Nutritional support of inland aquatic food webs by aged carbon and organic matter

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Abstract
Aged (typically tens to thousands of years old) forms of non-living carbon (C) and organic matter (OM) predominate in many inland water ecosystems. Advances in the methodologies used to measure natural abundance radiocarbon ($^{14}$C) have led to increased use of natural $^{14}$C as both a source and age tracer in aquatic ecosystem and food web studies. Here, we review (1) $\Delta^{14}$C values and ages of C and OM typically found in different inland water systems, (2) the mechanisms through which these materials enter inland water ecosystems, and (3) all available $^{14}$C data on aquatic consumers across a range of inland water ecosystem types. Using $\Delta^{14}$C values of aquatic consumers and their potential nutritional resources, we estimate contributions of aged C and OM to aquatic consumer biomass. We conclude that in nearly every case, one or more forms of aged C and/or OM contribute to aquatic consumer nutrition in inland water ecosystems.

Growing evidence indicates that aged and even ancient forms of carbon (C) and organic matter (OM) dominate in most inland waters (Raymond and Bauer 2001; Hosler and Bauer 2013; Spencer et al. 2014a). The sizes of Earth’s geologically aged C and OM reservoirs are orders of magnitude larger than modern to moderate-aged reservoirs (Hedges 1992). These aged C and OM sources are continuously mobilized to inland water (hereafter referred to as “aquatic”) ecosystems by various mechanisms (Butman et al. 2015; Marwick et al. 2015). However, the extent to which aged forms of C and OM contribute to consumer nutrition and food webs, and the factors controlling these contributions, are poorly understood and have not been accounted for in the vast majority of studies, leading to potentially major gaps in our conceptual and quantitative models of C and energy flow in aquatic ecosystems (Guillemette et al. 2017).

The goals of this review and synthesis are to (1) evaluate the literature on potential sources and inputs of C and OM to inland water ecosystems using natural abundance $^{14}$C as a tracer, (2) present representative $\Delta^{14}$C values and ages of particulate and dissolved organic C (POC and DOC, respectively) and dissolved OM reservoirs are orders of magnitude larger than modern to moderate-aged reservoirs (Hedges 1992). These aged C and OM sources are continuously mobilized to inland water (hereafter referred to as “aquatic”) ecosystems by various mechanisms (Butman et al. 2015; Marwick et al. 2015). However, the extent to which aged forms of C and OM contribute to consumer nutrition and food webs, and the factors controlling these contributions, are poorly understood and have not been accounted for in the vast majority of studies, leading to potentially major gaps in our conceptual and quantitative models of C and energy flow in aquatic ecosystems (Guillemette et al. 2017).

The goals of this review and synthesis are to (1) evaluate the literature on potential sources and inputs of C and OM to inland water ecosystems using natural abundance $^{14}$C as a tracer, (2) present representative $\Delta^{14}$C values and ages of particulate and dissolved organic C (POC and DOC, respectively) and dissolved
inorganic C (DIC) pools in streams, rivers, and lakes potentially available to aquatic consumers, (3) assess the $\Delta^{14}C$ values and apparent ages of aquatic consumer organisms in various systems, and (4) estimate aged C contributions to aquatic metazoan consumer biomass and nutrition.

**Background**

**Stable isotopes as tracers of aquatic C and OM nutritional sources**

Natural abundance stable isotopes (e.g., $^{13}$C, $^{15}$N, and $^2$H) have been used extensively for evaluating OM utilization by aquatic consumers and have been shown to be far more quantitative than, e.g., classical gut content analysis (Peterson and Fry 1987; Junger and Planas 1994; Leberfinger et al. 2011). However, in most aquatic systems $\delta^{13}$C and $\delta^{15}$N have relatively small dynamic ranges (tens of $\%_{oo}$ at most), and differentiation of multiple dietary and nutritional sources having overlapping $\delta^{13}$C and $\delta^{15}$N signatures can be challenging and non-definitive (Phillips and Gregg 2003). Stable isotope ratios of H ($\delta^2$H or $\delta^D$) have a much larger dynamic range than $\delta^{13}$C and $\delta^{15}$N in natural systems ($\geq ~ 100%_{oo}$), and when used in conjunction with $\delta^{13}$C and $\delta^{15}$N may provide greater (i.e., isotopically three-dimensional) differentiation of dietary and nutritional contributions (Deines et al. 2009; Cole et al. 2011; Tantet et al. 2017). $\delta^2$H has been increasingly used to distinguish between allochthonous and autochthonous sources of OM in aquatic systems because terrestrial vegetation can be enriched in $^2$H by $\sim 100%_{oo}$ or more over aquatic vegetation (Doucett et al. 2007; Finlay et al. 2010). A major limitation of using stable isotopes in aquatic dietary and nutritional studies is that they cannot easily differentiate C and OM from newly formed contemporary (i.e., modern) sources and those originating from mobilization of far more abundant aged (i.e., $10^2$ to $\geq 10^9$ year timescales) sources. Knowledge of the ages of C and OM contributing to consumer nutrition is also important for reassessing the paradigm that young, recently produced materials are more—or even exclusively—biologically available and reactive compared to aged sources (Caraco et al. 2010; McCallister and del Giorgio 2012; Guillemette et al. 2017).

**Natural $^{14}$C as a source and age tracer of C and OM in aquatic systems**

Natural abundance radiocarbon ($^{14}$C), the radioactive isotope of carbon ($t_{1/2} = 5568$ yr; Stuiver and Polach 1977), has at least a one to two order-of-magnitude greater dynamic range than $\delta^{13}$C, $\delta^{15}$N, and $\delta^2$H. Natural (“pre-bomb”) $^{14}$C values range over $\sim 950%_{oo}$, (i.e., from $-1000%_{oo}$ to $-50%_{oo}$; McNichol and Aluwihare 2007; Taylor 2016) and are even greater (up to $\sim 1900%_{oo}$ range) when anthropogenic $^{14}$C inputs such as thermonuclear weapons testing and nuclear reactors are considered (McNichol and Aluwihare 2007; Taylor 2016). In addition to being a highly sensitive source tracer, $^{14}$C uniquely allows for determination of C and OM ages in both non-living and living aquatic C-containing components.

Natural abundance $^{14}$C has historically been employed much less frequently than stable isotopes in aquatic and terrestrial food web studies. This was due in large part to the challenges in obtaining adequate sample C quantities, lengthy sample processing times, and analytical cost (McNichol and Aluwihare 2007). However, the advent of accelerator mass spectrometry (AMS) has resulted in increased use of natural $^{14}$C measurements in ecosystem studies. Prior to the development and widespread use of AMS, $^{14}$C analyses were conducted using $\beta$-decay methods such as gas proportional and liquid scintillation counting (McNichol and Aluwihare 2007; Taylor 2016). Decay counting requires grams of C and up to days of counting time per sample for an accurate assessment of $^{14}$C content, whereas AMS methods require only tens to hundreds of micrograms of C and minutes or less analysis times (McNichol and Aluwihare 2007; Taylor 2016).

Most inland water food web studies utilizing natural $^{14}$C have been conducted in subtropical, temperate, and subarctic North American and European systems, but a growing number are being conducted in Asian systems (Table 1). To our knowledge, no such studies have yet been carried out in desert (e.g., hypersaline), tropical, or arctic aquatic systems. While the use of natural $^{14}$C for estimating ages of C and OM utilized in aquatic food webs is in principal relatively straightforward, the influence of 1950s–1960s nuclear weapons testing (i.e., the so-called “bomb” $^{14}$C) must also be considered, especially for the mid-20th to early 21st century period (McNichol and Aluwihare 2007; Taylor 2016). In addition to the apparent ages of consumer organisms resulting from their utilization of non-living aged OM, live aquatic autotrophs consumed for nutrition may also possess apparent “age” due to fixation of aged DIC (as CO$_2$(aq)) commonly observed in inland waters (Ishikawa et al. 2013, 2014) and must be accounted for.

**Variability in the ages of carbon and organic matter in inland waters**

Modern-aged forms of carbon and organic matter in inland waters

Non-living C and OM in aquatic systems have been found to range from modern ($\Delta^{14}$C $\geq ~ 0%_{oo}$) to fossil ($\Delta^{14}$C $\leq -1000%_{oo}$) in age (Fig. 1; Supporting Information Table S1; Hedges et al. 1997; Raymond et al. 2004; Butman et al. 2012) and may be either autochthonous or allochthonous in origin. Living or recently living terrestrial vegetation is derived from modern-day CO$_2$ recently fixed from the atmosphere ($\Delta^{14}$C $> 0%_{oo}$; Fig. 1; Supporting Information Table S1; Garnett and Billett 2007; Gaudinski et al. 2009; Carbone et al. 2013). However, modern-aged terrestrial vegetation is a globally relatively small reservoir of organic C compared to moderately aged (i.e., that having $\Delta^{14}$C $< 0%_{oo}$ but $>-1000%_{oo}$) and fossil aged C reservoirs (i.e., those having $\Delta^{14}$C $\sim -1000%_{oo}$; Figs. 1, 2; Supporting...
Potential sources of newly formed autochthonous OM in aquatic systems include macrophytes, benthic algae, phytoplankton, cyanobacteria (Allan and Castillo 2007) and young OM-utilizing heterotrophic bacteria (Hall et al. 2000), and biogenic methane (Fig. 1; Supporting Information Table S1; Chanton et al. 1995; Grey 2016).

Moderate- to fossil-aged forms of carbon and organic matter in inland waters

Non-living aged autochthonous and allochthonous OM sources to aquatic systems include aquatic sediments, terrestrial soils, thermogenic and biogenic methane, and sedimentary rocks that have been stored in watersheds, streams, and rivers for decades to many millions of years since their deposition (Fig. 1; Supporting Information Table S1; Hedges 1992; Copard et al. 2007; Battin et al. 2009). Soil OM from leaf litter, roots, and woody debris derived from recent primary production are typically modern- or near-modern (i.e., decadal or less) aged (Fig. 1; Supporting Information Table S1; Bellamy et al. 2010, 2012; Caraco et al. 2010, 2012; Wang et al. 2014; Evans 2012; Fellman et al. 2015; Bellamy et al. 2016; Westfall et al. 2016; Bellamy et al. 2017; DelVecchia et al. 2016; Philippsen and Heinemeier 2013; Fernandes et al. 2012; Hagvar and Ohlson 2013 and Hagvar et al. 2016; Keaveney et al. 2015; Fernandes et al. 2013; Bellamy et al. 2016; Ishikawa et al. 2010, 2014, 2016).

Information Table S1). Potential sources of newly formed autochthonous OM in aquatic systems include macrophytes, benthic algae, phytoplankton, cyanobacteria (Allan and Castillo 2007) and young OM-utilizing heterotrophic bacteria (Hall et al. 2000), and biogenic methane (Fig. 1; Supporting Information Table S1; Chanton et al. 1995; Grey 2016).

**Table 1.** Inland water food web studies from the published literature that have employed natural abundance $^{14}$C analyses of consumer organisms.

| Study location | Study dates | Organisms studied | References* |
|----------------|-------------|-------------------|-------------|
| *North America* |             |                   |             |
| Great Basin Lakes, U.S.A. and Canada | 1950s | Fish, brine shrimp | 1 |
| Colville River/Beaufort Sea, Alaska, U.S.A. | 1980 | Fish, invertebrates, birds | 2 |
| Hudson River, New York, U.S.A. | 2000–2001 | Bacteria | 3 |
| Eastern Townships Lakes, Quebec, Canada | 2004 | Bacteria, cladocerans, copepods | 4 |
| Hudson River, New York, U.S.A. | 2004–2005 | Cladocerans and copepods | 5 |
| Everglades, Florida, U.S.A. | 2006–2007 | Fish | 6 |
| Lake Superior, U.S.A. and Canada | 2009 | Cladocerans and copepods | 7 |
| Pigeon River, Michigan, U.S.A. | 2011 | Ammocoetes (lamprey larva) | 8 |
| Clear Fork River, Ohio, U.S.A. | 2011 | Ammocoetes (lamprey larva) | 8 |
| Lake Superior, U.S.A. and Canada | 2012 | Cladocerans, copepods, mysids, amphipods, fish | 9 |
| Herbert River, Alaska, U.S.A. | 2012 | Macroinvertebrates and fish | 10 |
| Paint Creek, Ohio, U.S.A. | 2012–2013 | Macroinvertebrates | 11 |
| Muskingum River, River, Ohio, U.S.A. | 2013 | Freshwater mussels | 12 |
| Susquehanna River watershed, Pennsylvania, U.S.A. | 2011–2014 | Macroinvertebrates | 13 |
| Mohawk-Hudson, New York, U.S.A. | 2014 | Macroinvertebrates | 14 |
| Nyack Floodplain, Montana, U.S.A. | 2013–2015 | Stonellies | 15 |
| *Europe* |             |                   |             |
| Trave River, Germany | 2007–2010 | Fish, crayfish | 16 |
| Weibe River, Lake Rosenfield‡, Germany | 2009/2011 | Mussels | 17 |
| Midtdalsbreen Glacier, Norway | 2010/2014 | Fish, chironomids, aquatic beetles | 18 |
| Loughe Erne, Ireland | 2011 | Fish, cladocerans, copepods, mysids | 19 |
| Lakes Schwerin‡ and Ostorf‡, Germany | 2011 | Fish, eels, zebra mussels | 20 |
| Rivers Rebbe and Schloss Wilhelmsl†, Germany | 2013 | Fish | 21 |
| *Asia* |             |                   |             |
| Lake Biwa watershed, Japan | 2006–2008 | Macroinvertebrates and fish | 22 |
| Kano River watershed, Japan | 2011–2012 | Macroinvertebrates | 22 |

* References: 1- Broecker and Walton (1959), 2- Schell (1983), 3- McCallister et al. (2004), 4- McCallister and del Giorgio (2008, 2012), 5- Caraco et al. (2010), 6- Wang et al. (2014), 7- Zigah et al. (2012a), 8- Evans 2012, 9- Kruger et al. (2016), 10- Fellman et al. (2015), 11- Bellamy et al. (unpubl. data), 12- Weber et al. (2017), 13- Bellamy et al. (pers. comm.), 14- Bellamy et al. (2017), 15- DelVecchia et al. (2016), 16- Philippsen and Heinemeier (2013), 17- Fernandes et al. (2012), 18- Hagvar and Ohlson (2013) and Hagvar et al. (2016), 19- Keaveney et al. (2015), 20- Fernandes et al. (2013), 21- Fernandes et al. (2016), 22- Ishikawa et al. (2010, 2014, 2016).
† $^{14}$C of bacterial respired CO$_2$ was measured as a proxy for bacterial biomass.
‡ Due to small sample sizes from each of these lakes, data were grouped for Fig. 3 as “German Lakes.”
and sedimentary POM can also lead to moderately aged suspended POM commonly observed in aquatic systems, especially in turbulent flowing waters (Bianchi and Bauer 2011; Hossler and Bauer 2013). Biogenic methane, depending on the age of its OM source (e.g., peat) supporting methanogenesis, can also be moderately to highly aged (Fig. 1; Supporting Information Table S1; Chanton et al. 1995; DelVecchia et al. 2016).

Aquatic primary production in aquatic systems is typically assumed to be a source of modern- or near-modern aged OM (Zigah et al. 2011; Kruger et al. 2016). However, $^{14}$C is often depleted in DIC and CO$_2$(aq) in inland waters relative to atmospheric CO$_2$ (known as the freshwater reservoir offset; Keaveney and Reimer 2012; Philipsen and Heinemeier 2013; Fernandes et al. 2016) and can lead to $^{14}$C-depletion and apparent “age” in living autotrophic biomass. $^{14}$C-depleted DIC and CO$_2$(aq) in aquatic systems can originate from fossil carbonaceous rock weathering and respiration of non-living, aged water column soil and sediment OM (Fig. 1; Supporting Information Table S1; Broecker and Walton 1959; Butman and Raymond 2011; Keaveney and Reimer 2012; Ishikawa et al. 2014). This $^{14}$C-depleted living or recently living aquatic OM may thus serve as a source of “aged” nutrition to aquatic consumers (Broecker and Walton 1959; Ishikawa et al. 2013, 2014).

Ancient or fossil-aged ($\Delta^{14}$C $\leq$ $-1000\%$) OM reservoirs derive from materials stored on geological timescales in sedimentary rocks (e.g., shales, kerogens, coal, petroleum, and thermogenic methane; Figs. 1, 2; Supporting Information Table S1). Fossil aged C and OM are far more abundant in global reservoirs than contemporary or moderately aged C and OM by several orders of magnitude (Fig. 2; Hedges 1992). Thus, mobilization of even small amounts of these abundant and highly aged materials has the potential to contribute significantly to the amounts and $^{14}$C ages of C and OM in modern-day aquatic systems (Caraco et al. 2010; Hossler and Bauer 2013; Marwick et al. 2015).

Fig. 1. Representative ranges of $\Delta^{14}$C values and equivalent $^{14}$C ages of potential carbon (C) and organic matter (OM) sources to various inland water ecosystems from the published literature. For details of the values and associated references, see Supporting Information Table S1.

Fig. 2. Potential sources and ages of allochthonous carbon (C) and organic matter (OM) in aquatic systems, and their generalized transport pathways and global reservoir sizes. Values in parenthesis are $10^{18}$ grams C. Adapted from Hedges (1992) and Bauer and Bianchi (2011).
Aquatic systems and terrestrial landscapes are closely linked, and their connectivity facilitates hydrologic inputs of different sources of C and OM of varying age (Fig. 3; Aufdenkampe et al. 2011). While inland waters cover a small fraction of the earth’s surface (~ 3%; Raymond et al. 2013), C and OM yields from the surrounding landscape are large and can support ecosystem processes such as metabolism and secondary production, leading to inland waters being largely net heterotrophic (Cole et al. 2007; McCallister and del Giorgio 2012; Wilkinson et al. 2013). Geomorphology, lithology, climate (e.g., temperature, precipitation), and land use may all influence the forms and ages of C and OM mobilized to inland waters (Marwick et al. 2015).

Aged materials were historically not considered as significant components of aquatic C and OM pools, as they are known to be today (Hedges et al. 1986; Raymond and Bauer 2001). Human activities have dramatically further increased the mobilization of these materials from their storage reservoirs (Fig. 3; Regnier et al. 2013; Butman et al. 2015). Consequently, inputs of aged allochthonous materials to aquatic systems may impact aquatic food webs to an even greater extent as agriculture, urbanization, and other land use changes have expanded (Aufdenkampe et al. 2011; Butman et al. 2015; Fellman et al. 2015).

In addition, assumptions of the biological recalcitrance of allochthonous OM based on molecular structure and age are also changing (McCallister and del Giorgio 2012; Fellman et al. 2014; Marín-Spiotta et al. 2014).

Extensive aging of C and OM results from long-term storage in forms (e.g., sorbed to mineral particles) and environments (e.g., suboxic and anoxic) that inhibit or prevent their degradation and utilization by heterotrophic bacteria and higher organisms (Salmon et al. 2000; Lützow et al. 2006; Kleber 2010). However, when aged OM is released from its points of storage and protection, it may become increasingly susceptible to degradation and utilization (Petsch et al. 2001; Schillawski and Petsch 2008; Kleber and Johnson 2010). Interactions between terrestrial and aquatic environments via the hydrologic cycle therefore likely (1) increase rates and extents of aged C and OM processing (Blair and Aller 2012; Marín-Spiotta et al. 2014), and (2) modify the routes through which C and OM of differing sources and ages enter streams, rivers, and lakes (Fig. 3).
 Streams and rivers

Carbon and OM in streams and small rivers is typically dominated by allochthonous materials (Fig. 3) for two primary reasons. First, stream waters arise from surface and sub-surface runoff in watersheds, and these waters are in intimate contact with both living and non-living terrestrial biomass, as well as with soils and their associated terrestrial OM (Hope et al. 1997; Aitkenhead et al. 1999; Boix-Fayos et al. 2009). Second, shading by riparian vegetation and canopy cover can limit autochthonous production in streams and small rivers (Vannote et al. 1980; Finlay 2001).

Both natural and anthropogenic controls play important roles in the sources and ages of C and OM entering streams and rivers (Fig. 3; Hossler and Bauer 2013; Marwick et al. 2015). Natural controls include hydrogeomorphology, lithology, and climate, all of which may show dramatic temporal and spatial variability (Hossler and Bauer 2012, 2013). For example, steep small mountainous rivers (SMRs)—typically underlain by ancient kerogen-rich sedimentary rocks—are responsible for transport and delivery of large amounts of moderately to fossil aged C and OM to streams, rivers, and ocean margins (Kao and Liu 1996; Leithold et al. 2006; Hilton et al. 2008). Low-gradient watersheds containing fossil carbonates and shales may also introduce aged C and OM to aquatic systems (Fig. 3; Longworth et al. 2007; Marwick et al. 2015).

Human activities in watersheds are increasingly important controls on the sources and ages of C and OM in streams and rivers, and land use change may alter the ages of materials transported to aquatic systems (Fig. 3; Butman et al. 2015). Agricultural activity and/or removal of riparian vegetation may lead to higher inputs of moderately aged soil C to streams and rivers by destabilizing soils and accelerating erosion (Longworth et al. 2007; Restrepo et al. 2015). While global increases in watershed disturbance have been concentrated in developed countries, the intensity and timing of land use change has been highly variable across terrestrial regions (Goldewijk 2001; Vorosmarty et al. 2010; Lambin and Meyfroidt 2011). These and related factors may lead to variability in the delivery of aged C and OM to specific aquatic systems. In urbanized regions, petroleum hydrocarbons in runoff, wastewater treatment plants, and aerosol deposition can all transport aged C and OM to streams and rivers (Fig. 3; Griffith et al. 2009; Wozniak et al. 2012a,b).

Increasing stream water temperatures resulting from climate warming have also led to observed increases in aged C and OM inputs to streams and rivers, especially in high latitude regions associated with thawing permafrost soils and glaciers (Vonk et al. 2013; Hood et al. 2015).

Lakes

Depending upon their size, lakes are also integrated to varying extents with the terrestrial landscape and are influenced by many of the same factors that affect inputs of C and OM of different sources and ages to streams and rivers (Caraco and Cole 2004; Cole et al. 2007). However, in contrast to streams and rivers, lake basins can serve as significant storage reservoirs for imported C and OM from surrounding watersheds (Fig. 3; Cole et al. 2007; Tranvik et al. 2009). High stream flow may lead to increased delivery of younger-aged DOC (Fig. 3; Zigha et al. 2011, 2014; Spencer et al. 2014b). The surface area and volume of lakes further influence the sources and ages of C and OM that predominate in them (Wetzel 1990; Zigha et al. 2012a,b). Higher watershed:lake surface areas generally lead to greater inputs and amounts of allochthonous and aged C and OM in small lakes, whereas in large lakes modern-aged autochthonous production tends to be quantitatively more important (Zigha et al. 2012a; Wilkinson et al. 2013; Tanetzap et al. 2017). Aged C and OM may be more common in lakes in drainage basins having lithologies comprised of aged forms of C and OM, and in lakes at higher elevations and latitudes receiving glacial run-off and inputs from combustion-derived aerosol deposition (Broecker and Walton 1959; Keaveney and Reimer 2012; Spencer et al. 2014; Keaveney et al. 2015).

Anthropogenic activities such as fossil fuel combustion may lead to increased inputs of aged C and OM to watersheds and aquatic water bodies (Fig. 3; Wozniak et al. 2012a,b; Iavorivska et al. 2017; Mahowald et al. 2017). Glacial meltwaters are also increasingly recognized sources of aged C and OM from both natural and anthropogenic aerosol deposition (Xu et al. 2009; Spencer et al. 2014a,b). The ages of C and OM both in, and transported by, aquatic systems can thus vary widely, and will depend on the sources and ages of C and OM mobilized to their POC, DOC, and DIC pools.

Ages of POC, DOC, and DIC pools in streams and rivers

The majority of studies have found POC to be more highly aged than DOC and DIC in streams and rivers (Fig. 4A; Supporting Information Table S2). This is thought to result from inputs of moderately to highly aged OM from fossil aged bedrock and accreted soils (Raymond and Bauer 2001a; Leithold et al. 2006; Blair et al. 2010; Marwick et al. 2015). The generally young ages of stream and river DOC (Hossler and Bauer 2013; Marwick et al. 2015; Fig. 4A; Supporting Information Table S2) may be attributed to significant contributions from leachates of fresh surface soil litter and root exudates (Aitkenhead-Peterson et al. 2003; Dodds et al. 2017). More highly aged riverine DOC is commonly associated with human activities such as agricultural, wastewater treatment plant, and petroleum inputs (Wang et al. 2012; Butman et al. 2015; Marwick et al. 2015). Glacial melt water also contributes moderately aged DOC to subarctic streams and rivers (Hood et al. 2009, 2015). With the exceptions of some agricultural and high relief watersheds, stream and river DIC is generally modern or near modern in age (Fig. 4A; Supporting Information Table S2), suggesting that it is predominantly derived from respiration of recently produced forms of OM (e.g., terrestrial and aquatic plants; Marwick et al. 2015). Δ13C and δ13C values of DIC are often more challenging to
interpret than those of POC and DOC due to combined effects of respiration, dissolution of mineral carbonates, and both turbulent and diffusive atmospheric exchange (Finlay 2003; Raymond et al. 2004; Hossler and Bauer 2013).

**Ages of POC, DOC, and DIC pools in lakes**
Fewer studies have used natural $^{14}$C in lakes than in streams and rivers, but this number has been gradually increasing. The $\Delta^{14}$C values and ages of DIC, DOC, and POC in lakes studied to date are generally less variable than in streams and rivers (Fig. 4A,B; Supporting Information Tables S2, S3). Similar to streams and rivers, POC in lakes is generally more highly and variably aged than DOC or DIC (Fig. 4A,B; Supporting Information Tables S2, S3; Zigah et al. 2011, 2012a,b; McCallister and del Giorgio 2012; Fernandes et al. 2013; Keaveney et al. 2015). DOC and DIC in lakes are most often modern- to century-aged (Fig. 4A,B; Supporting Information Tables S2, S3) and thought to result from similar sources and processes as streams and rivers. Runoff from surrounding watersheds and inputs from groundwaters may also be important sources of aged DOC and DIC to lakes (Brady et al. 2009; Melymuk et al. 2014). We speculate that lake trophic status (i.e., nutrient loading) co-varies inversely with aquatic C and OM age, especially in fertilized agricultural and recently deforested watersheds.

**$\Delta^{14}$C values and apparent ages of aquatic consumer organisms**

**Utilization of aged C and OM by aquatic consumers**
When aged C and OM is mobilized from its points of preservation and storage to aquatic systems, it may be assimilated by aquatic bacterial and metazoan consumers as particulate and/or dissolved OM (POM and DOM, respectively; note that POC and DOC refer to only the carbon-containing components of POM and DOM; Supporting Information Fig. S1). POM and DOM can contribute to consumer diet both directly and indirectly. Metazoan consumers may directly ingest aged POM (which also includes aquatic primary producers that have fixed aged aquatic DIC), as well as flocculated aged DOM (Supporting Information Fig. S1; Wallace et al. 1997; Kerner et al. 2003; Zigah et al. 2012a). Some soft-bodied organisms (e.g., dreissenid mussels) also have the ability to take up DOM directly (Supporting Information Fig. S1; Roditi et al. 2000; Baines et al. 2005). In addition to being consumers of non-living and aquatic OM, heterotrophic bacteria may also “repackage” aged DOM and POM into bacterial biomass that can then be consumed by higher consumers directly or by grazing on protozoan bacterivores (Supporting Information Fig. S1; Cherrier et al. 1999; McCallister et al. 2004; Berggren et al. 2010).

**Bacterial utilization of aged C and OM in temperate and subtropical systems**
It is well-documented that heterotrophic bacteria play an important role in the degradation and utilization of fossil aged petroleum hydrocarbons in many aquatic systems (Atlas 1981; Heitkamp and Johnson 1984; Ahad and Pakdel 2013). Bacteria, through their enzymatic and hydrolytic activities, are also known to mediate the mobilization of C and OM to inland waters (Guillemette et al. 2016). Natural $^{14}$C measurements show that modern- to moderate-aged (i.e., non-fossil) OM contributes to heterotrophic bacterial biomass ($\Delta^{14}$C range $-153_{\text{iso}}$ to $214_{\text{iso}}$ yr to modern-aged, respectively; McCallister et al. 2004) and/or respired CO$_2$(aq) ($\Delta^{14}$C range $-172_{\text{iso}}$ to $94_{\text{iso}}$ yr to modern-aged, respectively; McCallister et al. 2004 and/or respired CO$_2$(aq) ($\Delta^{14}$C range $-172_{\text{iso}}$ to $94_{\text{iso}}$ yr to modern-aged, respectively).
aged, respectively; McCallister and del Giorgio 2012) in temperate lake systems (Table 1). Fellman et al. (2014) reported that in the Kimberley region of Western Australia stream DOC was increasingly enriched in $^{14}$C with increasing stream size. Use of a mass balance approach to estimate potential $^{14}$-enrichment of DOC from each stream suggested that one potential explanation for the $^{14}$C enrichment was that heterotrophic bacteria selectively metabolized older, $^{14}$C-depleted DOC components (Fellman et al. 2014).

Fossil DOC leached from shales in watersheds may also enter streams and rivers and be utilized by heterotrophic bacteria. Petsch et al. (2001) estimated that 74–94% of the lipid C in bacteria growing on watershed shales was derived from fossil ($\geq 50,000$ yr in age) OM. Rapid bacterial utilization of fossil DOC solubilized from shale OM (80% loss over 2-week incubation) further illustrates that highly aged forms of OM may be bioavailable once mobilized to aquatic systems (Schillawski and Petsch 2008).

**Metazoan utilization of aged C and OM in temperate and subtropical systems**

Invertebrates in inland waters play an important role in the processing of autochthonous and allochthonous C and OM through their feeding and metabolic activities (Anderson and Sedell 1979; Wallace et al. 2015; Tanentzap et al. 2017). They are also an important source of nutrition for higher consumers (e.g., vertebrates). Natural $^{14}$C measurements have been conducted on both primary aquatic consumers as well as secondary consumers and predators (Table 1), and studies suggest that aged C and OM can be transferred to progressively higher trophic levels (Schell 1983; Wang et al. 2014; Hagvar et al. 2016).

**Streams and rivers**

Natural $^{14}$C has been employed as a food web tracer in multiple stream and river systems (Table 1). $^{14}$C-depletion in living consumer biomass may result from direct consumption and assimilation of (1) non-living aged OM (Schell 1983; Caraco et al. 2010; Hagvar and Ohlson 2013; Wang et al. 2014) and/or (2) living $^{14}$C-depleted OM derived from fixation of $^{14}$C-depleted DIC and CO$_2$(aq) by aquatic primary producers (Figs. 1, 5; Supporting Information Table S1; Fernandes et al. 2012, 2016; Philipsen and Heinemeier 2013; Ishikawa et al. 2014).

Caraco et al. (2010) showed that cladoceran and copepod zooplankton from the Hudson River, New York, USA had a mean $\Delta^{14}$C of $-240_{\%o}$ (equivalent age of $\sim 2200$ yr B.P.) due to utilization of millennial-aged terrestrial soil OM (Fig. 5; Supporting Information Table S1). In this study, zooplankton mean $\Delta^{14}$C was nearly $200_{\%o}$ ($\sim 1790$ yr) lower than average phytoplankton $\Delta^{14}$C and about $300_{\%o}$ ($\sim 2870$ yr) lower than modern terrestrial plant biomass. Similar conclusions of aged OM assimilation by freshwater larval insect and mussel consumers were reached by studies in the Hudson-Mohawk watershed, New York and the Muskingum River, Ohio, respectively (Fig. 5; Weber et al. 2017; Bellamy et al. 2017). In the Hudson-Mohawk, aged soil OM contributions to macroinvertebrate nutrition were greater in streams with high vs. low agriculture watersheds, however, utilization of $^{14}$C-depleted “aged” algae by macroinvertebrates could also not be ruled out (Bellamy et al. 2017). Recent $\Delta^{14}$C measurements of stonesflies in the hyporheic zone of the Nyaack floodplain on the Middle Fork of the Flathead River, Montana, USA revealed that they were highly aged (up to 6900 yr B.P.; Table 1; Fig. 5, Supporting Information Table S1), suggesting they utilized methanotrophic bacterial biomass derived from aged biogenic, and possibly thermogenic, methane (DelVecchia et al. 2016).

In the Kanno River, Japan, deforestation was found to lead to inputs and autotrophic fixation of $^{14}$C-depleted DIC from fossil carbonate weathering, resulting in $^{14}$C-depleted aquatic primary producer biomass (Ishikawa et al. 2016) (Fig. 5). Similar to the Hudson-Mohawk system, modern-aged forms of OM contributed more to macroinvertebrate biomass from Kanno River streams in watersheds having less disturbance (Ishikawa et al. 2016). These authors further suggest that during forest reestablishment, root biomass of Cryptomeria japonica (Japanese cedar) increased bacterial respiration of soil OM to CO$_2$, and consequently led to greater carbonic acid weathering of soil fossil carbonates and inputs of $^{14}$C-depleted DIC to headwater streams (Ishikawa et al. 2016). Thus, various forms of watershed disturbance may facilitate the mobilization of aged C and OM into aquatic systems, and impact the $^{14}$C content and apparent ages of consumers (Caraco et al. 2010; Ishikawa et al. 2016; Fig. 3).

**Lakes and wetlands**

Fewer studies using natural $^{14}$C have been conducted in lake and wetland food webs than in streams and rivers (Table 1; Fig. 5, Supporting Information Table S1). In the lakes studied to date, fixation of aged DIC and CO$_2$(aq) by primary producers appears to be the dominant pathway through which consumers assimilate aged C (Keaveney and Reimer 2012; Fernandes et al. 2013; Keaveney et al. 2015). In Eastern Townships lakes (Quebec, Canada), cladocerans and copepods were used as proxies for autochthonous primary production by assuming that zooplankton would select algal over detrital components of the POM pool (McCallister and del Giorgio 2008). Zooplankton $\Delta^{14}$C values ranged from $-2_{\%o}$ to $40_{\%o}$ (modern in age) and overlapped with the $\Delta^{14}$C values of the DIC, thus appearing to confirm the validity of this assumption. Aged terrestrial OM was therefore deduced not to contribute significantly to zooplankton biomass in these lakes (Fig. 5; McCallister and del Giorgio 2008).

$^{14}$C-depletion ($\Delta^{14}$C = $-130_{\%o}$ to $33_{\%o}$; 1120 yr to modern-aged) in vertebrates and molluscs in German lakes (Lakes Roesnfeld, Schwerin, Ostorf, and Schloß Wilhelmsthal; Fig. 5) was also attributed to their consumption and utilization of $^{14}$C-depleted algae ($\Delta^{14}$C = $-44_{\%o}$; 270 yr equivalent age) and
macrophytes ($\Delta^{14}C = -33\%_{\text{op}}$, 360 yr equivalent age) fixing $^{14}$C-depleted DIC, which in Lake Schwerin, DIC was 240 yr in equivalent age ($\Delta^{14}C = -29\%_{\text{op}}$, Fernandes et al. 2013). However, mussel tissue collected from Lake Ostorf was more $^{14}$C-depleted ($\Delta^{14}C = -130\%_{\text{op}}$, 1120 yr equivalent age) than fish and mussels collected from Lake Schwerin. It was speculated by the authors that $^{14}$C-depleted, respired CO$_2$ from aged peat OM in the Lake Ostorf watershed contributed to lake DIC and phytoplankton (neither was measured directly) that served as a nutritional source to mussels there (Fernandes et al. 2013).

Extensive work in Lough Erne, Ireland has similarly shown algae to be $^{14}$C-depleted (mean $\Delta^{14}C = -59 \pm 9\%_{\text{op}}$, 490 yr equivalent age), suggesting a significant freshwater reservoir offset, presumably from inputs of weathered fossil carbonates and/or aged soil CO$_2$ to lake DIC (Keaveney et al. 2015a,b). In contrast, some consumers in Lough Erne were enriched in $^{14}$C (maximum invertebrate and fish $\Delta^{14}$C values were $0\%_{\text{op}}$ and $10\%_{\text{op}}$ respectively, and modern-aged; Fig. 5), suggesting that modern terrestrial OM contributed to their biomass (Keaveney et al. 2015a). POC was more $^{14}$C-depleted ($-122 \pm 61\%_{\text{op}}$, 1050 yr in age) than DIC or algae, suggesting that there was a moderately aged, but relatively non-utilized, source of OM in the lake. Copepods in Lough Erne apparently selected against significant amounts of this aged OM because their $\Delta^{14}$C values ($-50\%_{\text{op}}$ to $-4\%_{\text{op}}$, 410 yr to modern-aged; Fig. 5) closely matched algae and DOC (depending on season), while cladocerans ($\Delta^{14}C = -138\%_{\text{op}}$ to $-67\%_{\text{op}}$, equivalent ages of 1190–560 yr b.p.; Keaveney et al. 2015) derived some of their biomass from moderately aged detrital OM.

Natural $^{14}$C measurements of zooplankton and fish in the limited number of lakes studied to date suggest in the majority of cases that their utilization of aged C and OM is minimal (Fig. 5). Due to their greater watershed : lake surface area ratios, small lakes are more likely to receive relatively greater amounts of aged allochthonous materials than larger lakes (smaller watershed : lake surface areas) that contain relatively greater amounts of $^{14}$C-enriched modern-aged autochthonous C and OM (Zigah et al. 2012a,b; Wilkinson et al. 2013) from autotrophic utilization of $^{14}$C-enriched CO$_2$ from atmosphere-lake exchange. Studies in Lake Superior, the largest lake yet studied, showed that recently produced $^{14}$C-enriched aquatic OM was the preferred source of nutrition to cladocerans and copepods (Fig. 5). Zooplankton $\Delta^{14}$C values were slightly elevated ($\Delta^{14}C = 44\%_{\text{op}}$, 65\%_{\text{op}}$, modern-aged; Fig. 5) compared to POC and DOC ($\Delta^{14}C = 10 \pm 29\%_{\text{op}}$, 38 $\pm 21\%_{\text{op}}$ respectively; both modern-aged), and contributions of aged OM to the Lake Superior pelagic food web were minimal (Zigah et al. 2012a). More recent work in Lake Superior has shown that aged (940 yr equivalent age; $\Delta^{14}C = -117\%_{\text{op}}$, Li et al. 2013) sedimentary OM was assimilated by the benthic amphipod Diaporeia, which was more $^{14}$C-depleted (by $\sim 61\%_{\text{op}}$ in some cases) relative to non-benthic organisms (e.g., zooplankton and fish; Kruger et al. 2016). However, young recently produced OM contributed more than aged sources even to benthic consumer biomass in Lake Superior. Examination of the relationships between zooplankton $\Delta^{14}$C and hydrologic residence times (an indicator of lake size) suggested that allochthonous, and potentially aged resources, support more zooplankton biomass in smaller lakes (Zigah et al. 2012a). However, given that there have been limited studies utilizing $^{14}$C in large lakes, we cannot definitively conclude that aged C and OM are not important to consumers in large lakes. Additionally, the composition of vegetation and soil in lake catchments will...
strongly influence the sources and ages of C and OM in lakes, regardless of size (Sobek et al. 2007; Tanentzap et al. 2017).

In contrast to most lake consumers, the $^{14}C$ contents of wetland consumers (fish and aquatic invertebrates) in the Florida Everglades, USA examined by Wang et al. (2014) suggest that they rely to varying extents on aged OM. Large-mouth bass and sunfish collected from agriculturally impacted regions of the Florida Everglades were $^{14}C$-depleted ($\Delta^{14}C$ range: $-52_{\%}^{+5}$ to $-22_{\%}^{+6}$; 430–180 yr equivalent age; Fig. 5). The authors assumed that OM derived from exposed peat deposits (estimated at $\sim$ 2000 yr old) from nearby agricultural activity was exported to Everglades wetlands. This aged OM was assimilated by aquatic invertebrates, and in turn consumed by fish (Wang et al. 2014). Wetlands differ from most lakes in a number of important ways. Their generally shallower nature, smaller volumes, and proximity to abundant aged sources (e.g., peat) of OM may lead to greater reliance of wetland consumers on aged sources of C and OM.

**Bacterial utilization of aged C and OM in subarctic systems**

The most commonly used method of assessing the age of OM utilized by aquatic bacteria in subarctic waters has been to measure changes in the amounts and $\Delta^{14}C$ values of DOC before and after a period (generally days to weeks) of dark incubation (Hood et al. 2009; Vonk et al. 2013). Isotopic mass balance between the amounts and $\Delta^{14}C$ values of DOC at the start and end of the incubation is then used to infer the $\Delta^{14}C$ of the utilized DOC, and by association, of the biomass of bacteria utilizing it. Laboratory incubation experiments using this approach with DOC from streams discharging to the Gulf of Alaska revealed that there was a significant positive correlation between DOC degradation and $^{14}C$ age (i.e., older DOC was preferentially utilized; Hood et al. 2009). Separate incubations using DOC from thawing, millennial-aged permafrost from Siberian Yedoma deposits showed that highly aged (24,100 and 21,700 yr equivalent ages in 2010 and 2011, respectively), $^{14}C$-depleted ($-946 \pm 25_{\%}^{+25}$ and $-933_{\%}^{+33}$ for 2010 and 2011, respectively) DOC was utilized by stream heterotrophic bacteria from the Kolyama River, Siberia (Vonk et al. 2013). Thus, the information available to date from subarctic waters suggests that aged OM can be repackaged to bacterial biomass and potentially be available to higher consumer levels.

**Metazoan utilization of aged C and OM in subarctic systems**

Growing evidence suggests that aged C and OM from permafrost and melting glaciers can also contribute to higher aquatic consumers in downstream rivers and lakes in subarctic ecosystems (Schell 1983; Fellman et al. 2015). One of the earliest applications of natural $^{14}C$ in aquatic food web studies was conducted by Schell (1983) using $\beta$-decay counting in an Alaskan river-estuarine food web (Table 1; Fig. 5). Anadromous and riverine fish and oldsquaw ducks collected from the Colville River, Alaska, USA were significantly more $^{14}C$-depleted ($\Delta^{14}C = -153_{\%}^{+4}$ to $-55_{\%}^{+5}$; 1330–460 yr equivalent age) than marine fish from the Beaufort Sea ($\Delta^{14}C = 17_{\%}^{\pm}$ to 204$_{\%}^{\pm}$; modern-aged) due to the reliance of river invertebrate prey on millennial aged ($\sim$ 8700 yr b.p.) terrestrial peat (Schell 1983).

Recent studies in Norwegian subarctic systems found that tissues from predatory terrestrial invertebrates (spiders, harvestmen, and beetles) were moderately aged ($\Delta^{14}C = -130_{\%}^{\pm}$ to $-41_{\%}^{\pm}$; 1100–340 yr equivalent age), and gut content analysis revealed that aged chironomids ($\Delta^{14}C = -121_{\%}^{\pm}$; 1040 yr equivalent age; Fig. 5) were a primary nutritional resource (Hagvar and Ohlson 2013). Hagvar and Ohlson (2013) further postulated that chironomid larvae that relied on aged, glacier-derived OM were then consumed by terrestrial invertebrate predators. In the same study, a predaceous diving beetle larva and adult collected from a nearby dam had equivalent $^{14}C$ ages of 1200 ($\Delta^{14}C = -139_{\%}^{\pm}$) and 1130 yr ($\Delta^{14}C = -131_{\%}^{\pm}$), respectively (Fig. 5). Diving beetles were also likely consuming aged chironomid larvae (Hagvar and Ohlson 2013). Subsequent studies in small lakes in the same region of Norway confirmed these earlier studies’ original hypothesis that aged glacial C and OM was transferred through chironomid larvae before being transferred to terrestrial and aquatic predatory invertebrates (Hagvar et al. 2016).

Studies by Fellman et al. (2015) in the heavily glaciated Herbert River, Alaska, USA also provide evidence of direct assimilation of glacial meltwater-derived C and OM by aquatic consumers. In a non-glacial stream, $\Delta^{14}C$ values of macroinvertebrates and fish ranged from $-14_{\%}^{\pm}$ to 52$_{\%}^{\pm}$ (115 yr to modern-aged), while in the glacially impacted upper and lower Herbert River, macroinvertebrates and fish had $\Delta^{14}C$ values as low as $-171_{\%}^{\pm}$ (equivalent age of 1500 yr; Fellman et al. 2015; Fig. 5). Inputs of aged DOC from glaciers and thawing permafrost to inland waters are predicted to increase with increasing global temperatures (Hood et al. 2015; Spencer et al. 2015), and this aged OM could increasingly contribute to the secondary production of both bacteria and higher consumers (Fellman et al. 2015; Hagvar et al. 2016).

**Estimates of aged C and OM utilization by aquatic consumers**

The use of stable isotope mixing models in food web studies has provided important insights to the nutritional sources to consumers (Fry 2007; Moore and Semmens 2008; Layman et al. 2012). Consumers often rely on multiple nutritional sources, and use of isotopic values of organisms and their potential nutritional resources along with known isotopic fractionation factors in mixing models provide estimates of the relative contributions of the different potential
nutritional sources to the consumer (Phillips and Gregg 2003; Solomon et al. 2011; Phillips et al. 2014). Early linear mixing models relied on a system of algebraic equations (Phillips and Gregg 2001), whereas more recent models employ a Bayesian approach and allow for improved incorporation of uncertainty (Phillips and Gregg 2003; Moore and Semmens 2008). Use of multiple (2–4) isotopes in a mixing model can additionally improve estimates of source contributions to consumer biomass by further reducing estimate uncertainty, especially when multiple isotopically unique potential nutritional sources are being considered (Finlay et al. 2010; Phillips et al. 2014). In addition, when natural 14C is used in conjunction with stable isotopes in mixing models, estimates of the ages of potential nutritional resources to consumer biomass are also obtained.

In order to estimate maximum possible C contributions of varying ages to aquatic consumers in different aquatic systems where natural 14C has been used (Table 1), we used the most 14C-depleted values for consumer organisms from each study system shown in Fig. 5. For illustrative purposes, we employed a simple, linear two endmember mixing model approach with one fossil to millennial aged endmember (i.e., nutritional source) and one modern-aged endmember. Since we used only two potential nutritional sources, we employed the following mass balance equations to estimate the proportional contribution of each to the consumer (Phillips and Gregg 2001):

\[
\Delta_{\text{mix}} = p_A \cdot \Delta A + p_B \cdot \Delta B
\]

\[
p_A + p_B = 1
\]

where \( \Delta_{\text{mix}} \) represents the mixture (consumer), \( A \) and \( B \) each represent the two endmember nutritional sources, and \( p \) represents the proportional contribution of each nutritional source. \( \Delta_A, \Delta_B, \) and \( \Delta_{\text{mix}} \) are the \( \Delta^{14} \)C values of \( A, B, \) and the mixture, respectively.

Our calculations employed three different aged endmembers (\( A \)) in order to cover a realistic range of aged C and OM sources to aquatic systems and consumers. These consisted of: (1) a fossil-aged endmember (either carbonate or shale-derived OM; \( \Delta^{14} \)C = –10000 ‰, age \( \geq \) 50,000 yr B.P.), (2) a moderately aged soil C and OM endmember (\( \Delta^{14} \)C = –540 ‰, age \( \geq \) 6240 yr B.P.; Hossler and Bauer (2012)), and (3) a 50 : 50 mixture of the moderately aged soil endmember (\( \Delta^{14} \)C = –540 ‰) and a young soil C and OM endmember (\( \Delta^{14} \)C = 157 ‰, modern in age; Hossler and Bauer 2012), yielding an endmember of \( \Delta^{14} \)C = –192 ‰ (age = 1710 yr B.P.). In their analysis of the \( \Delta^{14} \)C and \( \delta^{13} \)C values of POC, DOC, and DIC in eight northeast U.S. rivers, Hossler and Bauer (2012) determined that river OM was dominated by varying combinations of young and moderately aged soil endmembers. The results of the calculations using the three different aged endmembers are shown in Table 2.

**Streams and rivers**

Using the fossil endmember (\( \Delta^{14} \)C = –10000 ‰), our model estimates indicate that fossil C could potentially contribute maximums of 9–58% to consumer biomass in all streams and rivers included in our study (Table 2). When moderately aged, and a combination of moderately and young aged, soil endmembers were used, the maximum estimates of aged nutritional contributions to consumers increased correspondingly. Using the moderately aged soil C and OM endmember resulted in maximum nutritional contributions to consumers of this material ranging from 17% to >100% (Table 2). Employing a combined moderately/young aged soil endmember yielded a maximum contribution to consumers of 48% to >100% (Table 2). Estimates > 100% indicate that contributions from the moderately and combined moderately/young aged materials are not adequate to account for the entirety of aged consumer biomass. Therefore, utilization by consumers of more highly aged (i.e., greater amounts of fossil and/or moderately aged) materials are necessary to attain their observed \( \Delta^{14} \)C values (Table 2).

Only some of the studies reviewed here (Caraco et al. 2010; Ishikawa et al. 2014, 2016; Fellman et al. 2015; DelVecchia et al. 2016) provided estimates of the contributions of aged C and OM to consumer biomass. Differences between our fossil endmember estimates and those of the studies of Fellman et al. (2015), Ishikawa et al. (2014, 2016), and DelVecchia et al. (2016) may be explained by the fact that the glacially derived C, periphyton C, and methane endmembers that each study used, respectively, were not as 14C-depleted as our fossil C endmember. Use of soil C and OM endmembers (both moderately aged and a 50 : 50 mixture of moderately and young aged) in our model led to estimates closer to those calculated by Fellman et al. (2015) and Ishikawa et al. (2014, 2016). However, for DelVecchia et al. (2016), our moderately aged soil endmember was not old enough to explain their observed stonelike ages (see Supporting Information Section 2 for additional details regarding our vs. published estimates of aged nutritional contributions). Our estimates based on C and OM of moderately aged and 50 : 50 mixture of moderately and young aged soil may be more realistic because, as noted by Hossler and Bauer (2012), most rivers contain admixtures of moderately and young aged soil C and OM. This is consistent with our predicted contributions of the sources and ages of materials to consumer biomass for previous studies.

**Lakes and wetlands**

Our model indicates that maximum contributions of fossil, moderately aged, and a mixture of moderately/young aged soil C and OM to consumer biomass, were highly variable across all temperate and subtropical lake and wetland systems (Fig. 5). Maximum contributions of fossil, moderately aged, and mixed moderately/young aged material ranged from 0–17%, 0–31%, and 0–86%, respectively (Table 2). These estimates suggest that temperate and subtropical lake food webs may be less...
reliant on moderately and fossil aged C and OM, and more reliant on younger nutritional resources, than streams and rivers. In contrast, lentic food webs in agricultural watersheds, as well as those in subarctic regions, were supported by aged C and OM in the forms of soil-derived and glacial-derived material, respectively (Hagvar and Ohlson 2013; Hagvar et al. 2016; Wang et al. 2014). Estimates of aged C and OM contributions to consumer biomass were also not always provided for all lake studies (Table 1). However, discrepancies between estimates when provided and our estimates are likely due to differences in $\Delta^{14}C$ values and ages of the authors and our selected endmembers (see Supporting Information Section 2 for additional details).

### Table 2. Maximum estimated contributions of fossil aged C and OM, moderately aged soil C and OM, and a 50:50 mixture of moderately aged and young aged soil C and OM endmembers to metazoan consumer biomass as calculated in the present study for lakes, wetlands, streams, and rivers in which organism $\Delta^{14}C$ was measured. Also provided are the original contributions of aged C and OM estimated in each of the studies.

| System type                  | Fossil aged (%) | Moderately aged soil (%) | Moderately and young aged soil (%) | Original estimates (%) |
|------------------------------|-----------------|--------------------------|-----------------------------------|------------------------|
| **Streams and rivers**       |                 |                          |                                   |                        |
| Lake Biwa watershed, Japan   | 18              | 33                       | 93                                | 41–62¶                |
| Kanno River, Japan           | 20              | 36                       | >100||                           | ~45–90¶               |
| Susquehanna River, Pennsylvania | 50            | 92                       | >100||                           | 5–76#                 |
| Pigeon River, Michigan       | 11              | 21                       | 58                                | ND                     |
| Clear Fork River, Ohio       | 11              | 21                       | 58                                | ND                     |
| Muskingum River, Ohio        | 15              | 29                       | 80                                | 7–27¶                 |
| Colville River, Alaska       | 15              | 28                       | 80                                | ND                     |
| Hudson River, New York       | 35              | 65                       | >100||                           | 21–57¶                |
| Mohawk-Hudson, New York      | 11              | 21                       | 60                                | 0–93#                 |
| Weibe and Rebbe Rivers       | 13              | 24                       | 69                                | ND                     |
| Trave River                  | 19              | 35                       | 97                                | ND                     |
| Herbert River, Alaska        | 9               | 17                       | 48                                | 25–36¶                |
| Nyack Floodplain, Montana    | 58              | >100§                    | >100||                           | 26.2–69.2¶            |
| **Lakes and wetlands**       |                 |                          |                                   |                        |
| Everglades, Florida          | 7               | 13                       | 38                                | 31¹                   |
| Norway Glacier               | 33              | 62                       | >100¹                             | ND                     |
| Great Basin Lakes            | 17              | 31                       | 86                                | ND                     |
| Eastern Townships lakes, Quebec | 0             | 0                        | 0                                 | ND                     |
| Lake Superior                | 0               | 0                        | 0                                 | 3³                     |
| Lough Erne                   | 14              | 26                       | 72                                | ND                     |
| German Lakes                 | 13              | 24                       | 68                                | ND                     |

* Fossil C and OM endmember $\Delta^{14}C = -1000_{\text{iso}}$ (equivalent $^{14}C$ age $\geq 50,000$ yr B.P.).

† The moderately aged soil material is defined as “passive aged” soil C and OM by Hossler and Bauer (2012). Estimated $\Delta^{14}C$ values for the passive soil C and OM pool were $-538_{\text{iso}}$ (equivalent $^{14}C$ age $= 6200$ yr B.P.) for Inceptisols and $-541_{\text{iso}}$ (equivalent $^{14}C$ age $= 6260$ yr B.P.) for Ultisols according to Hossler and Bauer (2012). We used the average of these two values for a passive aged soil OC endmember $\Delta^{14}C$ value of $-540_{\text{iso}}$ (equivalent $^{14}C$ age $= 6240$ yr B.P.). See text for calculation details.

‡ The young aged soil material is defined as “slow turnover” soil C and OM ($\Delta^{14}C = 157_{\text{iso}}$ modern-aged) by Hossler and Bauer (2012).

§ 50:50 mixture of moderately aged soil C and OM (mean $\Delta^{14}C = -540_{\text{iso}}$; equivalent $^{14}C$ age $= 6240$ yr B.P.) and young aged soil C and OM ($\Delta^{14}C = 157_{\text{iso}}$; modern-aged) pools, giving a mean $\Delta^{14}C$ value of $-192_{\text{iso}}$ (mean equivalent $^{14}C$ age $= 1710$ yr B.P.). See text for calculation details.

¶ Mean contribution.

# Median contribution.

k Estimates $> 100\%$ indicate that inputs of C and OM having $\Delta^{14}C < -192_{\text{iso}}$ are required (i.e., lesser amounts of young aged soil material and greater amounts of moderately or fossil aged materials).

Conclusions and future directions

The ages of nutritional resources utilized by aquatic consumers have generally not been evaluated in the vast majority of aquatic food web studies. However, in every inland water study that has so far measured natural abundance $^{14}C$ in consumers, consumer biomass is supported by one or more forms of aged C or OM, with the exception of the largest lakes (Table 2). Minimal contributions of aged C and OM to the food webs of very large lakes like the Lake Superior may be explained by their relatively low watershed : lake surface areas and the greater abundance and importance of modern-aged aquatic primary production to consumer nutrition (Zigah et al. 2012a; Kruger et al. 2016). Collectively these
studies indicate that modern aquatic food webs rely not only on living or recently produced sources of nutrition, but are also supported by geologically aged reservoirs of C and OM.

The two primary mechanisms by which aged C can enter aquatic food webs are utilization of (1) aged OM directly (derived, e.g., from soils, sedimentary rocks, petroleum hydrocarbons, etc.) or bacterially “repackaged” biomass and (2) living or recently living biomass from photosynthetic fixation of aged DIC and CO$_2$(aq) by aquatic primary producers. While these mechanisms are independent from each other, both still represent contributions of moderately to geologically aged C to present-day aquatic food webs. Future studies using natural $^{14}$C to assess contributions of aged C and OM to aquatic consumer biomass must better distinguish between the aged OM and aged DIC pathways as entry points of aged C to aquatic food webs. While both are sources of “aged” nutrition, the former represents remobilization of truly aged and previously sequestered OM sources while the latter represents recent photosynthetic fixation of dissolved CO$_2$ derived from some combination of (1) fossil carbonates mobilized via weathering and (2) respiration of aged OM. Use of novel methods including compound-specific $^{14}$C analysis (Ishikawa et al. 2015; Kruger et al. 2016) and whole-system $^{13}$C-DIC labeling (Cole et al. 2002; Pace et al. 2004) may help delineate these different mechanisms.

A number of important research questions remain to be addressed on the importance of the ages of C and OM contributing to aquatic food webs. These include: (1) does utilization of aged C and OM augment or displace modern-aged sources of nutrition?; (2) do contributions of aged C and OM to aquatic consumer nutrition alter food web community structure, ecosystem function, and biogeochemical cycling relative to aquatic systems that do not contain aged C and OM or utilize aged nutritional resources? and (3) what are the dominant watershed- and larger-scale factors (e.g., changes in land use, hydrology, climate [i.e., temperature, rainfall, etc.]) influencing the mobilization of aged C and OM to aquatic systems and how might they alter food web nutrition and dynamics?

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