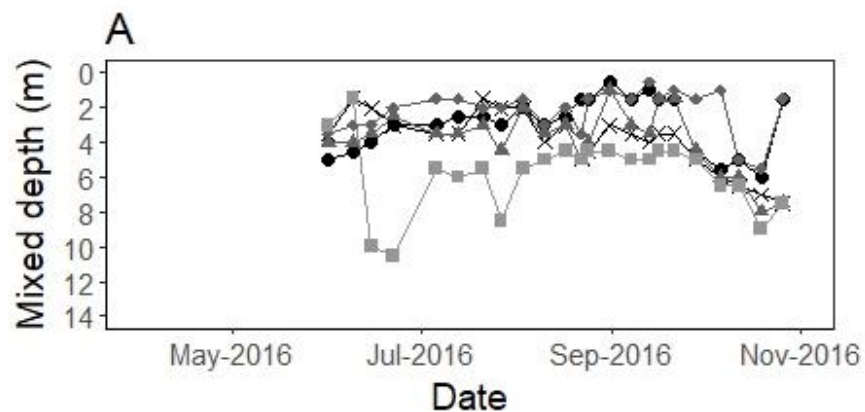
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Figure 2. Mixed depth estimates using Method 1a (density threshold; black square) and Method 1b (temperature threshold; grey diamond) in (a) 2016 and (b) 2017.



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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.

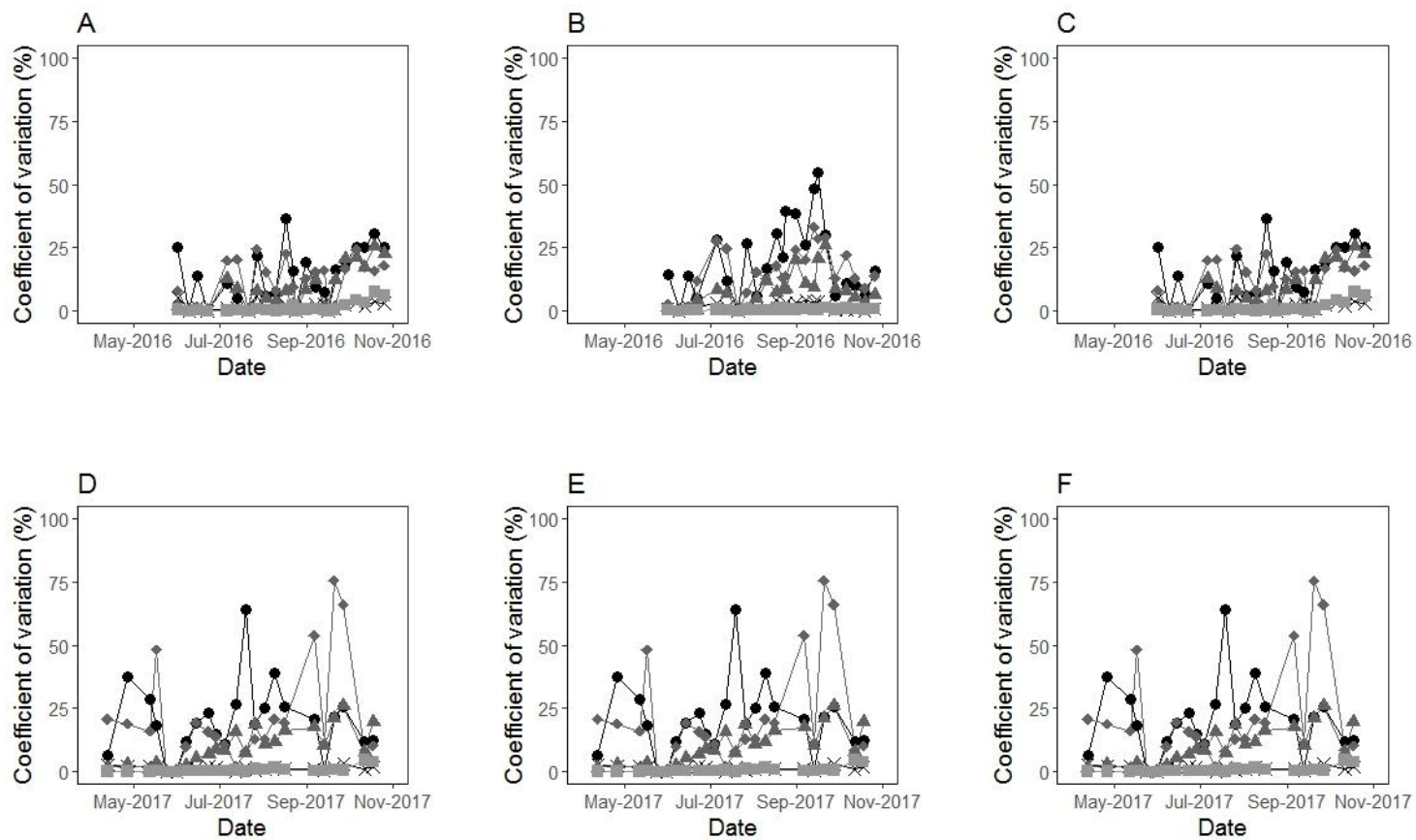


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647 Figure 4. Depth of the mixed layer calculated from density (*), chlorophyll-*a* (●), oxygen (▲), pH (◆) and specific
 648 conductivity (■) for (a) 2016 using Method 2, (b) 2016 Method 3 (c) 2017 Method 2 and (d) 2017 Method 3.

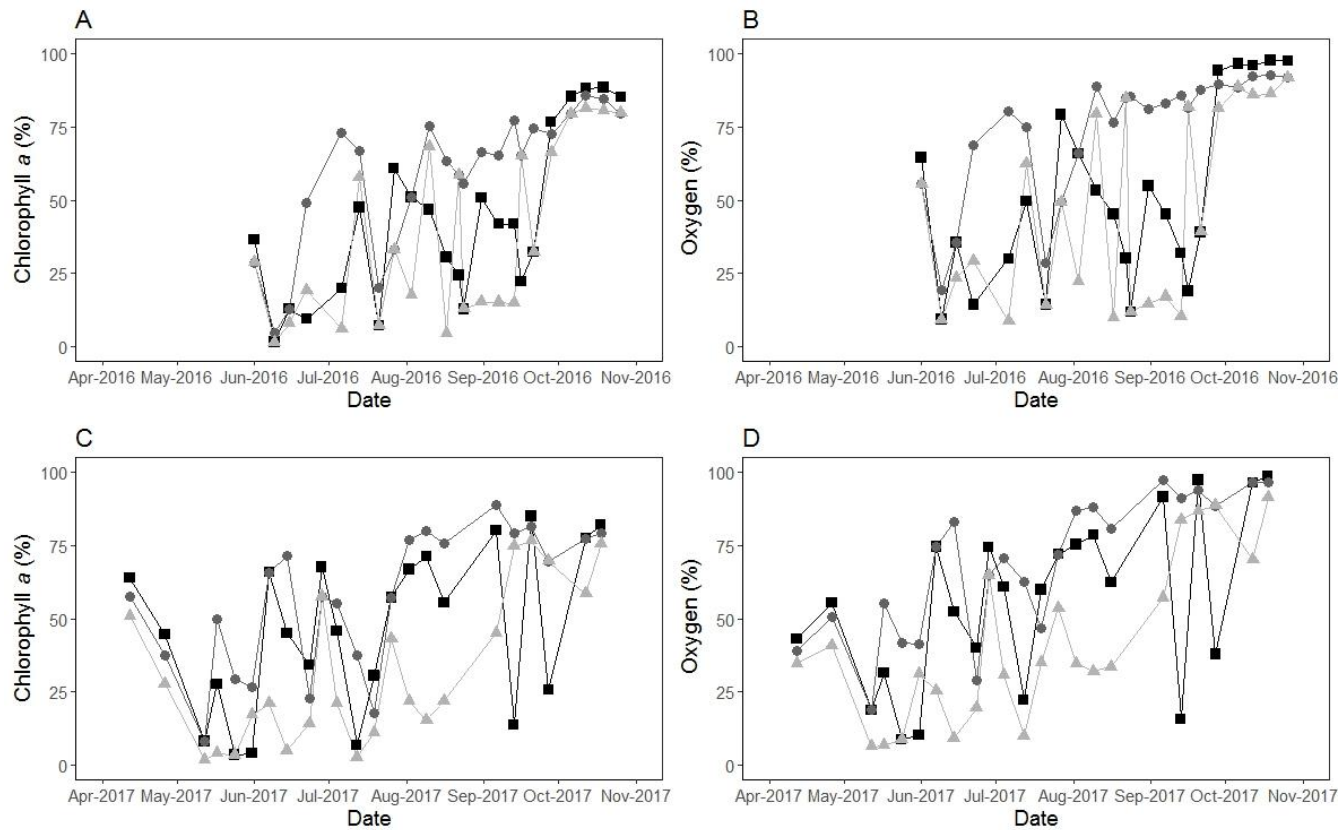
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651 Figure 5. The coefficient of variation in the mixed layer for temperature (\times), chlorophyll *a* (\bullet), oxygen (\blacktriangle), concentration of
 652 hydrogen ions (pH) (\blacklozenge) and specific conductivity (\blacksquare) for (a) 2016 Method 1a, (b) 2016 Method 2 , (c) 2016 Method 3, (d) 2017
 653 Method 1a, (e) 2017 Method 2 and (f) 2017 Method 3.

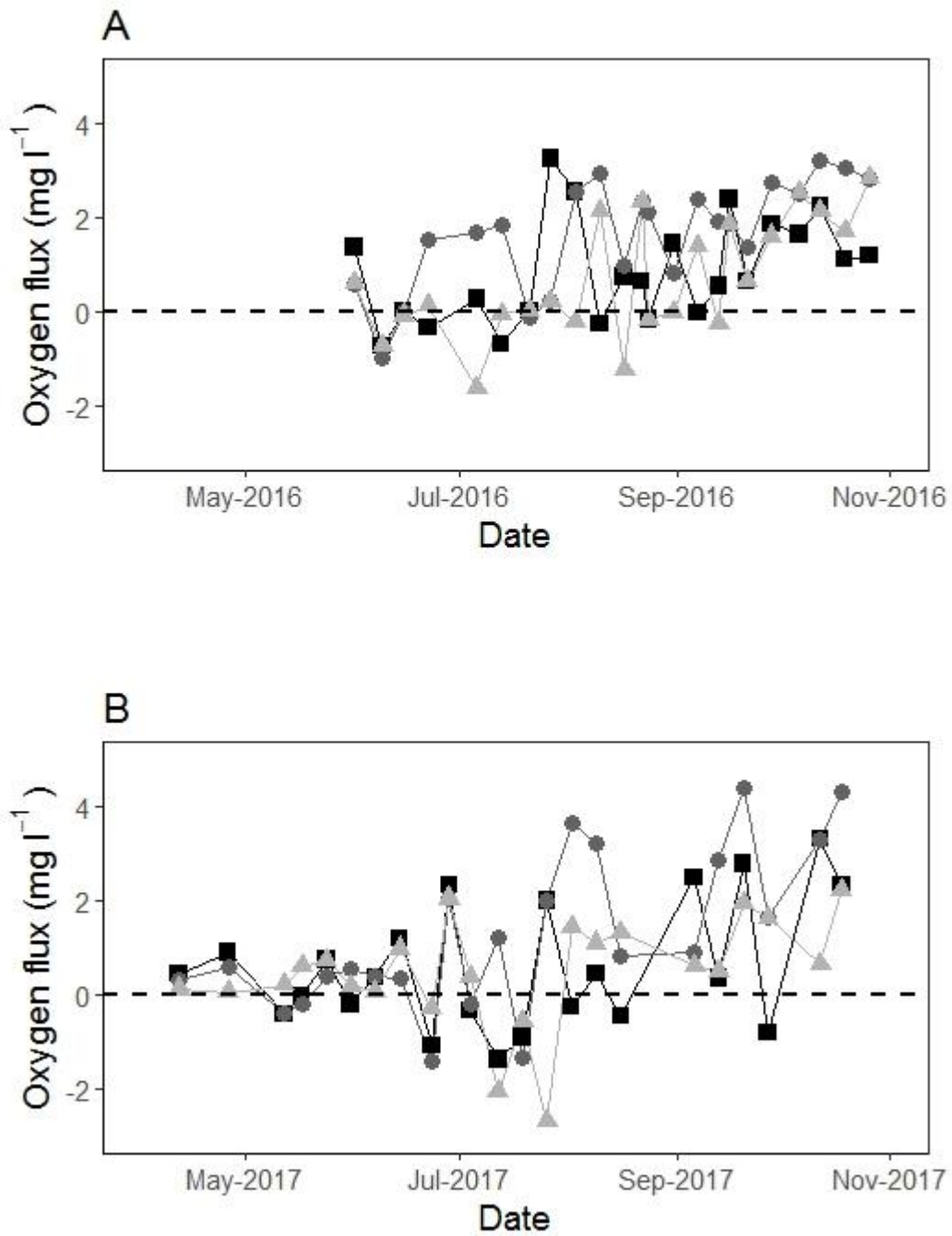
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656 Figure 6. The percentage of chlorophyll *a* and oxygen within the mixed layer using mixed depth estimates calculated using Method 1a (black
 657 square), Method 2 (grey circle) and Method 3 (light grey triangle) for (a) chlorophyll *a* in 2016, (b) oxygen in 2016, (c) chlorophyll *a* in 2017
 658 and (d) oxygen in 2017

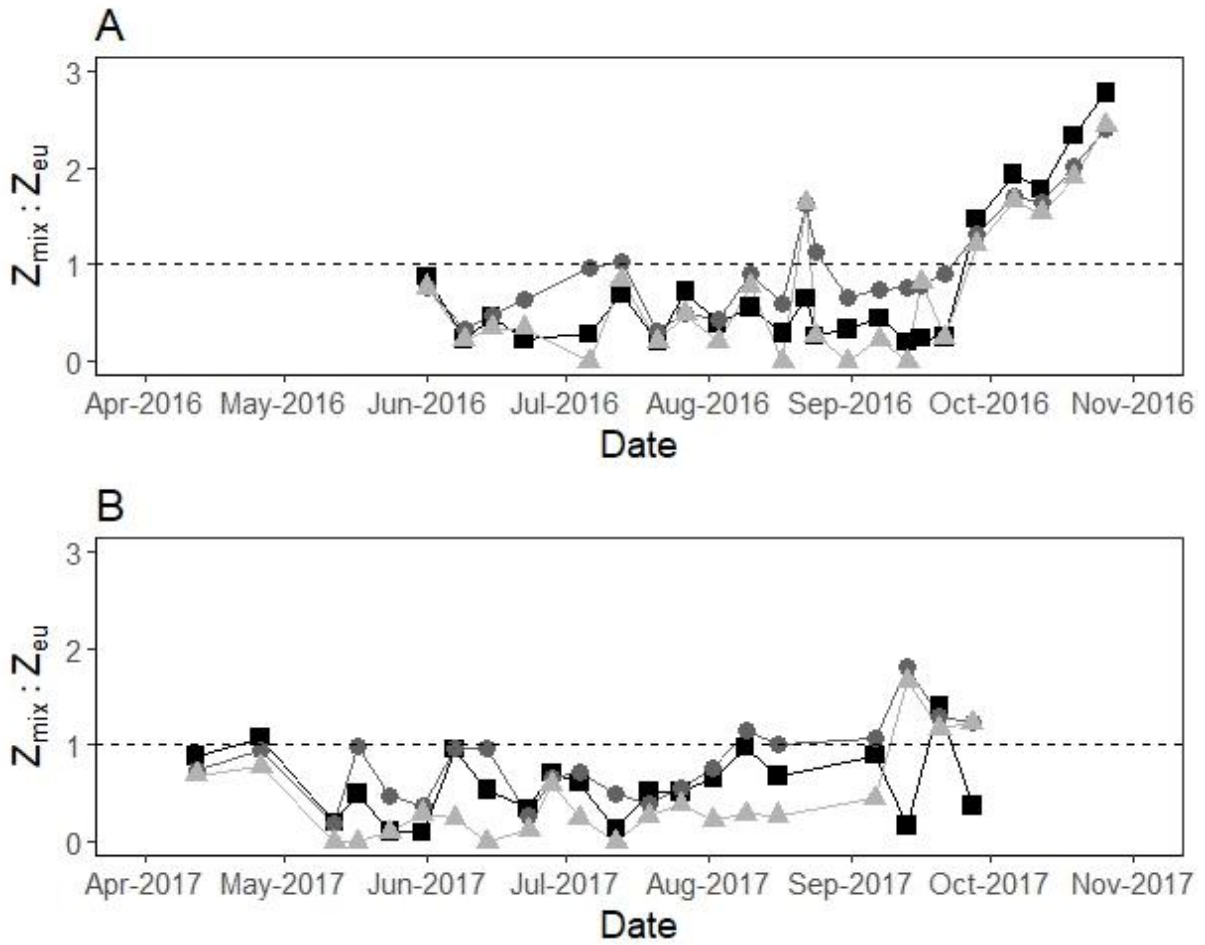
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661 Figure 7. The difference in the concentration of oxygen within the mixed layer compared to
 662 the concentration in the layer 0.5 m below using mixed depth estimates calculated from
 663 Method 1a (black square), Method 2 (grey circle) and Method 3 (light grey triangle) for (a)
 664 2016 and (b) 2017 .

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668 Figure 8. The $z_{mix}:z_{eu}$ ratio calculated using density derived mixed depth estimated using
 669 Method 1a (black square), Method 2 (grey circle) and Method 3 (light grey triangle) for (a)
 670 2016 and (b) 2017. Values below the horizontal y intercept line at 1 $z_{mix}:z_{eu}$ mark when
 671 mixed depths are shallower than the euphotic depth and vice versa for values above.

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