Review for manuscript bg-2016-49:

"Variations in triple isotope composition of dissolved oxygen and primary production in a subtropical reservoir" by:
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In this manuscript the authors present results of O_2/Ar ratio measurements, as well as of stable oxygen isotopes in dissolved oxygen (δ^{17}O and δ^{18}O) to estimate the ^{17}O excess ($^{17}\Delta$), and in water (δ^{D} and δ^{18}O). Water samples were taken over a year (from May 2014 to July 2015), at different depths in the vertical water column of the Feitsui Reservoir, Taiwan. The authors used the oxygen measurements to estimate the net and gross production (NP and GP, respectively) to evaluate the reservoir's metabolic state and seasonal variability. This is the first time that the triple oxygen isotopes technique is applied in a freshwater enclosed system. The authors gathered a nice data set that can help to understand the fast changes of the metabolic balance in the reservoir, and prove the ability of the method to capture them. The manuscript is generally well written, however the structure has to be modified slightly, as well as the main focus of the paper. It lacks of strong linkages between the dominating physical factors in the reservoir (vertically and horizontally) and changes of $^{17}\Delta$ and O_2/Ar. Furthermore, it contains major flaws on the data processing of the data, such as corrections on the isotopic analysis of samples as well as in the calculation of GP from $^{17}\Delta$; this is in the first place not correctly done, and in the second place, I don't think this estimation can be applied to the reservoir due to its fast changing vertical water column dynamics which are not considered in the calculations. Therefore, at this point, I cannot accept this manuscript for publication. From my opinion, there are major changes that need to be done before this work can be considered for publication in Biogeosciences. Next, I list a summary of my major concerns:

1) The definition of mixed layer depth used by the authors is provided. This is a very relevant concept because it defines the physical limit for the NP and GP estimations based on O_2/Ar and triple oxygen isotopes. Consideration of vertical transfer for GP and NP calculation cannot be neglected. Furthermore, in the way is given now, it makes totally irrelevant the calculation of GP since Feitsui Reservoir seems to have a complex vertical and horizontal water structure. The triple oxygen isotopes of dissolved oxygen, as well as the stable isotopes of water, can be powerful proxies that can be better used to understand the dynamics of the reservoir linked to their physical characteristics, and this is not sufficiently done in the manuscript in its current form.

2) Their statistical interpretation of the data lacks rigor and understanding of the method. The authors should express their precision and uncertainties in a better way. Also there is a lot of missing information in regard to the isotopic data correction due to interferences and imbalance between sample and reference side during the MS analysis, for example.

3) There seem to be a lack of explanation to the relevance of measuring aliquots of laboratory prepared equilibrated water. These serve as standard to estimate the reproducibility of the method during sample preparation and isotopic analysis by MS in the absence of samples duplicates, more detailed information is needed here.

4) After an improvement of the definition of mixed layer depth and interpretation of their changes, the GP calculation should be corrected to use δ^{18}O and δ^{17}O directly instead of $^{17}\Delta$. There is published evidence showing that due to numerical inaccuracies, this practice has to be changed.

5) Due to the fast changing physical dynamics in the reservoir, the authors should be careful in the GP estimation from triple oxygen isotopes, and this simply cannot be done in the same way as done until now for ocean basins.

Detailed information on these concerns, as well as a list of minor recommendations, are given below:

Major comments:

There is no place in the manuscript in which the authors mention their chosen criteria to define their mixed layer depth. It seems it follows nicely the Chl a vertical distribution in Fig. 2b, but this might be only and artifact of the colors in the figure. Whichever criteria the authors chose to define their mld seems just wrong and not necessarily useful for the observations in $^{17}\Delta$ values and estimation of mixed layer-GP. The timescale of processes that influence the vertical mixing in lakes and reservoirs depends on the basin size and stratification. I think here it is more complex to define a mixed layer depth that suits to the concept of the estimation of GP from oxygen isotopes. There seem to be a permanent and a temporal mixed layer, with overturning and convective cooling occurring at faster orders of magnitude than what can be estimated with a standard calculation for the gas exchange coefficient. The vertical displacement of primary producers and adaptation should be evaluated and taken into account together with the stable isotopes data. The mld definition for applications of GP from oxygen isotopes and NP from O_2/Ar ratios must represent closely the metabolic state of the water column within the productive zone. A definition based on oxygen as done in Castro-Morales and Kaiser, 2012, could potentially help to define a better mld for GP and NP estimates based on oxygen measurements.

For this reason, I don't think here it is appropriate to apply the estimates of GP from the triple oxygen isotopes method for lakes and reservoirs as done for the ocean until now.

Besides: calculating GP from $^{17}\Delta$ should be avoided. This was the standard calculation and the approximation may be still fine for low, typically oceanic $^{17}\Delta$ values. However, higher values will lead to a larger error in the GP, to avoid this, GP should be instead calculated from the measured δ^{17}O and δ^{18}O as demonstrated by Prokopenko et al., 2011 and Kaiser, 2011. Since this are lake samples and some of the results show high $^{17}\Delta$ values, the authors should consider re-calculating their GP using directly the δ^{17}O and δ^{18}O, but this should be done only if the authors find an agreement on defining a mixed layer adequate to the fast changes occurring in the water column of the reservoir. Furthermore, the presentation of $^{17}\Delta$ is a better practice in this case and should be presented and discussed in the manuscript as a proxy variable of GP.

The high oxygen supersaturation in the entire water column between May and June 2014 could be due to strong vertical mixing with most of the oxygen from

atmospheric source, this is also shown by the very low ${}^{17}\Delta$ and $\delta O_2/Ar$ values. Rain season?
Which physical processes (horizontal or vertical transfer) occur in the reservoir for the productivity to increase in July at depth? How relevant here are seiches? Was that specifically evaluated?

P4:
L10 – this part requires major explanation on the in vitro dissolved oxygen measurements, how many samples and at which depths were collected to calibrate the CTD data? What is the precision of the in vitro oxygen measurements? Which technique was used for the detection of the reduced oxygen species in the sample after titration?
L24 – This paragraph needs a reference for the extraction and collection method into the molecular sieve pellets. Did the authors follow Abe, 2009 (*Rapid Commun. Mass Spectrom.* 2008, 22, 2510) or Keedakkadan and Abe, 2015 (*Rapid Commun. Mass Spectrom.* 2015, 29, 775–781)?. If there was a modification to any of these two suggested extraction procedures, or it was used a different one (?) then the authors should specify in which way this was done.

P5:
L10-11 – this statistical analysis doesn't make sense. A student's t-test can be only applied for the comparison of normally distributed data sets. Here it is simply the average of the repetition of measurements (cycles) of the same sample (not duplicates or triplicates), which only gives the analytical precision or mean standard error in the measurement of one sample. It is clear that the more acquisitions with more cycles each will reduce the error in the measurement. The two-sigma outlier removal, why they were done? Were they the source of an error/contamination in the sample or in the IR measurement?. Statistically it doesn't mean anything to give an average of the standard error for all samples, since each sample is independent to each other in space and time, I encourage the authors to delete the sentence in L10-11.
Furthermore, the reproducibility and performance of the samples preparation and the MS analysis must be evaluated with the standard error of the air-equilibrated water samples mentioned only until the discussion section (P11, L23-25). This should be moved to section 2.3, see more comments below on this regard. Uncertainties in the O_2/Ar ratio and $\delta^{18}O$ and ${}^{17}\Delta$ from the air-equilibrated water aliquots with respect to air should be also provided.
L20-21 – The explanation of this correction is not very clear. Did the authors corrected $\delta^{17}O$ due to N_2 interference in the analysis?
Also did the authors made corrections due to differential gas depletion between the sample and reference sides during the IR analysis? See Stanley et al., 2010 for this, and report if this was done or not.
A correction to $\delta^{18}O$ due to fractionation has to also be done, did the authors checked for this?
L17 – did the $\delta O_2/Ar$ were normalized to air? There is also no explanation regarding the correction for the residual gas in water sample after equilibration, the authors should have been done that in order to obtain their $([O_2]/[O_2]_{eq})_{bio}$ in eq. 3, but this is not stated in the manuscript. See also my comments below for P7 and Fig. 5.

P8:

L19 – I am missing more information from the results of the isotopic composition of water. I would have expected more insights on the different sources of water in the reservoir; it is possible to differentiate here rainwater and standing water? Is it possible to see here the extent of rainwater after typhoon events in the reservoir? How about possible horizontal contribution of water with potentially different characteristics?

L23 - Do the authors assume here that the $^{17}\Delta$ from JUL14 and AUG14 represent only biological production and these are selected to represent the end member $^{17}\Delta_{\text{bio}}$? The typical value used here was 249 per meg derived experimentally and shown in Luz and Barkan, 2000, and controversy on the values for the isotopic signatures of photosynthetic activity ($\delta^{17}O_p$ and $\delta^{18}O_p$) have been discussed in literature (e.g. Kaiser, 2011, Luz and Barkan, 2011 *Global Biogeochem. Cycles*, see also the comment by D.P. Nicholson (doi: 10.5194/bg-8-2993-2011) on the Kaiser (2011) paper for detailed discussion on this regard. The most recent values for $\delta^{17}O_p$ and $\delta^{18}O_p$ are shown in Luz and Barkan, 2011b (*Geophys. Res. Lett.*) and also in Barkan and Luz (2011, *Rapid Commun. Mass Spectrom.*) and in Kaiser and Abe (2012). The authors are encouraged to revise this literature and select a value for $\delta^{17}O_p$ and $\delta^{18}O_p$ in case they find a better definition of mixed layer depth for their GP estimates. In any case, the authors should stop using their $^{17}\Delta_{\text{bio}}$ for this.

P9:

L09 – By DEC14 thermal stratification nearly disappeared" but mld became deeper? How is mld defined? It follows the color code of Chl a in Fig. 2 but this might be an artifact of the figure. How vertical mixing is high but a very strong marked mld? What happened from SEP14 to OCT14 that the stratification broke, which physical process dominated for this change in the water column? (horizontal or vertical transfer? Why? Wind speed increase? Rain?), increase in atm O_2, but you cannot see that in $\delta O_2/Ar$, I recommend using $\Delta O_2/Ar$ instead.

L30 – is the nutrient availability also so high as in 100 m in spring 2015? Which physical process may dominate in the reservoir to achieve this?

L32 – How common are seiches in Feitsui Reservoir? This seems to be an important process that dominates the distribution of nutrients and gases vertically in the water column of the reservoir. More information is needed on this regard and the authors should discuss more this process in the context of their findings.

Which other external forcing processes the authors meant there?

P10:

L1 – were precipitation events recorded during the sampling periods (not only the typhoon events)?

L2-5 – here the authors suggest that vertical processes are relevant for the distribution of the $^{17}\Delta$ signal in the reservoir, it

L11-12 – most of the production seems to take place below the mld,

L12 – again this is arguable because of their definition of mld

P11:

L6 – how was that lifetime of O_2 estimated? Was based on mld and gas transfer coefficient?

L7 – I disagree that the vertical mixing and advection are negligible for $^{17}\Delta$ and ultimately GP determinations in the reservoir. I think the authors underestimate here the vertical transfer of biological O_2 (vertical displacement of primary producers within the reservoir) and their definition of mixed layer depth is by no means helpful for their budget model. The fact that there is high $^{17}\Delta$ in subsurface and deep waters defines the reservoir as a full column activity water system with marked seasonality and strong vertical influence, possibly due to wind and rain. It is hard to apply there the GP concept from δ^{17}O and δ^{18}O considering the shallow mixed layer depth. This is irrelevant to calculate here, I would be more focused on explaining the physical driving forces to the vertical distribution of $^{17}\Delta$, and how this changes in short periods of time the metabolic balance of the reservoir.
L8 – but most part of the sampling period the bottom limit of the euphotic zone lies below the mixed layer, so again the authors should revisit their definition of mld for GP calculations
L24-25 – more information is needed on the preparation of the air-equilibrated aliquots

P12:
L09-10 – I agree, but then why the authors wrote lines 10-12 in P. 11? This is contradictory to what is stated here. To actually compare productivity values based on ^{14}C and from oxygen isotopes, the sampling scheme had to be designed for this purpose with duplicate analysis and samples for ^{14}C also at depth.

Minor comments:
Throughout the manuscript, leave a space between the quantity and the unit in % and $^{o}/_{oo}$, and also for ^{o}C
P1, L15 – add "water" reservoirs
P2, L11 – change "confining it to a small volume" to "confining them into a small volume"
P2, L16 – replace the symbol "&" by the word "and" here and all the citations throughout the manuscript where it is used
Modify to "introduced the triple oxygen-isotopes technique, …"
P2, L17 – change to "The ^{17}O excess is defined as:"
P2, L19 (eq. 1) – here and elsewhere all variables must be italicized, this is particularly the case of all delta symbols in: $^{17}\Delta$, δ^{17}O and δ^{18}O, as well as K, C_o introduced in eqs. 2 and 4, and throughout the manuscript.
P3, L3 – change "large" to "largely"

P4:
L1 – add comma between "quality" and "the watershed"
L1 – add "the" before Feitsui Reservoir
L2 – pluralize "area"
L2 – add "are" between "active" and "prohibited"
L3 – add "the" before Feitsui Reservoir
L4 – since when the meteorological station near Feitsui Reservoir has been active?
L5 – change from "processing" to "preparation"
L9 – change to "using a Sea-Bird CTD…", what was the vertical resolution of the CTD measurements?
L14 – leave a space between the number and the units (15 μL)

L19 – "…for removal of water vapor at liquid nitrogen temperature. The extracted gases…"
L21 – The GC is to separate N_2 and CO_2 from O_2 and Ar, please delete "contaminants". Please correct and complete the sentence by adding that only O_2 and Ar remain the main components in the gas mixture.
L22 – "During the separation … "
L25 – I suggest here to add a new section (2.3) that corresponds to the "Stable isotope analysis in water". Also an opening sentence to explain why this was done is needed, for example: "To identify the source of water in the reservoir, the δ^D and $\delta^{18}O$ in the H_2O molecule of reservoir water was analyzed. For this, water samples were collected in 15 ml …."
L30- change uL to mL and to "an aliquot of 5 mL of water sample was converted to O_2 by injecting it to a CoF_3 reaction tube…". Leave also a space between 370 and ^oC.

P5:
L3 – Do you mean that a set of duplicates of standard water samples were measured every 80 water samples analysis?
L8 – change to "O_2 from the purified oxygen-argon mixture (as explained in section 2.2)…"
L9 – change to "12 cycles each. Thus, the reported …"
L18 – this precision is for δO_2/Ar in repetitions of atmospheric air measurements?
L23 – the correction is not to achieve high precision, it is simply a correction of the measurement due to interferences. Delete this sentence.
L26 – remove the second "of" (…"and for obtaining more precise results…")
L29 – How many samples represent one set or trip to the reservoir? Are all the black dots plotted in a single vertical profile in Fig. 5 representing one set of samples? They were not always the same number isn't?

P6:
L2-8 – what is written in this paragraph is only true for a system at steady state. This should be stated.
L16 – Co as expressed by the authors is not simply the O_2 solubility, but the O_2 concentration at saturation, or at equilibrium with the atmosphere, using the solubility coefficients from Benson and Krause, 1984, and the standard term to express this is $[O_2]_{sat}$.
L17 – did the daily wind speed measurements were collected from the meteorological station? Were they corrected to represent wind speed 10 m above sea level? Why averaged over 1 week? What is the residence time of the gas in the mixed layer depth of the reservoir as calculated from the gas transfer coefficient and the mixed layer depth?
L22-25 – this paragraph should be moved to another section maybe below section 2.2, stating specifically how the ^{14}C analysis was done.
L29 – change to "… Ar supersaturaion in water …"

P7:
L2, Eq. 3 –The term on the left hand side of Eq. 3 is misleading and doesn't represent what is really expressing. The biological O2 saturation should be expreseed as ΔO_2/Ar as in many past works that use this method (e.g. Cassar et al., 2011, Castro-Morales et al., 2013). Please avoid introducing new ways for terms and variables. The new

community using this method should make use of the same variables to express the terms to avoid confusion and to keep consistency.
L6-7 - here it should be stated that the authors corrected $\delta O_2/Ar$ for the residual gas in water sample after equilibration in order to obtain their $([O_2]/[O_2]_{eq})_{bio}$ (that should be $\Delta O_2/Ar$) in eq. 3.
L21 – I wouldn't call it permanent but seasonal stratification
L22 – the temperatures above 30 ^{o}C are only in the top 10 m.
L25 – here it should be defined which criterion the authors used to define mixed layer depth.
Is only the change in atmospheric temperature what makes the temperature of the water reservoir to change? Is there no evidence of vertical or horizontal water transport? As mentioned later in the manuscript, other lake processes as the presence of seiches (P9, L32) or other external influences such as wind or water input from precipitation can also alter the temperature of the reservoir. Discuss this here in the context of this factors possibly contributing to the change in the water temperature. Of particular interest is what happens at depth in the reservoir, away of the direct atmospheric influence.
L26 – which processes occur within the reservoir to shallow the mixed layer depth in summer? Only warming by atmospheric influence at the surface?
L27 – in all other cases where mixed layer was present was also defined, only shallower
L28 – where are these sediments from? From the bottom or from lateral transport within the reservoir? Which other lake processes?

P8:
L13-16 – during late spring in 2014, O_2 supersaturation also at the bottom of the reservoir is seen, what is the origin of this? It should be then a very strong vertical mixing in the reservoir at this period of time, this is the indication of a vertical transfer also of potentially biological O_2. Why is so fast changing this to a very shallow O_2 supersaturation by end of April?
L22 – complete the sentence as: "showed more depleted values during autumn at the top 60 m…"
L23 – what do the authors mean with "selected waters"?

P9:
L5 – wouldn't be better JUL14 than JUN14?
L7 – Also in JUL14
L8-9 – the thermal stratification nearly disappeared, but the mixed layer depth just became deeper, how it is defined mld?
L22 –how much is the annual mean?

P10:
P10, L17-18 – remove one dot at the end of the sentence (it has two) and the dot at the end of line 18 should only have the comma.
L6-7 – change to "…we briefly discuss our results in the context of typhoon events."
L18 – higher wind speeds can also explain higher GP rates at depth of the reservoir?

References
- The reference of Barkan and Luz, 2005 is missing

Figures

Fig. 2, is one monthly band of data representing only a once in a month sampling? So this is not really an entire month of data but only few days (maybe only one day) in which sampling a set of samples took place? The interpolated figures as shown in Fig. 2, 3 and 5 are then very misleading, since the vertical data cannot be put sequentially one after the other and do a horizontal interpolation with them. There is a gap of about 29 days between them, and as it looks now in the figures, “fast” changes occur between one month and the other. I will be careful in the way the data is presented in this figures. I would rather do simply vertical profiles or not put together the bands. It is unclear to see how much of the information on the figure is the result of interpolation artifacts.
Also, the mixed layer depth and limit of euphotic zone should be drawn also from May 2014.

Fig. 3, what happened with the data from May to August 2014?
The lower δ^{18}O and δ^{D} from the surface to about 60 m from October to December 2014 is related to the first typhoon according to the authors, However, at the time of the second typhoon there is also a different d18O signal at the surface (top 20 m in May-July 2015), the authors must explain these differences and linkages to δ^{18}O and δ^{D} from dissolved oxygen as shown in Fig. 5a for the second typhoon.

Fig. 5,
5b, the low $^{17}\Delta$ in the water column from 20 m down during May-June 2014 is coincident with very high O_2 saturation, which indicates a strong vertical mixing from surface air saturated water down. This is also evidenced in the $\delta O_2/Ar$ signal. However, the authors claim that the high $^{17}\Delta$ seen at the depth during July-October 2014 also originates from vertical transfer from the surface, however, there is lower $^{17}\Delta$ signal in the top <10 m. could it be local O_2 photosyntetically produced? or horizontal transfer? Which process actually causes breaking down the high $^{17}\Delta$ in the entire column between 40 and 60 m from July-November 2014?
First signal of high $^{17}\Delta$ at the bottom (80-100 m) in March-May 2015 it seems is a different water mass, this is also seen in the δ^{18}O and $\delta O_2/Ar$, what is its origin? It looks lateral transport.

5c, why is this third depth point at around 20 m in August 2014 so high in O_2/Ar? Most of $\delta O_2/Ar$ is below zero. It is hard to see the biological and atmospheric contribution in this ratio. A better way to express this is as $\Delta O_2/Ar$ (biological O_2 saturation) in % (this is their $([O_2]/[O_2]_{eq})_{bio}$). I recommend the authors to plot instead $\Delta O_2/Ar$ in Fig. 5 panel c.

Fig. 6, are the $^{17}\Delta$ GP shown there is only the surface values?