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

Emma Gray [a, b, *], Eleanor B. Mackay [a], J. Alex Elliott [a], Andrew M. Folkard [b], Ian D. Jones [c]

[a] Centre for Ecology and Hydrology, Lancaster, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK
[b] Lancaster Environment Centre, Library Avenue, Lancaster University, Lancaster, LA1 4YQ, UK
[c] Biological and Environmental Sciences, University of Stirling, Stirling, FK9 4LA, UK

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ABSTRACT

The mixed layer, or epilimnion, is a physical concept referring to an isothermal layer at the surface of a water body. This concept is ubiquitous within limnology, is fundamental to our understanding of chemical and ecological processes, and is an important metric for water body monitoring, assessment and management. Despite its importance as a metric, many different approaches to approximating mixed depth currently exist. Using data from field campaigns in a small meso-eutrophic lake in the UK in 2016 and 2017 we tested whether different definitions of mixed depth resulted in comparable estimates and whether variables other than temperature could be assumed to be mixed within the layer. Different methods resulted in very different estimates for the mixed depth and ecologically important variables were not necessarily homogenously spread through the epilimnion. Furthermore, calculation of simple ecologically relevant metrics based on mixed depth showed that these metrics were highly dependent on the definition of mixed depth used. The results demonstrate that an idealised concept of a well-defined fully mixed layer is not necessarily appropriate. The widespread use of multiple definitions for mixed depth impairs the comparability of different studies while associated uncertainty over the most appropriate definition limits the confirmability of studies utilising the mixed depths.

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 (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 °C 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 °C, 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

* Corresponding author. Centre for Ecology and Hydrology, Lancaster, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK.
E-mail address: emma.gray9229@gmail.com (E. Gray).

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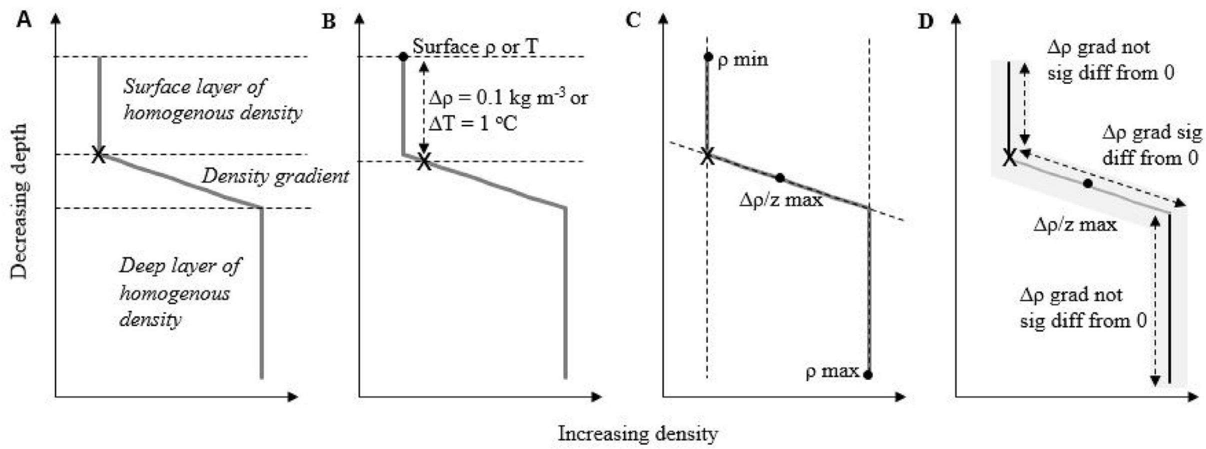

Fig. 1. Diagram of density profiles marking the mixed depth (X) for (a) a theoretical mixed depth; (b) estimating the mixed depth using a 0.1 kg m-3 or 1 °C difference from the surface (Surface ρ or T) (Methods 1a and b); (c) estimating the mixed depth using Method 2 where lines are extended from the depth of the maximum gradient (Δρ/Δz 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 (Δρ/Δz max) and a change in the density gradient (Δρ grad) significantly different from zero marking the mixed depth and the top of the hypolimnion, respectively, the grey shading marks the profile confidence intervals.

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 through turbulence (8%) or calculated using a secondary variable (23%). The latter method could be categorised into temperature (Coloso et al., 2008) or density gradients (Staehr et al., 2012), temperature (Wilhelm and Adrian, 2007), or density differences (Winder et al., 2009) and isotopic (Imboden et al., 1983) or chemical tracers (Maiss et al., 1994). Temperature gradients were most commonly used to define the mixed depth, followed by density gradients, temperature thresholds and density thresholds. There are, however, at least 20 different thresholds and gradients of temperature or density currently being applied to estimate the mixed depth (Table 1).

Implicitly, the common usage of such a wide variety of methods suggests that each one is assumed to define approximately the same depth of mixed layer. If the vertical profiles of a lake match the idealised concept, then this should be true, but any discrepancies from an idealised profile could lead to different methods producing different estimates for the mixed depth. This would make a cross comparison of mixed layer depths between different studies meaningless and poses difficulties for the understanding and quantification of linkages to biological or chemical processes.

These methodological caveats are of particular concern when using the mixed depth as an explanatory or predictive variable in chemical and ecological studies. For example, the mixed depth can control the vertical distribution of phytoplankton and therefore the light climate to which they are exposed (Diehl et al., 2002). The ability for a phytoplankton community to grow and maintain biomass depends on the ratio of the mixed depth to the euphotic depth (Huisman, van Oostveen and Weissing, 1999) in addition to the loss of cells due to sinking and the motility and light affinity of the species in the community (Diehl et al., 2002; Huisman et al., 2002; Jäger et al., 2008). Mixing that encroaches into the hypolimnion during stratification can also incorporate nutrients into the mixed layer increasing their availability for phytoplankton near the surface (Kunz and Diehl, 2003) and mix oxygen into the hypolimnion potentially reducing future internal loading (Mackay et al., 2014). Having a robust estimate of mixing is therefore required to understand the vertical positioning and composition of phytoplankton taxa within a lake, along with the mechanisms of bloom formation (Cyr, 2017) and the associated water quality impacts (Dokulil and Teubner, 2000; Jaša et al., 2019).

Similarly, the vertical pattern of productivity in the water column is influenced by the mixed depth and water clarity (Obrador et al., 2014); therefore lake metabolism studies require a robust mixed depth estimation. The depth of surface mixing determines how much of the water column has regular contact with the atmosphere, influencing the depth of oxygen penetration. This is particularly important in stratified, productive systems where incomplete mixing can result in anoxia in the hypolimnion due to the oxidation of organic matter by bacteria (Nürnberg, 1995). The direction of the flux of oxygen into and out of the mixed layer will also vary depending on the vertical distribution of primary

Table 1
Examples of temperature and density thresholds and gradients used in existing 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 et al., 2004	0.5 kg m^{-3} m^{-1}

producers in the water column relative to the mixed depth (Obrador et al., 2014; Peeters et al., 2016; Staehr et al., 2012, 2010).

Despite the widespread use of the mixed depth concept and the large number of methods used to estimate mixed depth, there is a lack of research evaluating the consistency among methods of mixed depth estimation and the implications of using different estimates when interpreting ecological and chemical data. This study therefore aims to: (1) determine if different methods of calculating the mixed depth produce comparable estimates; (2) evaluate the extent to which ecological and chemical parameters are homogenously distributed throughout the mixed depth; (3) evaluate how the choice of mixed depth definition may influence the calculation of simple example metrics relevant to studies of phytoplankton dynamics and metabolism. Analysis of vertical profiles of physical, chemical and ecological parameters collected from a small meso-eutrophic lake in the UK were used to address these aims.

2. Materials and methods

2.1. Site description

Blelham Tarn is a small (surface area 0.1 km^2), moderate depth lake (mean depth 6.8 m, maximum depth 14.5 m) (Ramsbottom, 1976), which stratifies typically for seven to eight months each year between spring and autumn. It is located in north-west England, UK (54°24′N, 2°58′W) and lies on the meso-eutrophic boundary (mean total phosphorus 24.5 mg m^{-3}) (Maberly et al., 2016).

2.2. Field methods and data collection

Vertical profiles of oxygen, chlorophyll *a* (measured via fluorescence as a proxy for phytoplankton biomass), temperature, specific conductivity and pH were measured using a YSI EXO2 multi-parameter sonde. Given the limitations of chlorophyll *a* fluorescence profiles (Gregor and Maršálek, 2004), water samples for chemical determination of chlorophyll *a* were taken at metre intervals in the water column (1–10 m) using standard methods (Mackereth et al., 1979). Vertical profiles of chlorophyll *a* obtained using both methods were compared visually and statistically using linear regression ($R^2 = 0.53$, $p < 0.001$). The probes were calibrated every six weeks according to manufacturer specifications. Profiles were measured weekly between 9:30 a.m. and 11 a.m. during the stratified period (46 sample days), defined here as when the density difference from the surface to the bottom was greater than 0.1 kg m^{-3}, at 0.5 m intervals in the water column from 1 m to 13 m (2016) and 0.5 m–13 m (2017).

A LI-COR underwater quantum cos-corrected sensor was also used to measure photosynthetically active radiation (PAR); measurements were taken just below the surface and then at 1-m intervals from 1 m to 9 m. The natural logarithm of the PAR measurements were regressed with depth and the slope of the equation was used to estimate the extinction coefficient (*k*) for each sample day. The euphotic depth (z_{eu}) was then defined as the depth where only 1% of the surface measurement of PAR remained:

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

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

Four methods of mixed depth estimation were tested for consistency, the first two methods used threshold changes in density (Method 1a) and temperature (Method 1b) from surface values to determine the depth of the mixed layer whereas Methods 2 and 3 determined the depth of the mixed layer statistically.

2.3.1. Method 1a: density threshold

The baseline mixed depth for this study was calculated as the depth at which the density first became 0.1 kg m^{-3} greater than the density at the surface (e.g. Andersen et al., 2017) (Fig. 1b). Water density was calculated using water temperature and salinity from equations within Lake Analyzer (Read et al., 2011). Salinity was calculated from conductivity using the GibbsSeaWater (GSW) Oceanographic Toolbox (McDougall and Barker, 2011).

2.3.2. Method 1b: temperature threshold

Temperature is frequently used instead of density to define the mixed layer, therefore a 1 °C difference in temperature from the surface was used, roughly equating to a 0.1 kg m^{-3} density difference at moderate water temperatures. Below these temperatures the density difference will be smaller and vice versa for higher temperatures (Fig. 1b).

Equivalent and directly comparable threshold methods cannot be applied to chemical and ecological variables due to their different units of measure. Therefore, two statistical methods 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 (Simpson, 2018). The section of the profile that contained the depth of the maximum gradient was identified, with the upper and lower values of this section being the mixed depth and the top of the hypolimnion, respectively (Fig. 1d).

2.4. Comparison of mixed depth method estimations

To compare the differences in mixed depth estimates, the mean difference (including the directional sign of the difference i.e. shallower or deeper), mean absolute difference (not including the directional sign), root mean square error and the range were calculated for the different estimates of mixed depth for each sample day. The relative shift in the mixed depth (shallowing, deepening or no change) was calculated between sample days as well as the percentage of instances in which the methods were consistent. Initial comparisons were made between temperature and density thresholds (Methods 1a and 1b), followed by comparing Method 1a with the two statistical methods (Methods 2 and 3).

Statistical models were then used to determine if the depth of the mixed layer calculated from density using Method 2 was a good predictor for the depth of the mixed layer calculated by Method 2 from the other variables. A similar assessment was carried out using Method 3. This was initially assessed by linear regression of the density-derived mixed depth against the depth of the mixed layer derived from chlorophyll *a*, oxygen, pH and specific conductivity profiles. The residuals from each regression were visually inspected for normality, homoscedasticity, autocorrelation, and the influence of outliers with no issues found. Non-linearity was initially assessed visually and then each model was fitted with a quadratic density-derived mixed depth term to optimise the model fit. The density-derived mixed depth as a predictor of the mixed depth calculated from oxygen and specific conductivity profiles was better described using a quadratic model whereas the equivalent for chlorophyll *a* and pH were best described using a linear model based on the *F*-test.

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

The coefficient of variation (expressed as a percentage) and the range of values for temperature, chlorophyll *a*, oxygen, specific conductivity and pH within the mixed layer were calculated for each method of mixed depth estimation and compared to the equivalent variation for the whole water column.

2.6. Calculation of example metrics using different mixed depth estimates

The following metrics were calculated for each sample day using mixed depth estimates for Method 1a, Method 2 and Method 3: (a) the percentage of oxygen and chlorophyll *a* within the mixed layer and whether more than 50% of chlorophyll *a* and oxygen were contained within the mixed layer, (b) the directional flux of oxygen, that is, the sign of the difference in the mean concentration of oxygen in the mixed layer compared to the concentration 0.5 m below and, (c) the ratio between the mixed depth and euphotic depth.

3. Results

3.1. Comparing mixed depth estimates

3.1.1. Methods 1a and 1b

Mixed depth estimates calculated using temperature were on average 0.7 m deeper than estimates calculated from the density baseline, equivalent to an increase of 70%. The RMSE was 1.1 m. The differences differed temporally (Fig. 2) with the maximum daily range in values being 5.5 m.

3.1.2. Methods 1a, 2 and 3

There were large differences between the density-derived estimates of mixed depth calculated using the three different methods (Fig. 3). Method 2 estimates were shallower than Method 1a by 0.8 m on average, whereas Method 3 estimates were deeper by 0.6 m (Table 2). The daily differences in the estimates had no consistent systematic pattern (Fig. 3), with the largest daily range in

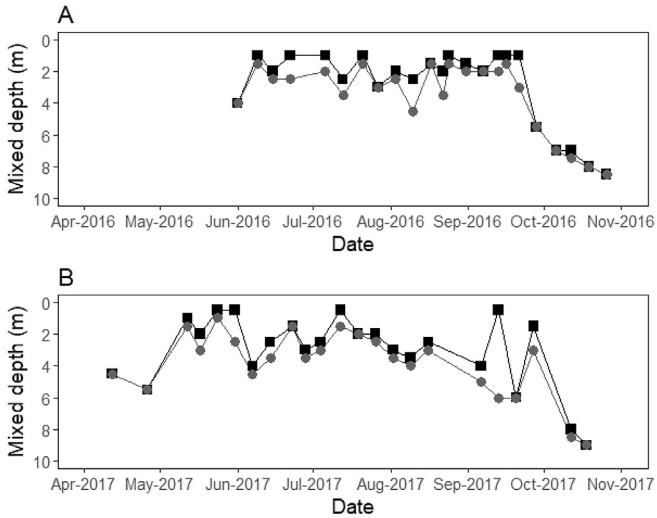

Fig. 2. Mixed depth estimates using Method 1a (density threshold; black square) and Method 1b (temperature threshold; grey diamond) in (a) 2016 and (b) 2017.

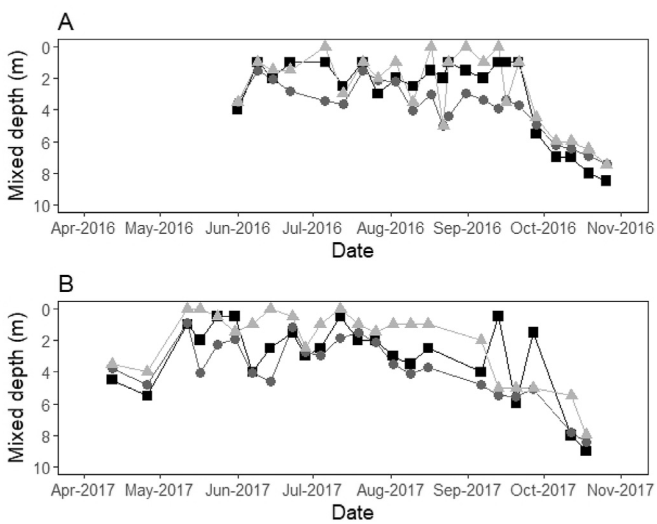

Fig. 3. Density-derived mixed depth estimates using Method 1a (black square), Method 2 (grey circle) and Method 3 (light grey triangle) (a) 2016 and (b) 2017.

values (5 m) occurring between Method 1a and Method 2. The methods were also inconsistent on whether there was shallowing, deepening or no change in the mixed depth between sample days with methods only being directionally consistent for 51% of sample days (one method disagreed for 42% of sample days and three different answers occurred for 7% of sample days).

Table 2
The mean difference, root mean square error (RMSE) and range in mixed depth estimates as calculated using Methods 1a, 1b, 2 & 3. Negative values indicate that the latter 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

3.2. Using the density-derived estimate as a predictor for ecological and chemical derived estimates of mixed depth

Mixed depths calculated using ecological and chemical parameters were varied and dissimilar from the estimates calculated from density (Fig. 4). The density-derived estimate was found to be a poor predictor for the estimates using chlorophyll *a*, pH and specific conductivity profiles, with low *F*-statistic values and weak or insignificant r^2 and *p*-values (Table 3). A significant relationship was found between the depth of the oxygen derived mixed depth and the density derived mixed depth using a quadratic model. Further statistical testing, however, demonstrated that at depths shallower than 4.5 m the density derived mixed depth was a poor predictor for the equivalent oxygen derived mixed depth.

Mixed depth estimates were also a poor predictor of the chlorophyll *a* maxima for 2016 and 2017 and a good predictor for the depth of the oxygen maxima during 2016 using Method 3 but not during 2017 when no significance was found (Table 3).

3.3. Determining the homogeneity of limnological variables within the mixed layer

As expected, temperature had a small coefficient of variation and range of values within the mixed layer compared to the whole water column suggesting a homogenous distribution of heat within the mixed layer (Fig. 5; Table 4). The coefficient of variation and range of values in the mixed layer for specific conductivity were also small relative to the whole water column suggesting homogeneity (Fig. 5; Table 4). Though the coefficient of variation was relatively low for oxygen in the mixed layer, values could differ by up to 2.4 mg/L at times suggesting that oxygen concentrations were not always homogenous (Fig. 5; Table 4). Chlorophyll *a* and the concentration of hydrogen ions demonstrated the largest coefficients of variation and range of values in the mixed layer relative to the water column (Table 4) and therefore had a heterogeneous distribution in the mixed layer for much of the stratified period (Fig. 5).

3.4. The impact of using different mixed depth estimates when calculating example metrics

3.4.1. The percentage of chlorophyll a and oxygen within the mixed layer

The mean percentage of chlorophyll *a* in the mixed layer during the stratified period differed between methods. Even the proportion of days when the majority (>50%) of chlorophyll *a* was contained within the mixed layer varied greatly depending upon the mixed layer estimation method (Fig. 6). For 2016 the proportion of days when the majority of chlorophyll *a* was contained within the mixed layer was 35%, 74% and 39% for Methods 1a, 2 and 3 respectively, whereas for 2017 the values were 48%, 65% and 30%. The methods only all agreed for 50% of sampling days on whether the majority of chlorophyll *a* was contained within the mixed layer (Fig. 6).

The mean percentage of oxygen in the mixed layer for the whole of the stratified period also differed depending on the definition used for mixed depth (Fig. 6). The proportion of days when the percentage of oxygen in the mixed layer was greater than 50% varied between methods (Fig. 6). For 2016 the proportion of days when the majority of oxygen was contained within the mixed layer was 43%, 83%, and 43% for Methods 1a, 2 and 3 respectively whereas for 2017 the values were 61%, 74% and 35%. The methods all agreed on whether the majority of oxygen in the water column was in the mixed layer for less than half (46%) of the sampling days (Fig. 6).

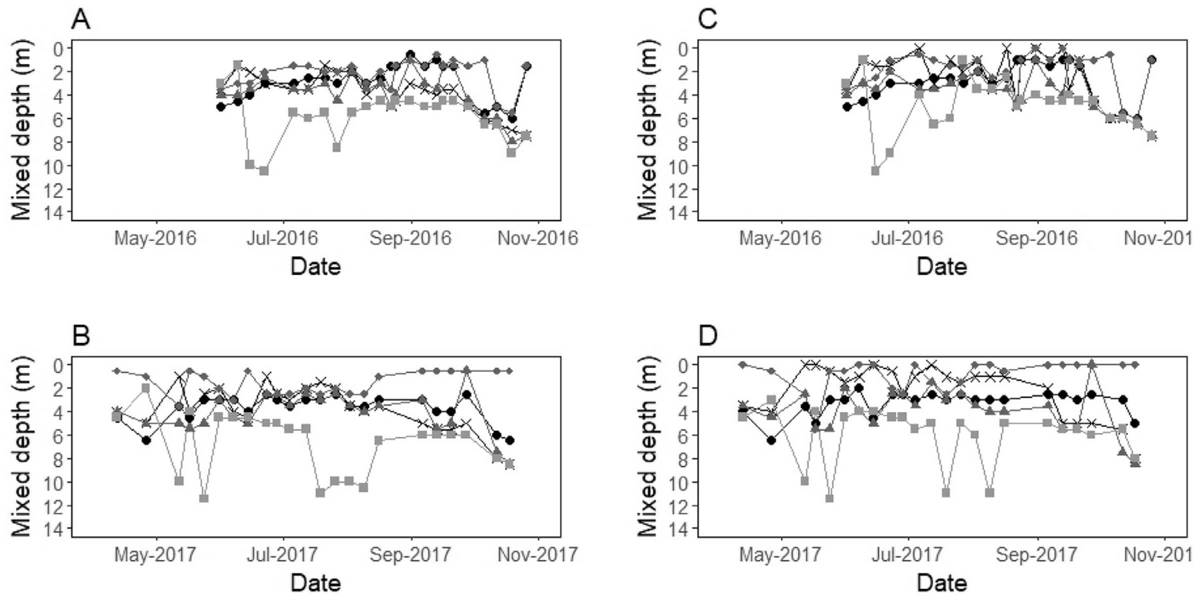

Fig. 4. Depth of the mixed layer calculated from density (×), chlorophyll-a (●), oxygen (▲), pH (◆) and specific conductivity (■) for (a) 2016 using Method 2, (b) 2016 Method 3 (c) 2017 Method 2 and (d) 2017 Method 3.

Table 3
Statistical model coefficients and adjusted R^2 values for the depth of the density-derived mixed depth compared with the mixed depth calculated from chlorophyll-*a*, oxygen, specific conductivity and pH as well as the depth of the chlorophyll *a* and oxygen maxima for Method 2 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 used for oxygen and specific conductivity whereas linear models were used for chlorophyll *a*, chlorophyll *a* maxima, oxygen maxima and pH, 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

3.4.2. The directional flux of oxygen

The direction of the flux of oxygen between the mixed layer and the layer below, as determined by whether concentration was greater within or beneath the mixed layer, was not always consistent between methods with contradictory results occurring 24% of the time (Fig. 7). Even when the direction of the oxygen flux was consistent between methods the size of the gradient between the mixed layer and the water directly underneath was markedly different (Fig. 7). Thus, both the direction and magnitude of the flux of oxygen between the mixed layer and the thermocline were highly dependent on how the mixed layer depth was defined.

3.4.3. Mixed layer to euphotic layer depth ratio

The ratio of mixed depth to euphotic depth was very different depending on which method was used to calculate mixed depth (Fig. 8). The mean ratio calculated using Method 2 (0.9) was typically greater than that using Method 1a (0.7), which was itself greater than that using Method 3 (0.6). As well as the systematic differences there was also a lot of temporal variation between the consistency of the estimates (Fig. 8). The mean difference between the mixed depth to euphotic depth ratio between Method 1a and Method 2 was 0.32 and between Method 1a and Method 3 was 0.90, with methods being contradictory as to whether the euphotic or the mixed depth was deeper for 20% of sample days (Fig. 8).

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

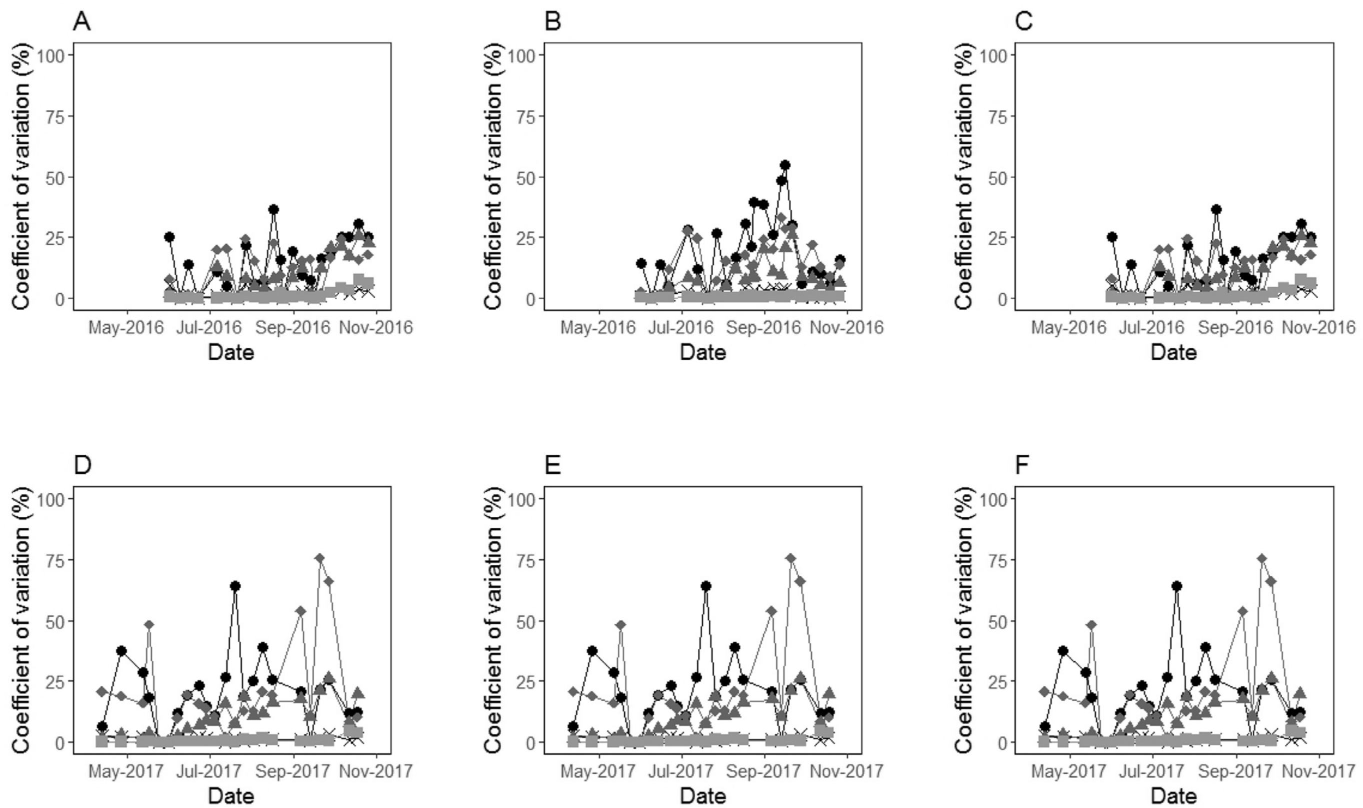

Fig. 5. The coefficient of variation in the mixed layer for temperature (—×—), chlorophyll *a* (—●—), oxygen (—▲—), concentration of hydrogen ions (pH) (—◆—) and specific conductivity (—■—) for (a) 2016 Method 1a, (b) 2016 Method 2, (c) 2016 Method 3, (d) 2017 Method 1a, (e) 2017 Method 2 and (f) 2017 Method 3.

Table 4
The coefficient of variation (COV) and the range of temperature, oxygen, chlorophyll *a*, concentration of hydrogen ions (exponential of 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), percentage values in brackets depict the percentage variation in the mixed layer relative to the whole water column variation.

Variable	*Mean coefficient of variation (COV) (%)*				*Mean Range*			
	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%)

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 surface layer in one property does not guarantee that it will be homogenous in another property (Table 4, Fig. 5). Studies interested in identifying homogenous distributions of phytoplankton, for example, for which gradients of light and nutrients as well as turbulence are controlling their distribution (Huisman et al., 1999; Kunz and Diehl, 2003), could be inaccurate if a density definition of mixed layer was used. That the depth of the mixed layer is highly dependent on the definition, and that not all properties will be evenly distributed within it, necessitates caution when analysing vertically resolved limnological data. Even the analysis of simple metrics relating to the distribution of chlorophyll *a* and oxygen demonstrates that the choice of mixed depth definition could

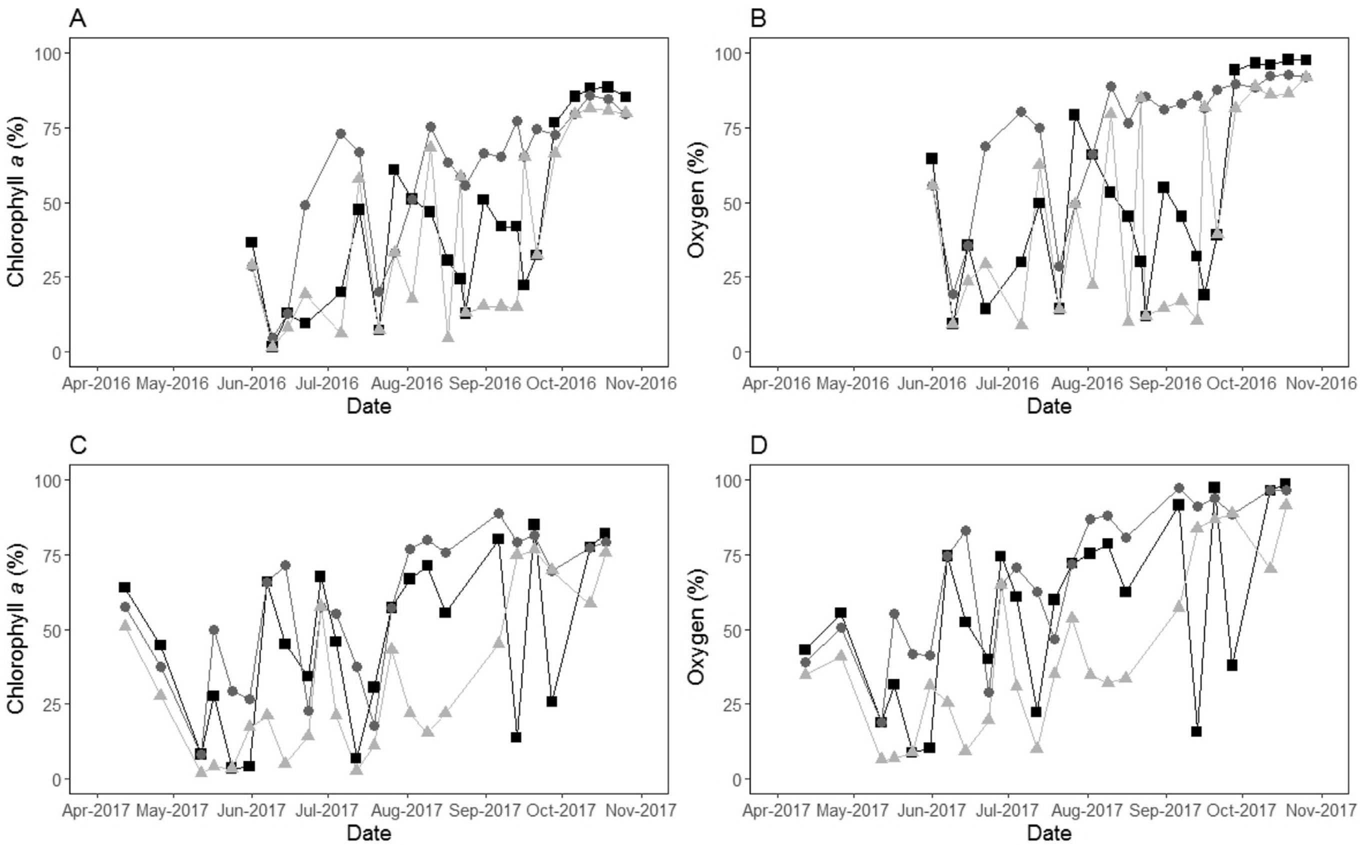

Fig. 6. The percentage of chlorophyll *a* and oxygen within the mixed layer using mixed depth estimates calculated using Method 1a (black square), Method 2 (grey circle) and Method 3 (light grey triangle) for (a) chlorophyll *a* in 2016, (b) oxygen in 2016, (c) chlorophyll *a* in 2017 and (d) oxygen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

influence the interpretation of results (Figs. 6–8). Thus, where phytoplankton samples are integrated over the epilimnion for assessing water quality (Noges et al., 2010) the assessment could be influenced by the definition of mixed layer adopted. Similarly, whether phytoplankton maxima are within or beneath the mixed layer will depend on the definition chosen. The oxygen flux into and out of the mixed layer is important for metabolism studies (Obrador et al., 2014), but the magnitude of the oxygen gradient between layers, and therefore the magnitude and direction of the oxygen flux, is highly dependent on the definition of mixed depth (Fig. 7). Nutrient fluxes will be similarly dependent on definition, which may have consequences for water quality determination and restoration responses (Hupfer et al., 2016; Read et al., 2014; Schauser et al., 2003). In general, the accuracy of flux estimated will be limited without turbulence measurements. The widely used ratio of the mixed depth to euphotic depth was also dependent on the definition of mixed depth used (Fig. 8). This is consequential, when explaining the formation of sub-surface phytoplankton maxima, which are thought to occur in eutrophic systems when the euphotic depth is deeper than the mixed depth (Hamilton et al., 2010; Leach et al., 2018; Mellard et al., 2011).

The interrogation and interpretation of vertical profiles is a fundamental and burgeoning area of limnological study (Brentrup et al., 2016; Hamilton et al., 2010; Leach et al., 2018; Obrador et al., 2014) and will require careful consideration of how best to use mixed depth as a predictive or explanatory variable or as a determinant of water quality monitoring. One approach is to assess the impact of using different mixed depth estimates when analysing results. For example, the Giling et al. (2017) study on metabolism found that halving or doubling the threshold density gradient used to estimate the mixed depth changed the estimated thickness of the metalimnetic depth zone by 22%. For the study, this inconsistency was deemed relatively insignificant to the findings, however the authors highlighted that this would become problematic when aggregating metabolic rates to the metalimnion and hypolimnion (Giling et al., 2017). Another approach is to examine systematically which method or methods are more consistently useful than others for approximating a mixed depth.

5. Conclusions

By testing three methods of mixed depth and using them to calculate simple ecological and chemical metrics this study has demonstrated that methods of mixed depth estimation are inconsistent and influence the interpretation of chemical and ecological results. Based on these findings we recommend that future studies should:

- Favour density over temperature for estimating the mixed depth
- Not assume homogeneity of other variables within the mixed layer
- Assess the sensitivity of the findings of the study to mixed depth definition or
- Examine several methods to choose the most consistent and useful method for the study

Ultimately, any method adopted for estimating mixed depth from standard limnological data should be used cautiously and

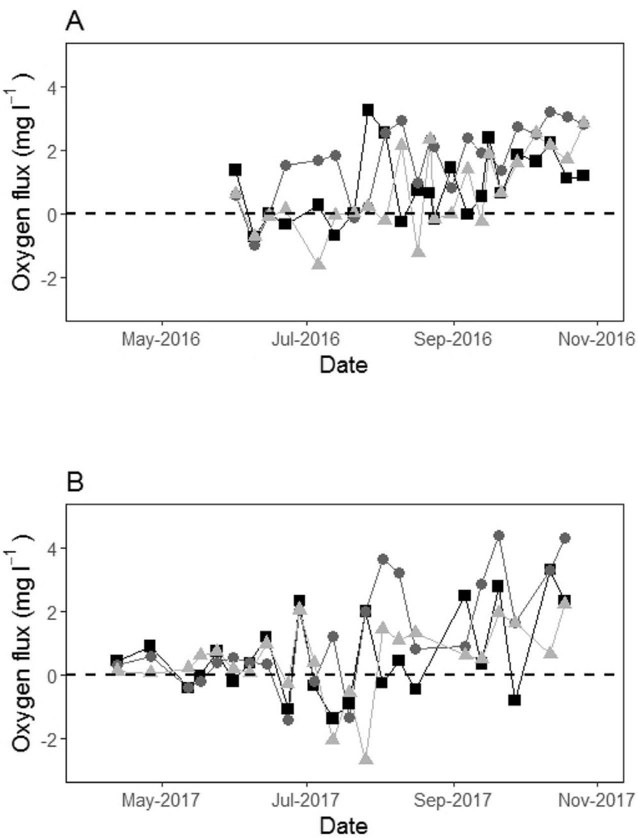

Fig. 7. The difference in the concentration of oxygen within the mixed layer compared to the concentration in the layer 0.5 m below using mixed depth estimates calculated from Method 1a (black square), Method 2 (grey circle) and Method 3 (light grey triangle) for (a) 2016 and (b) 2017.

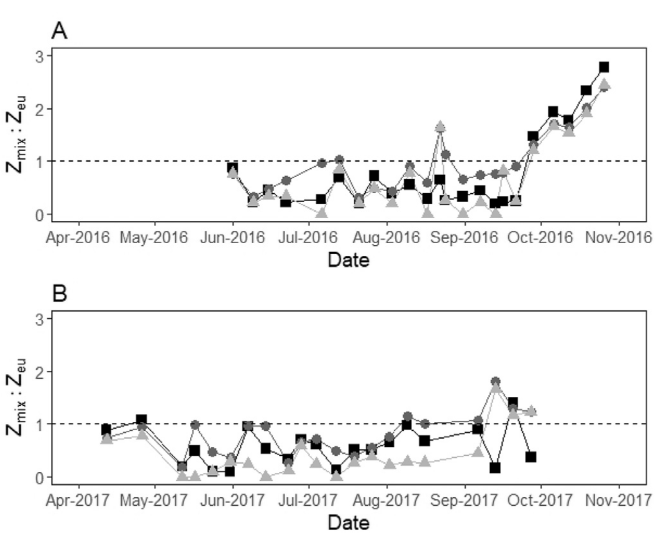

Fig. 8. The zmix:zeu ratio calculated using density derived mixed depth estimated using Method 1a (black square), Method 2 (grey circle) and Method 3 (light grey triangle) for (a) 2016 and (b) 2017. Values below the horizontal y intercept line at 1 zmix:zeu mark when mixed depths are shallower than the euphotic depth and vice versa for values above.

with awareness of the potential deviation of observed profiles from idealised ones.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2019.115136.

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