Response to Comment: Terrestrial support of pelagic consumers in unproductive lakes—Uncertainty and potential in assessments using stable isotopes

The use of stable carbon isotopes ($\delta^{13}C$) has played a key role in estimation of the proportion of aquatic consumer biomass derived from terrestrial organic matter (OM; i.e., allochthony; Meili et al. 1996; Grey et al. 2001; Pace et al. 2004). However, the use of $\delta^{13}C$ for assessing allochthony has shortcomings because of the small natural separation between terrestrial and aquatic isotopic end members and the difficulty in physically separating autotrophic phytoplankton for $\delta^{13}C$ analysis from other components of particulate organic carbon (POC). These shortcomings are especially problematic in unproductive lakes where the phytoplankton are dominated by small and mixotrophic species, and where the internal photosynthesis is low compared to the input of terrestrial OM (Algesten et al. 2004; Jansson et al. 2008). Several alternative analyses and approaches have been tested to overcome these methodological limitations, including compound-specific analyses of phytoplankton biomarkers (Pace et al. 2007; Van Den Meersche et al. 2009; Berggren et al. 2014), manipulation of phytoplankton $\delta^{13}C$ by addition of ^{13}C-labeled dissolved inorganic carbon (Pace et al. 2004; Taipale et al. 2008), addition of ^{13}C-enriched OM (Karlsson et al. 2007; Bartels et al. 2012), and various mass balance and modeling approaches (Marty and Planas 2008; Mohamed and Taylor 2009; Berggren et al. 2010). Still, a generally applicable method is lacking, implying that the problems with assessing $\delta^{13}C$ of phytoplankton is a major limitation in the use of $\delta^{13}C$ for estimating allochthony with the accuracy needed for detailed understanding of food web dynamics.

As a consequence, researchers have started to use alternative isotopic tracers and especially the stable hydrogen isotope (δ^2H). The terrestrial and aquatic end-member δ^2H values have been shown to be clearly separated (Doucett et al. 2007), and the method has been used to quantify allochthony of consumers in lakes where the use of conventional $\delta^{13}C$ analysis has been problematic (Cole et al. 2011; Solomon et al. 2011; Wilkinson et al. 2013a). Although issues related to, e.g., dietary water and analytical procedures need to be carefully addressed to reduce uncertainties in estimates of allochthony using δ^2H (Solomon et al. 2009; Soto et al. 2013), the technique has a large potential for advancing our understanding of patterns and magnitude of allochthony in consumers of many aquatic systems. In Karlsson et al. (2012), data on metabolism, δ^2H, and stable nitrogen isotopes were used to show that terrestrial OM supported all parts of the food web in a humic lake (Upper Bear Lake in boreal Sweden). These results suggest preferential use of autochthonous-based resources and that there is an upper level of terrestrial support of higher trophic levels in lakes. They then went on to compare these results with those concurrently obtained

using $\delta^{13}C$ analyses. Brett (2014) questions the $\delta^{13}C$ of phytoplankton used for estimates of consumer allochthony in Upper Bear Lake and, thereby, also the "key results" of the study. However, Karlsson et al. (2012) based the key results on δ^2H and further explained the choice of δ^2H over $\delta^{13}C$ by the fact that the $\delta^{13}C$ approach has limitations due to small separation between terrestrial and aquatic primary producers and the fact that the $\delta^{13}C$ of phytoplankton is variable and difficult to measure. The Karlsson et al. (2012) paper then compared the findings regarding allochthony based on δ^2H with allochthony estimated from $\delta^{13}C$ data using previously published end-member $\delta^{13}C$ values from similar lake types in boreal northern Sweden. Only zooplankton was included in this comparison because it is not possible to estimate allochthony for zoobenthos and fish by using the $\delta^{13}C$ data. Karlsson et al. (2012) did not attempt to estimate the $\delta^{13}C$ of end members in Upper Bear Lake but rather compared values with results obtained using the end-member $\delta^{13}C$ used in previous studies. Such a comparison we believe is of interest for the scientific community. The comparison shows a close relationship between the two methods. Thus, the point by Brett (2014), i.e., that allochthony may be overestimated by assuming too high ^{13}C depletion in phytoplankton, cannot be reconciled with the fact that the δ^2H data show similar results as the ^{13}C data.

Brett's comment criticizes the method in which Karlsson et al. (2012) used an unusually depleted phytoplankton $\delta^{13}C$ value, which was taken from Karlsson et al. (2003). Brett (2014) points out that Karlsson et al. (2003) used a too-high ^{13}C fractionation factor ($\varepsilon_p = -23.7‰$ for forested lakes) for phytoplankton photosynthesis compared to the "contemporary literature" (the Karlsson et al. [2012] article did not speculate on the ε_p). We are aware that the ε_p in Karlsson et al. (2003) is high when compared with many other studies, but we also note similarities with a recent study of 18 boreal clear-water and humic lakes in Canada, where ε_p was estimated from $\delta^{13}C$ of phytoplankton biomarker fatty acids to $-21.8‰ \pm 3.6‰$ (Berggren et al. 2014). However, the $\delta^{13}C$ of phytoplankton is not only dependent on ε_p, but also, to a large extent, on the $\delta^{13}C$-CO_2, which varies largely between lakes. Our point here is that $\delta^{13}C$ of phytoplankton is expected to be especially low in unproductive humic lakes, such as the Upper Bear Lake, due to relatively low $\delta^{13}C$-CO_2 and relatively high ε_p. A majority of the data used in Brett (2014) originates from studies of lakes in North America with characteristics that are significantly different from those used by Karlsson et al. (2012), i.e., temperate lakes with generally higher to very much higher chlorophyll a (Chl a) compared to Upper Bear Lake (Drakare 2002). Furthermore, in unproductive humic lakes, the CO_2 concentration is most often highly supersaturated (Sobek et al. 2003), and small-sized phytoplankton with low growth rates usually

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Corresponding author: jan.karlsson@emg.umu.se

dominate (Jones 1992; Jansson et al. 2012). These factors likely result in relatively high availability of CO_2 relative to phytoplankton demands (Jansson et al. 2012) and, consequently, a high ^{13}C discrimination (Laws et al. 1995; Burkhardt et al. 1999). In Smyntek et al. (2012), ε_p was shown to increase with CO_2 concentration of the water, up to $-18‰$ at $[CO_2]$ of around 70 μmol L^{-1} (which is low compared to our lake; M. Klaus unpubl.). In one of the few unproductive lakes (Loch Ness) where phytoplankton $\delta^{13}C$ has been directly measured, the ε_p was estimated to $-18‰$ in large-sized diatoms (Grey et al. 2001; Jones et al. 2001). Grey et al. (2001) also point out that the abundant small-sized taxa of Loch Ness (which were not possible to separate) probably had even higher ε_p.

Another important factor that Brett (2014) does not address in his analysis is that acidic (mean summer pH for 1996–98 = 4.7 in Upper Bear Lake; Drakare 2002) humic lakes, with high respiration of terrestrial OM and net heterotrophic conditions (gross primary production (GPP) : community respiration (R) = 0.03 in Upper Bear Lake; Karlsson et al. 2012), also tend to have particularly low $\delta^{13}C$ of the dissolved CO_2 available for phytoplankton (Striegl et al. 2001; Bade et al. 2004). In the comment by Brett (2014) the $\delta^{13}C$-CO_2 was set to $-17.5‰ \pm 0.8‰$, which likely introduced substantial additional problems in the analysis. The low GPP : R ratio in Upper Bear lake (Karlsson et al. 2012) suggests that the $\delta^{13}C$-DIC in the lake water is rather around $-25‰$ (which agrees with data from the lake; M. Klaus unpubl.). The $\delta^{13}C$-CO_2 would be even lower (Mook et al. 1974). Using a $\delta^{13}C$-CO_2 of $-25‰$ in the calculations presented by Brett (2014) makes the phytoplankton similarly or more depleted in ^{13}C than what was assumed in Karlsson et al. (2012). For example, adopting the mean $\delta^{13}C$-CO_2 of $-25‰$ and the range of ε_p values used by Brett (2014) in scenario 1 ($-27‰$ to $-12‰$) gives phytoplankton $\delta^{13}C$ of $-37‰$ and $-52‰$, respectively, and adopting the ε_p value used by Brett (2014) in scenario 3 ($-11‰ \pm 6‰$) results in a phytoplankton $\delta^{13}C$ of $-36‰ \pm 6‰$. Thus, the $\delta^{13}C$ of phytoplankton might be "extremely" [13]C depleted in Upper Bear Lake when using ε_p values more similar to those observed in more productive, less humic lakes. In fact, a ε_p of $-16.2‰$ is enough to obtain the assumed $\delta^{13}C$ of phytoplankton ($-41.2‰$) in Karlsson et al. (2012). This ε_p is similar to newly published data on ε_p of $-14.5‰ \pm 2.6‰$ based on physical separation of phytoplankton in 10 temperate lakes (Wilkinson et al. 2013a) but lower compared to direct estimates from other unproductive systems (Jones et al. 2001).

The problems with Brett's (2014) assumptions of ε_p and $\delta^{13}C$-CO_2 are demonstrated by the data presented in his (Brett 2014) Figure 1c, where almost all simulations resulted in zooplankton allochthony values that were unrealistic on both the negative and positive ends (i.e., far below and above the 0–100% range). The true values of these model outcomes are not shown in his (Brett 2014) Figure 1c because they are misleadingly grouped into bars representing -5% to 0% or 100% to 105%. The mixing model gives negative values of zooplankton allochthony when the phytoplankton $\delta^{13}C$ is higher than that of zooplankton (after accounting for zooplankton fractionation) but lower than $\delta^{13}C$ of terrestrial OM. For

cladocerans ($\delta^{13}C = -32.8‰$), this occurs when phytoplankton $\delta^{13}C$ is between $-27‰$ and $-33.2‰$. The allochthony for these data is, e.g., -95% at a phytoplankton $\delta^{13}C$ of $-30.2‰$ and -3000% at a phytoplankton $\delta^{13}C$ of $-27.2‰$. Further, zooplankton allochthony becomes $> 100\%$ when phytoplankton $\delta^{13}C$ is higher than that of terrestrial OM (i.e., $> -27‰$ to $-24.8‰$). For example, at phytoplankton $\delta^{13}C$ of $-24.8‰$, the allochthony of cladocerans will be 384%. The calculated allochthony by using Brett's assumptions would be even more unrealistic for the calanoid copepods (*Eudiaptomus* sp.), which had the lowest $\delta^{13}C$ ($-37.3‰$) of all zooplankton groups. Furthermore, Brett (2014) excludes the calanoid copepods from his analyses. This is problematic as the δ^2H data presented in Karlsson et al. (2012) suggest that calanoid copepods have very low allochthony and hence are mainly relying on phytoplankton OM. If Brett (2014) had included these data, the assumed $\delta^{13}C$ values of phytoplankton in his comment would be even more out of the $\delta^{13}C$ range of the food resources for zooplankton, suggesting that phytoplankton OM are not important for the calanoid copepods in the lake.

The comment by Brett (2014) was also based on a compilation of literature data on $\delta^{13}C$ of POC and the difference in $\delta^{13}C$ between zooplankton and POC, to argue for uncertainties in the $\delta^{13}C$ of phytoplankton used in Karlsson et al. (2003). He concludes that the difference between publications from northern Sweden and other places is "the assumptions that have been used to obtain phytoplankton $\delta^{13}C$." However, one cannot exclude the possibility that phytoplankton do have a low $\delta^{13}C$ value based on this comparison. It is difficult to understand the rationale for his comparison when considering that the phytoplankton share of POC (Wilkinson et al. 2013b) and the $\delta^{13}C$ of phytoplankton (mentioned above) vary between lakes, and that the zooplankton use of phytoplankton vs. terrestrial OM varies between lakes and also between different zooplankton groups (Grey et al. 2001; Solomon et al. 2011; Wilkinson et al. 2013a). Thus, comparisons of $\delta^{13}C$ of POC and of the difference in $\delta^{13}C$ between zooplankton and POC between lakes do not permit speculations on phytoplankton $\delta^{13}C$ values.

To summarize, in order to advance the understanding of lake ecosystems, we need to continue to develop better techniques for quantifying food web dynamics and the extent to which different resources are used by consumers. The introduction of stable isotope techniques using $\delta^{13}C$ has opened up new doors. However, there has been a substantial lack of practically feasible approaches for detailed quantification of allochthony in pelagic consumers using $\delta^{13}C$ data, and especially so for unproductive humic lakes. We are convinced that the use of δ^2H represents a promising step towards a deeper understanding of aquatic food web dynamics, not the least in studies of terrestrial carbon influences on energy transfer in food webs. The major take home messages in Karlsson et al. (2012) are (1) the major strength of using δ^2H over $\delta^{13}C$ in studies of aquatic food web dynamics, and (2) the fact that allochthony decreases with trophic position. For reasons outlined herein, we find little in Brett's comment (Brett 2014) that can change our view in these respects. We look

forward to future scientific discussions on the allochthony concept and welcome more scientists to develop and explore this challenging field of aquatic research.

J. Karlsson,[1] M. Berggren,[2] J. Ask,[1] P. Byström,[1] A. Jonsson,[1] H. Laudon,[3] and M. Jansson[1]

[1] Department of Ecology and Environmental Science, Umeå University, Umeå, Sweden
[2] Department of Physical Geography and Ecosystem Science, Lund University, Lund, Sweden
[3] Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, Lund, Sweden

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