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2 The incredible lightness of being methane-fuelled: stable isotopes
3 reveal alternative energy pathways in aquatic ecosystems and beyond

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18 **Abstract**

19 We have known about the processes of methanogenesis and methanotrophy for over 100
20 years, since the days of Winogradsky, yet their contributions to the carbon cycle were
21 deemed to be of negligible importance for the majority of that period. It is only in the last two
22 decades that methane has been appreciated for its role in the global carbon cycle, and stable
23 isotopes have come to the forefront as tools for identifying and tracking the fate of methane-
24 derived carbon within food webs, especially within aquatic ecosystems. While it is not
25 surprising that chemosynthetic processes dominate and contribute almost 100% to the
26 biomass of organisms residing within extreme habitats like deep ocean hydrothermal vents
27 and seeps, way below the reach of photosynthetically active radiation, it is perhaps
28 counterintuitive to find reliance upon methane-derived carbon in shallow, well-lit, well-
29 oxygenated streams. Yet, apparently, methane-derived carbon contributes to varying degrees
30 across the spectrum from point sources to extremely diffuse sources. Certainly a good
31 proportion of the evidence for methane-derived carbon contributing to freshwater food webs
32 comes from somewhere in the middle of that spectrum; from studies of seasonally stratifying
33 lakes (mono- or dimictic) wherein, there is a defined gradient or boundary at which anoxic
34 meet oxic conditions and consequently allows for close coupling of methanogenesis and
35 methanotrophy. However, even seemingly well-mixed (polymictic) lakes have a contribution
36 of methane-derived carbon contributing to the benthic biomass, despite an almost continual
37 supply of photosynthetic carbon being delivered from the surface.

38 Aside from the fundamental importance of identifying the carbon sources fuelling biomass
39 production, stable isotopes have been integral in the tool box of palaeolimnologists seeking to
40 identify how contributions from methane have waxed and waned over time. Here, we

41 synthesise the current state of knowledge in the use of stable isotopes to trace methane-
42 derived carbon in primarily freshwater ecosystems.

43

44 Running head:

45 Identifying and tracking methane in food webs

46

47 Keywords:

48 Methanotrophy; chironomids; fatty acids; zooplankton; carbon; hydrogen; production; food
49 webs; greenhouse gas; biogeochemical cycling; trophic transfer

50

51

52 *A brief synopsis on the global importance of methane in aquatic systems, and particularly in*
53 *freshwaters*

54 The global carbon cycle was considered, until relatively recently, to be solely the flux and
55 storage of carbon between the atmosphere, and terrestrial and oceanic pools. Within the total
56 carbon budget, it has been noted that despite their relatively small area, inland freshwaters
57 make a considerable contribution to the global methane (CH₄) budget with emissions of CH₄
58 from freshwaters being at least comparable to the terrestrial CH₄ sink (Battin *et al.*, 2009).
59 However, there is a considerable bias toward data from lakes and other wetlands, and the role
60 of rivers remains poorly defined (Bastviken *et al.*, 2011). Emissions of CH₄ may be small in
61 terms of carbon, but one must consider that CH₄ is a more potent greenhouse gas than CO₂
62 over century time scales; (Bastviken *et al.*, 2011) estimated that global CH₄ emissions
63 expressed as CO₂ equivalents correspond to at least 25% of the estimated terrestrial
64 greenhouse gas sink. Our understanding of the global carbon cycle will only be complete if
65 we include the flux of carbon through inland freshwaters (Battin *et al.*, 2009); (Cole *et al.*,
66 2007); Trimmer *et al.*, 2012); getting to grips with methane-fuelling of food webs is an
67 interesting and important component of this. Indeed, (Cole, 2013) noted that “the role of
68 methane in supporting food webs in lakes, and perhaps even beyond their shores, has come as
69 a surprise” and that “the notion that lake methane partially supports higher organisms in
70 surrounding terrestrial environments fundamentally changes our understanding of how
71 aquatic food webs work”.

72 Methanogenesis is a universal terminal degradation process of organic matter in anoxic
73 aquatic sediments when inorganic oxidants such as nitrate, ferric iron or sulphate are depleted
74 (Conrad, 2005). Hence, in marine systems where there is typically a high concentration of
75 sulphate, the sulphur cycle tends to dominate chemosynthesis, but in freshwaters where
76 sulphate concentrations are typically lower (Hobbie, 1988) then methanogenesis dominates.
77 Stable isotopes have been an incredibly useful tool in the identification and quantification of
78 methanogenic and methanotrophic pathways (Conrad, 2005) and further identifying the
79 constituents of the complex microbial community that is actively involved via stable isotope
80 probing (SIP; e.g. (He *et al.*, 2012), but those aspects are not the focus of this review.
81 Methane may be lost directly from the system via ebullition or the recently hypothesised
82 micro-bubble pathway, stochastic processes notoriously difficult to quantify (Prairie and del
83 Giorgio, 2013) or be effectively ‘piped’ to the surface via plants (Bergstrom *et al.*, 2007);
84 (Sanders *et al.*, 2007). Alternatively, or in addition, it may subsequently serve as an energy
85 and C source for methanotrophs (methane oxidising bacteria; MOB), typically at oxic-anoxic
86 boundaries (if anaerobic CH₄ oxidation is excluded) in the sediment, or in the water column
87 (Rudd and Taylor, 1980; (Kankaala *et al.*, 2007). It is essentially from this point in the cycle
88 that stable isotopes have been key in tracing the use of methane-derived carbon (MDC) into,
89 and through, food webs, particularly in freshwaters (Jones and Grey, 2011). A schematic of
90 potential routes by which CH₄ produced in anoxic freshwater sediments may either by-pass
91 or become incorporated into food webs is shown in Figure 1.

92

93 *Could a methane pathway be important to secondary production in food webs?*

94 Anoxic water and sediments are typically rich in organic matter compared to the overlying
95 oxic water, and anoxic metabolism may account for a substantial part (20–60%) of the carbon

96 metabolism and the heterotrophic microbial production within freshwater environments
97 (Hessen and Nygaard, 1992). Methanogenesis in lakes has been reported corresponding to
98 30-80% of the anaerobic mineralisation in waters and sediments (Bastviken, 2009). While
99 seasonal variability in CH₄ oxidation is known to be considerable, especially in dimictic
100 lakes, between 30-94% of the CH₄ reaching oxygenated layers is reputedly oxidised (Casper
101 et al., 2000); (Morana *et al.*, 2015). In essence then, CH₄ is a major product of the C
102 mineralisation in lakes, and a large proportion may be converted to microbial biomass
103 equivalent in some instances to the total C fixation by heterotrophic bacteria and a significant
104 proportion of primary production (Hessen and Nygaard, 1992; Bastviken *et al.*, 2003). Again,
105 data from rivers are lacking, but across 15 rivers, in late summer, i.e. when one might expect
106 the greatest contribution from photosynthesis, (Shelley *et al.*, 2014) conservatively calculated
107 that net methanotrophy was equivalent to between 1% and 46% of benthic net photosynthetic
108 production within the gravel beds of chalkstreams. Couple this to the apparently high (50%)
109 carbon conversion efficiency of methanotrophs (relative to 10-30%, typical for bacteria in
110 detrital-based food webs), regardless of marked spatial and temporal changes in ambient
111 methane concentration, and it suggests that methanotrophs can sustain net production
112 throughout the year (Trimmer *et al.*, 2015)).

113 The importance of a CH₄ pathway to food webs might yet increase further under climate
114 change. Increases in temperature forecast for the coming decades may have profound
115 implications for the cycling of carbon in aquatic ecosystems due to the differential
116 temperature dependencies of carbon fixation by gross primary production (GPP) and carbon
117 mineralisation by ecosystem respiration (ER). For example, (Yvon-Durocher et al., 2010)
118 showed that warming of 4°C reduced the carbon sequestration capacity of freshwater
119 mesocosms by 13%, shifting them towards net heterotrophy (i.e. net sources of CO₂ to the
120 atmosphere) because ER responded more strongly to temperature than GPP. They also found
121 that methanogenesis responded even more strongly than ER or GPP, with 20% more of the
122 GPP being accounted for by CH₄ emissions with 4°C of warming (Yvon-Durocher *et al.*,
123 2011). Benthic community structure and how that contributes to a host of ecosystem
124 processes, including microbial and macrofaunal decomposition rates, was also clearly
125 affected by such warming (Dossena *et al.*, 2012). If it is assumed that delivery of organic
126 matter does not change but temperature increases as predicted, then for example, the
127 increased mineralisation will equate to a 4-27% (0.9-6.4 Tg C y⁻¹) decrease in organic carbon
128 burial in boreal lakes (Gudasz *et al.*, 2010). However, very recent work in rivers suggests that
129 methanotrophy has the potential to match methanogenesis enhanced by warming (Shelley *et*
130 *al.*, 2015). How climate change might impact upon food web mediation of methane-derived
131 carbon will be returned to later.

132

133 *Why are stable carbon and hydrogen such useful tracers of methane?*

134 Isotopic signatures of environmental CH₄, both ¹³C/¹²C and ²H/¹H, have been compiled by
135 (Whiticar *et al.*, 1986) and (Bréas *et al.*, 2001) amongst others. An important characteristic of
136 biogenic methane is that its carbon stable isotope composition is typically markedly ¹³C-
137 depleted compared to other putative basal resources in a food web. So, for freshwater lakes,
138 CH₄ δ¹³C may be as low as -110‰ to -50‰ dependent upon formation pathway; (Whiticar,
139 1999); (Deines and Grey, 2006); (Taipale *et al.*, 2007) relative to either allochthonous

140 terrestrial plant detritus ($\delta^{13}\text{C}$ value from C3 plants typically -28‰ to -26‰ (Peterson and
141 Fry, 1987); or autochthonous phytoplankton ($\delta^{13}\text{C}$ typically between -35 to -25‰; (Grey et
142 al., 2000, Vuorio et al., 2006) but acknowledging that components of the phytoplankton such
143 as their fatty acids may be ~10‰ further ^{13}C -depleted e.g. (Taipale et al., 2015)). However,
144 CH_4 $\delta^{13}\text{C}$ values reported from sediments are not necessarily linked to the $\delta^{13}\text{C}$ values of
145 sedimentary organic matter; instead they may be strongly influenced by the quality of the
146 organic matter substrate and/or the predominant methanogenic pathway (Rinta et al., 2015),
147 and of course to a certain extent as to whether some of the CH_4 has already been oxidised by
148 MOB prior to analysis (Coleman et al., 1981). In marine hydrocarbon seep communities,
149 $\delta^{13}\text{C}$ has been the primary isotope value examined, used to differentiate between animals with
150 chemoautotrophic symbionts (-40‰ to -20‰) from those with methanotrophic symbionts
151 (≤ -40 ‰) (Brooks et al., 1987) and to identify the source CH_4 pool as either thermogenic
152 ($\delta^{13}\text{C} = -45$ ‰ to -40‰) or biogenic ($\delta^{13}\text{C} < -45$ ‰) CH_4 (Sassen et al., 1999).

153 Isotopic fractionation during the use of CH_4 by MOB typically leads to further ^{13}C -depletion
154 (by 0-30‰; (Summons et al., 1994, Templeton et al., 2006). For example, CH_4 -consuming
155 archaea isolated from anoxic marine sediments have been reported with $\delta^{13}\text{C}$ values as low as
156 -96‰ (Orphan et al., 2001), while biomarkers (e.g., archaeol and hydroxyarchaeol) from such
157 archaea within a CH_4 -supported benthic microbial community in cold-seep sediments
158 exhibited $\delta^{13}\text{C}$ values as low as -111‰ (Werne et al., 2002). Hence, the MOB biomass
159 available to consumers has a strikingly low $\delta^{13}\text{C}$ and, because stable carbon isotope ratios
160 differ little between consumers and their diets, assuming no selective assimilation or
161 substantial biosynthesis (McCutchan Jr et al., 2003, Grey, 2006), this should allow its
162 contribution to consumer biomass to be rather readily traced.

163 Hydrogen isotope effects during methanogenesis of methylated substrates can lead to
164 deuterium depletions as large as -531‰, whereas, bacterial D/H discrimination for the CO_2 -
165 reduction pathway is significantly less (-250‰ to -170‰; Whiticar, 1999). Very little is
166 known regarding the δD values of MOB. However, when compared to typical values of
167 autochthonous (-290‰ to -215‰) and allochthonous (-160‰ to -125‰) resources, there is
168 still great scope for the use of δD to trace CH_4 -derived production (Doucett et al., 2007, Estep
169 and Dabrowski, 1980), especially when in combination with $\delta^{13}\text{C}$ (e.g. (Deines et al., 2009)).
170 The more distinct the sources, and indeed, the more tracers used, the more confidence can be
171 assigned to estimates of contribution to diet derived from any of the recently published
172 mixing models (e.g. (Parnell et al., 2013)). Problems arise using isotopic tracers when a
173 relatively minor contribution from MDC results in δ values that could be arrived at via
174 alternative pathways (see 'The zone of contention' section below).

175

176 *Methane use across a spectrum of sources*

177 As appreciation of the possibility of MDC providing an alternative energy source to food
178 webs has grown, so the emphasis on research has shifted from point sources to ever more
179 diffuse sources, and less intuitively obvious locations where it might be relevant. The
180 proportion of MDC contributing to food webs at more diffuse sources may well be smaller
181 (but still of significance); as such, there is likely to be greater ambiguity in the stable isotope

182 signal, and so the importance of MDC might have been overlooked in many of these systems
183 (Figure 2).

184 It is perhaps unsurprising that the use of CH₄ (amongst other chemosynthetic production) is
185 strongly evident at point sources such as deep-sea vents and seeps, whale, kelp and wood
186 falls, and some sewage outflows, typically far beyond the direct reach of photosynthetically
187 active radiation (although of course photosynthetic production can ‘fall-out’ of the water
188 column to benthic communities). The potential for chemosynthesis to fuel entire animal
189 communities in the ocean was first noted around 35 years ago (e.g. (Rau and Hedges, 1979)).
190 Early attention focussed on megafaunal or epifaunal taxa such as molluscs or pogonophorans
191 but there was a broad suite of smaller infaunal deposit feeding and omnivorous invertebrates
192 whose mode of nutrition remained largely ignored until the application of stable isotope
193 analyses by the likes of (Van Dover and Fry, 1994), (Colaço et al., 2002) and (Levin and
194 Michener, 2002). For example, Levin and Michener (2002) looked at a variety of sites
195 including CH₄ seeps in the Gulf of Alaska, on the Oregon margin, and on the northern
196 California slope and found that seep macrofauna exhibited lighter δ¹³C (and δ¹⁵N) values
197 than those in non-seep sediments. Significant contributions were found from MDC to
198 macrofaunal biomass from sediments of pogonophoran fields (32%–51%) and clam beds
199 (12%–40%) in the Gulf of Alaska, and in microbial mat sediments on the Oregon
200 margin (20%–44%). Some polychaetes exhibited extremely low values of δ¹³C (-90.6‰) at
201 these point sources (see Figure 2a).

202 Within the last 15 years, research on MDC and food webs primarily focussed on lakes,
203 particularly stratifying lakes in temperate and boreal systems, and much of this work has been
204 extensively reviewed by Jones & Grey (2011). Tube-dwelling chironomid larvae appear key
205 in lake sediments. Field studies from lakes across Alaska, England, Finland and Germany
206 (amongst others) have demonstrated that chironomids can assimilate MDC extensively (up to
207 70% of larval biomass; (Jones et al., 2008). The degree to which they do may vary within
208 lakes on a temporal (Deines et al., 2007c, Grey et al., 2004c) or spatial (Deines and Grey,
209 2006; (Gentzel et al., 2012) scale, or by taxa (Jones and Grey, 2004, Kelly et al., 2004, Jones
210 et al., 2008), and among lakes with ‘strength’ of stratification (Grey et al., 2004c, Deines et
211 al., 2007b, Hershey et al., 2015). Chironomid larvae are bioengineers; they bioturbate the
212 sediment while ‘digging’ and maintaining their burrows and draw down oxygenated water,
213 bringing it into contact with anoxic sediment. The sediments on the burrow walls have been
214 shown to exhibit higher methane oxidation rates and higher densities of MOB than the
215 surrounding bulk or surficial sediments (Kajan and Frenzel, 1999); (Gentzel et al., 2012).
216 Larvae thereby appear to create the perfect micro-niche for the coupling of methanogenesis
217 and methanotrophy (Deines et al., 2007a); (Kelly et al., 2004); see route 3 in Figure 1).

218 It was assumed from field studies that the low δ¹³C values for taxa such as *Chironomus*
219 *plumosus* (e.g. -70‰ to -50‰; (Jones et al., 2008)) reflected ingestion of the MOB on their
220 burrow walls (Deines et al., 2007a) akin to ‘gardening’ by trichopteran caddis flies on the
221 biofilms that develop on caddis cases (also studied by using stable isotopes; (Ings et al.,
222 2012). By using ¹³C-labelled CH₄ additions directly into sediments housing chironomid
223 larvae under controlled experimental settings, Deines et al., (2007a) have elegantly
224 demonstrated that larvae assimilate MDC via MOB; this was further supported by
225 phospholipid fatty acids diagnostic for MOB and significantly enriched by the ¹³C-labelled
226 methane being detected in the larval tissues. In a series of parallel experiments, they showed

227 that larvae could also obtain MDC via ^{13}C -labelled Type II MOB introduced into the water
228 column above sediments. Type I and Type II MOB use different pathways for formaldehyde
229 assimilation (ribulose monophosphate and serine, respectively) and typically favour different
230 environmental conditions; Type I appear to be dominant in environments in which CH_4 is
231 limiting and combined nitrogen and copper concentrations are relatively high, whereas Type
232 II appear where there are high CH_4 concentrations, low dissolved oxygen, and limiting
233 concentrations of combined nitrogen and/or copper (Hanson and Hanson, 1996). The ability
234 to access MDC via two discrete routes might account for some of the incredible inter-
235 individual variability that has been observed in chironomid stable isotope ratios (e.g. (Grey et
236 al., 2004b); Figure 2).

237 When stratification of the water column becomes too pronounced, generally in duration, and
238 the benthic sediments become inhospitable even for the hypoxic tolerant chironomid larvae,
239 Jones & Grey (2011) hypothesised that MDC is more likely to be taken up in the water
240 column at the oxic-anoxic boundary by zooplankton. Again, evidence for this is mostly
241 derived from the field from small Finnish boreal lakes with marked oxyclines (e.g. (Jones et
242 al., 1999); (Taipale et al., 2007, Taipale et al., 2008), but see (Bastviken et al., 2003); (Santer
243 et al., 2006); (Schilder et al., 2015a). Pelagic zooplankton $\delta^{13}\text{C}$ values are typically not as low
244 as those reported from similar lakes for benthic chironomids, perhaps again reflecting the
245 more diffuse nature of the source CH_4 , and / or the more mobile feeding capability of
246 zooplankton in the water column relative to tube dwelling chironomids in the sediments.
247 Some of the lowest values reported are for *Daphnia* spp. from small, strongly stratifying
248 lakes with anoxic hypolimnia; for example, -47‰ in a kettle lake, Plußsee (Harrod and Grey,
249 2006), or -46‰ from Mekkojarvi (Taipale et al., 2008). Laboratory support for zooplankton
250 uptake of MOB is sparse, but (Kankaala et al., 2006) measured growth rates of *Daphnia* in
251 replicated cultures fed microbial suspensions with or without addition of CH_4 and found that
252 their $\delta^{13}\text{C}$ values indicated consumption of ^{13}C -depleted MOB, as have (Deines and Fink,
253 2011) using ^{13}C -labelling of CH_4 .

254 Evidence of MDC contributions to biomass in polymictic (permanently mixed) lakes is rarer.
255 Such lakes are often shallow and contain considerable stands of macrophytes; while
256 methanogenesis is certainly proceeding in the sediments, much of the CH_4 produced might be
257 routed via the plant stems and via ebullition (routes 1&2 in Figure 1) and hence, side-step
258 incorporation into the food web (although see reference to (Agasild et al., 2014), below).
259 Since the whole water column is well oxygenated, there is no distinct boundary where MOB
260 will accumulate and thus it is unlikely that zooplankton will feed heavily upon MOB (Jones
261 and Grey, 2011). In the benthos, there is also typically a more consistent supply of ^{13}C -
262 enriched phytoplankton production from above which will 'swamp' the lower $\delta^{13}\text{C}$ values
263 from MOB. Examples of such lakes with permanently oxic sediment surface layers in which
264 MDC has been shown to make only a limited (maximum ~20%) or negligible contribution to
265 chironomid biomass include Großer Binnensee and Schöhsee in north Germany (Grey et al.,
266 2004c, Deines et al., 2007c), Lough Neagh and Rostherne Mere in the UK (Kelly et al.,
267 2004), Izunuma in Japan (Yasuno et al., 2012), and Võrtsjärv in Estonia (Agasild et al., 2014,
268 Cremona et al., 2014). Interestingly, the latter lake was sampled at various sites and it was
269 only at one particular site dominated by vegetation that low $\delta^{13}\text{C}$ values were recorded in
270 both zooplankton and chironomids. Agasild et al. (2014) postulated that the stands of
271 macrophytes prevented wind mixing from disturbing the sediments, and that dissolved

272 oxygen in the water column was reduced by the restricted circulation of water and gas
273 exchange between the water surface and the atmosphere and by increased oxygen demand
274 from the decomposition of organic matter; all processes which would lead to greater MDC
275 being available to the food web.

276 Within the last five years has come the first convincing evidence of MDC contributing to
277 food webs in free-flowing, well oxygenated streams and rivers, where because of the
278 turbulent nature, the source of CH₄ could be considered to be most diffuse. One of the first
279 studies claiming a river food web to be fuelled by MDC was by (Kohzu et al., 2004) who
280 reported *Helodes* sp. beetle larvae and adults with $\delta^{13}\text{C}$ values as low as -69.8‰ but these
281 were from stagnant backwater pools akin to stratifying lakes, and while these may be
282 important habitats on some lotic systems, they were not from the free flowing, main-stem
283 river food web. Since then, considerable research on the chalk streams of the UK, highly
284 productive, ground water fed systems has revealed that trichopteran larvae may play a similar
285 role to chironomids in lakes, the main conduit for MDC to route into the wider food web (e.g.
286 (Trimmer et al., 2009, Trimmer et al., 2010). In contrast, (Mbaka et al., 2014) studied small
287 inline impoundments with extremely short residence times on a river system in Germany but
288 could find negligible evidence of MDC contributing to chironomids from the sediments there.
289 How MDC might contribute significantly to river food webs clearly requires more research.

290 Unless there is almost 100% trophic transfer of MDC higher into the food web, then
291 obviously mixing with non-MDC food sources results in a dilution of the indicator isotope in
292 question, and the ability to trace MDC further using stable isotopes alone is weakened (see
293 below). An apparent gradient is thus evident from point to diffuse source of methane. For
294 example, on a species-specific basis, some mobile benthic predators (eels, sea stars, and
295 predatory snails) have been shown on the basis of their low $\delta^{13}\text{C}$ (and $\delta^{15}\text{N}$ & $\delta^{34}\text{S}$) values to
296 obtain close to 100% of their nutrition from CH₄ seep production in the Gulf of Mexico
297 (MacAvoy et al., 2002). From stratifying lakes, (Harrod and Grey, 2006) and (Ravinet et al.,
298 2010) have found isotopic evidence of MDC contributing (up to ~12%) to bream (*Abramis*
299 *brama*) and to ruffe (*Gymnocephalus cernuus*), respectively, while in a shallow, well-mixed
300 Pantanal (tropical) wetland lake (Sanseverino et al., 2012) could trace MDC into various fish
301 species. Even from the very shallow lake Vörtsjärv, Agasild *et al.* (2014) reported that at sites
302 amongst the macrophytes where zooplankton and chironomid larvae were most ¹³C-deplete,
303 there was a corresponding decrease in $\delta^{13}\text{C}$ for roach (*Rutilus rutilus*), perch (*Perca*
304 *fluviatilis*) and the apex predator, pike (*Esox lucius*), indicative of trophic transfer of MDC to
305 the very top of the food web. To date, evidence from rivers has not been reported, but given
306 the extremely abundant nature of the primary consumers (particularly cased caddis flies) that
307 appear key to linking MOB into the food web in such systems, the pathway is certainly in
308 place (Trimmer et al., 2012). Evidence of the transfer of MDC across ecosystem boundaries
309 is still limited. Aquatic invertebrates such as *Helodes* sp., Chloroperlidae spp., Leuctridae
310 spp. and *Sialis* sp. have all been recorded from Malaise traps on stream banks, i.e. post
311 emergence, with $\delta^{13}\text{C}$ values from -69.8 to -51.8‰ (Kohzu et al., 2004) but there has still
312 been only one study quantifying transfer of MDC and that was into riparian spiders (up to
313 18% of their biomass) mediated by emerging chironomid imagos from stratifying lakes
314 (Jones and Grey 2011). The potential is clear to see for vertebrate predators as well, such as
315 barn swallows (*Hirundo rustica*) which, using stable isotopes, have been identified as
316 prioritising such abundant prey at specific times of the year (Parnell et al., 2013). Of course,
317 we should also consider how alteration of a food web, for example by introduction of a top
318 predator for recreation or as a function of range expansion might cause cascading effects

319 down to biogeochemical cycling near the base of a food web. By experimentally
320 manipulating fish density in a previously fish-less lake, (Devlin et al., 2015) showed that a
321 trophic cascade from fish to microbes affected methane efflux to the atmosphere and reduced
322 the amount of MDC assimilated into the biomass of zooplankton that remained (assessed
323 from *Daphnia* $\delta^{13}\text{C}$ values). It may well be that such improved quantitative understanding of
324 the influence of higher trophic consumers on carbon budgets creates future opportunity for
325 management and policy to identify and implement new options for mitigating greenhouse gas
326 release at regional scales (Schmitz et al., 2014).

327

328 *The zone of contention*

329 Various authors (e.g. (Deines et al., 2009)) have acknowledged that confidence in the use of
330 isotopic tracers of MDC from field studies must be tempered where/when alternative
331 explanations for such isotope values can arise. The 'zone of contention' for $\delta^{13}\text{C}$ from
332 consumers in freshwater lakes for example typically occurs between -40‰ and -30‰.
333 Chironomid larvae could exhibit such a value if they assimilated: a) a small percentage from
334 very low $\delta^{13}\text{C}$ MOB and a greater percentage from relatively high $\delta^{13}\text{C}$ phytoplankton (e.g.
335 (Grey et al., 2004b)); b) alternative chemosynthetic sources of carbon such as sulphur
336 bacteria (e.g. (Deines et al., 2009); (Roach et al., 2011)); or c) phytoplankton with very low
337 $\delta^{13}\text{C}$. It should be remembered that these scenarios are not mutually exclusive. Scenario c
338 may arise because a substantial part of the dissolved CO_2 pool may originate from respiration
339 of autochthonous and allochthonous organic matter and have low $\delta^{13}\text{C}$ (from -20‰ to -15‰:
340 (Lennon et al., 2006); (Kankaala et al., 2010)). The degree of fractionation of that CO_2 by
341 phytoplankton is uncertain and extremely variable, but in lakes might range from 0‰ to 15‰
342 (with values near the upper end of the range probably most widespread; (Bade et al., 2006)).
343 Therefore, it is not uncommon to find $\delta^{13}\text{C}$ values for lake phytoplankton of < -30‰ (e.g.
344 (Grey et al., 2000); (Vuorio et al., 2006)), and anything feeding selectively on ^{13}C -depleted
345 phytoplankton (or assimilating selectively from components thereof such as fatty acids) will
346 show correspondingly low $\delta^{13}\text{C}$ values (Pel et al., 2003). The same has been shown for rivers
347 (Finlay et al., 1999). The situation is even more complex when a proportion of the low $\delta^{13}\text{C}$
348 values for CO_2 could have originated from the oxidation of CH_4 , and hence in effect, be an
349 indirect contribution from MDC (Route 5 in Figure 1). Further dilution of the MDC signal
350 with trophic transfer up the food web has already been mentioned.

351 In such scenarios, only with the addition of alternative but complementary tracers can the
352 assimilation of MDC be assigned with confidence. Hence, the addition of further stable
353 isotopes such as δD (e.g. (Belle et al., 2015, Deines et al., 2009, van Hardenbroek et al.,
354 2015), $\delta^{34}\text{S}$ (Grey and Deines, 2005), and to a certain extent $\delta^{15}\text{N}$ (Grey et al., 2004b,
355 Stephen et al., 2002); see later discussion), have proved useful in ascertaining the use of
356 MDC. Radio isotopes might offer some support under certain situations; for example.
357 (Opsahl and Chanton, 2006) studied the food webs of troglobitic organisms in the Upper
358 Floridian aquifer and found that crayfish trapped from remote sinkhole conduits were not
359 only on average $\sim 10\%$ ^{13}C -depleted relative to their counterparts at accessible springs at the
360 surface but that there was a strong correlation with radiocarbon ($\Delta^{14}\text{C}$) depletion relative to
361 modern values, indicative of a chemosynthetic food source. Concurrent analysis of
362 phospholipid fatty acids (PLFAs) which are diagnostic for MOB, as well as compound-
363 specific analysis of the isotope ratios of those PLFAs has also been invaluable. For example,
364 (Taipale et al., 2009) demonstrated a strong relationship between the $\delta^{13}\text{C}$ values of *Daphnia*

365 and the proportion of MOB-specific PLFAs in *Daphnia*. These methods have also
366 highlighted the indirect route via methane-oxidation and uptake of the resulting ^{13}C -depleted
367 CO_2 by autotrophs (Route 5 in Figure 1). For bog-pool food webs in Estonia, (Duinen et al.,
368 2013) suggested that the most parsimonious explanation for relatively low $\delta^{13}\text{C}$ values of
369 algae-derived polyunsaturated fatty acids found in insects was that MOB were creating CO_2
370 from the oxidation of CH_4 which was assimilated by their direct algal 'neighbours' within the
371 biofilm community. (Sanseverino et al., 2012) used the presence of MOB-diagnostic PLFAs
372 in various benthivorous and detritivorous fishes to support claims of MDC assimilation in
373 Brazilian wetlands as the fish $\delta^{13}\text{C}$ values were $<-36\text{‰}$; low relative to the other food web
374 components in question but clearly not the marked ^{13}C -depletion classically associated with
375 CH_4 . Further correlative evidence may be provided by concurrent assessment of the
376 methanogen / methanotroph community by molecular methods. (Eller et al., 2005) reported
377 zones of aerobic and anaerobic CH_4 oxidation in the water column of a strongly stratifying
378 lake, where high cell numbers of MOB were also detected by fluorescence *in situ*
379 hybridisation techniques. It was around this depth in the same lake that (Santer et al., 2006)
380 found that one of the cyclopoid copepod species, *Diacyclops bicuspidatus*, consistently
381 maintained highest density and exhibited $\delta^{13}\text{C}$ values $\sim 10\text{‰}$ lower than epilimnetic species
382 and the photosynthetic particulate organic matter sampled during the same time interval, and
383 proposed the role of MDC in its diet.
384

385 *Looking back: hindcasting*

386 A particular area of research related to CH_4 -fuelling of food webs that has emerged most
387 recently aims to identify or determine past 'methane environments', predominantly in lakes.
388 Insight into past variations of CH_4 availability in lakes would further our understanding of the
389 timing and magnitude of the response of lake CH_4 production and emissions to changing
390 environmental conditions. Palaeolimnologists have long recognised that recalcitrant remains
391 of organisms such as the strongly sclerotized head capsules of chironomids or the ephippia of
392 daphniids, can be found in abundance and well preserved in most lake sediment records.
393 Chironomid remains, especially the larval head capsules, can be found abundantly in lake
394 sediments. Indeed, exoskeleton fragments originating from moulting and deceased larvae, or
395 zooplankton resting eggs, are preserved for tens to hundreds of thousands of years at a quality
396 which allows microscopic identification usually to genus, or species morphotype, but
397 sometimes also to species level (van Hardenbroek et al., 2011). Since lake sediments can be
398 dated using radiometric and / or other dating methods, these remains can be used to
399 reconstruct historical community composition and by inference the water quality, ambient
400 temperature, or a particular habitat structure (Eggermont and Heiri, 2012). Head capsules and
401 exoskeletons comprise mainly chitin and proteins and, on the basis that their chemical
402 composition does not seem to be strongly affected by decomposition processes, they can be
403 used to develop palaeo-environmental reconstructions based upon stable isotope composition
404 (Perga, 2010, Perga, 2011); (Heiri et al., 2012).

405 (Heiri et al., 2012) recently reviewed the available stable isotope studies based on fossil
406 chironomids (which had mainly examined the elements C, N, H and O), and identified four
407 key areas: (1) developing the methodology for preparing samples for isotopic analysis, (2)
408 studies rearing chironomid larvae under controlled laboratory conditions to determine those
409 factors affecting stable isotope composition, (3) ecosystem-scale studies relating stable
410 isotope measurements of fossil chironomid assemblages to environmental conditions, and (4)

411 developing the first down-core records describing past changes in the stable isotope
412 composition of chironomid assemblages. In a relatively short period of time since that
413 review, a number of publications have arisen expanding upon those areas, further
414 demonstrating the usefulness of the technique, and including other complementary tracer
415 evidence to support suppositions when the stable isotopes alone might prove ambiguous.

416 Firstly, it is important to determine whether there is any isotopic offset between the
417 recalcitrant parts of organisms recovered from palaeolimnological samples and the whole
418 body that is typically analysed for the study of contemporary relationships in food webs. It is
419 also important to determine whether the ‘clean up’ protocols that palaeo-samples typically
420 require have any significant effect upon isotopic integrity. To answer both of these questions
421 for chironomid head capsules, (van Hardenbroek *et al.*, 2010) trialled various commonly used
422 chemical methods for sediment processing and found that treatment with 10% KOH, 10%
423 HCl, or 40% HF showed no detectable effect on $\delta^{13}\text{C}$, whereas, perhaps unsurprisingly, a
424 combination of boiling, accelerated solvent extraction and heavy chemical oxidation resulted
425 in a small but statistically significant decrease in $\delta^{13}\text{C}$ values (0.2‰). Then, using a
426 modification of the culturing experimental protocol by (Deines *et al.*, 2007a), they
427 demonstrated with MOB grown on ^{13}C -labelled methane, that methanogenic carbon is
428 transferred into chironomid head capsules (van Hardenbroek *et al.*, 2010). (Frossard *et al.*,
429 2013) have also looked at head capsule to whole organism isotopic offsets for chironomid
430 larvae and reported from experimental rearing on three different diets that the head capsules
431 were ^{13}C -depleted by 0.9‰ relative to whole biomass. For zooplankton, Perga (2010) has
432 shown that the C and N stable isotope compositions of the daphniid exoskeleton and those of
433 the whole body are strongly correlated. Exoskeleton $\delta^{13}\text{C}$ values were similar to those of the
434 whole body but were strongly depleted in ^{15}N (-7.9‰), reflecting its derivation from
435 excretory ammonia of dietary origin, known to be ^{15}N -depleted compared with dietary
436 organic nitrogen (Schimmelmann, 2011). Further elegant experiments have shown that the
437 stable isotopic composition of *Daphnia* ephippia provides information on that of the parent
438 *Daphnia*, and of the food and water they were exposed to during formation. (Schilder *et al.*,
439 2015b) demonstrated that there were only small offsets between *Daphnia* and ephippia
440 relative to the range of variation in *Daphnia* stable isotopic composition reported from down-
441 core studies. Interestingly however, their work also indicated that temperature may have a
442 minor influence on the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of *Daphnia* body tissue and ephippia
443 which has implications for water temperature reconstruction work using oxygen isotopes, as
444 well as highlighting the care with which controlled feeding experiments need to be conducted
445 (*sensu* (Perga and Grey, 2010). The suite of organism remains has been further extended
446 recently, as it now appears bryozoan statoblasts and zooids have the potential to act as
447 indicators of MDC (van Hardenbroek *et al.*, 2015).

448
449 Prior to the interest in palaeo-reconstruction, site-specific, and hence, differing CH_4
450 production potential and oxidation had only been linked to living chironomid larvae (e.g.
451 (Deines and Grey, 2006). More confidence in the potential of recalcitrant remains to provide
452 information about past changes in CH_4 availability in lakes using sediment records has arisen
453 since studies have been conducted across lake types and actually using remains from surficial
454 sediments i.e. reflecting the most recent CH_4 history that can be measured concurrently. In a
455 study of seven Swedish lakes, (van Hardenbroek *et al.*, 2012) observed significant negative
456 correlations between the $\delta^{13}\text{C}$ of Chironomini and both CH_4 fluxes at the lake surface, and
457 CH_4 releases from the sediment. That dataset was built upon by incorporating samples from
458 10 Siberian lakes and expanding the suite of remains to include those of *Daphnia* and

459 Tanytarsini; the $\delta^{13}\text{C}$ of all three groups were correlated significantly with diffusive CH_4 flux
460 in the combined Siberian and Swedish dataset suggesting that $\delta^{13}\text{C}$ in the biomass of these
461 invertebrates was affected by CH_4 availability (van Hardenbroek *et al.*, 2013). (Schilder *et al.*,
462 2015a) measured *Daphnia* ephippial $\delta^{13}\text{C}$ values from the surface sediments of 15 small
463 European lakes, and found a strong correlation to the late summer aqueous CH_4 concentration
464 in both the surface water and above the sediment.

465 Down-core work is providing some tantalising evidence of past CH_4 variability over time.
466 Adding to their proof-of-concept work on which invertebrate remains are useful tracers of
467 methane-derived carbon (van Hardenbroek *et al.*, 2013) went on to measure the $\delta^{13}\text{C}$ of
468 invertebrate remains from a sediment record (covering the past ~1000 years) of a shallow
469 thermokarst lake in northeast Siberia. Those taxa most sensitive to CH_4 availability
470 (Chironomini, Tanytarsini, and *Daphnia*) exhibited the lowest $\delta^{13}\text{C}$ values in sediments
471 deposited from ca AD 1250 to ca AD 1500, and after AD 1970, which coincided with periods
472 of warmer climate (indicated by an independent local temperature record). As a consequence,
473 the discrepancy in $\delta^{13}\text{C}$ between CH_4 -sensitive taxa and bulk organic matter was higher in
474 these sections than in other parts of the core, whereas the $\delta^{13}\text{C}$ of other invertebrate taxa did
475 not show the same trend. They concluded that there was higher CH_4 availability in the study
476 lake during warmer periods and that the energy sources of some key benthic invertebrates
477 changed accordingly. (Wooller *et al.*, 2012) managed to reconstruct the CH_4 history of
478 Qalluuraq Lake, a shallow Alaskan tundra lake, over a period ~12,000y in this manner, and
479 similar work has been conducted on large, deep sub-alpine lakes, particularly in France. A
480 change from oligotrophic status associated with anthropogenic nutrient enrichment over the
481 last 150 years was examined for associated shifts in the basal resources available to the
482 benthic food web (Frossard *et al.*, 2015). Chironomid head capsule $\delta^{13}\text{C}$ values started to
483 decrease with the onset of eutrophication in both Lake Annecy and Lake Bourget; the
484 estimates of the MDC contribution to chironomid biomass ranged from <5% prior to the
485 1930s to nearly 30% in recent years.

486 To date, values for chironomid head capsules have not been reported as ^{13}C -depleted as for
487 live organisms. This is in part a frustrating function of the requirement for multiple head
488 capsules to be pooled to provide sufficient material for elemental and isotopic analysis. It is
489 also likely associated with the fact that the sampling of the remains of organisms at a specific
490 location (depth) might not truly reflect the location where the animal assimilated its diet, due
491 perhaps to resuspension of sediments and/or focussing of material (Battarbee, 1999). Hence,
492 the ‘strength’ of a MDC signal that one can find in a contemporary sample derived from fresh
493 larvae with values for individuals <-70‰, will always be dampened (i.e. less ^{13}C -depleted)
494 by pooling and/or dilution effects in palaeolimnological samples. As a consequence, the
495 usefulness of $\delta^{13}\text{C}$ alone as a tracer deteriorates (see *the zone of contention* section above).
496 One very promising approach is the analysis of ancient DNA (aDNA) from the methanotroph
497 community. (Belle *et al.*, 2014) has elegantly demonstrated how aDNA can be used to
498 complement stable isotopes in a study of a sediment core from the deepest zone of Lake
499 Narlay, representing the last 1500 years of sediment accumulation. A significant change was
500 noted since ca AD1600, with an increase in the proportion of MOB in the total bacteria
501 community, and a corresponding decrease in chironomid head capsule $\delta^{13}\text{C}$. These trends
502 suggest that assimilation of MOB may account for up to 36% of chironomid biomass, with
503 evidence for preferential assimilation of methanotroph type I and the NC10 phylum. Parallel

504 strands of evidence are clearly required whenever there is ambiguity in stable isotope data,
505 and the development of aDNA will surely grow in this particular field.

506

507 *Looking forward: knowledge gaps*

508 To date, the majority of studies on CH₄ in food webs have solely concentrated on the stable
509 carbon isotopes as a tracer. However, equally evident to the very low and varying $\delta^{13}\text{C}$ values
510 in consumers part-fuelled by biogenic CH₄ have been low and highly variable $\delta^{15}\text{N}$ values;
511 indeed, one of the most striking patterns to emerge from studies involving chironomids and
512 CH₄ is the strong, positive relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Grey et al., 2004a) which
513 appears to have some species-specific basis (Kelly et al., 2004). These relationships appear
514 consistent and widespread (Figure 3) and while most likely linked to assimilation of MOB, a
515 test of the potential mechanisms underpinning such low $\delta^{15}\text{N}$ values in consumer tissues is
516 currently lacking.

517 In Grey *et al.* (2004a), it was postulated that nitrogen within chironomid tubes may be
518 continuously cycled between the larva and microbial consortia; for example, chironomids
519 excrete nitrogen in the form of ammonium directly into their tubes and the overlying water
520 (Devine and Vanni, 2002), and via essential fractionation of ammonia, any microbial
521 community taking up that nitrogen source would be ¹⁵N-depleted (Macko et al., 1987, Ings et
522 al., 2012). More specifically, both Type I and II MOB can fix atmospheric N₂ into
523 ammonium and share similar pathways to oxidize ammonia/ammonium as autotrophic
524 ammonium oxidizing bacteria (Lee and Childress, 1994) and thus, are likely to exhibit
525 correspondingly low $\delta^{15}\text{N}$ values. However, ammonium oxidation rates are typically low and
526 high ammonium concentrations may inhibit CH₄ oxidation. In addition, some MOB can
527 convert nitrate back to N₂ and such denitrifying methanotrophs may outcompete other MOB
528 in nitrogen-rich, low oxygen environments (Stein and Klotz, 2011), which are characteristic
529 of many of the lakes where low $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in chironomids have been found (Jones
530 et al., 2008). To examine the underlying causal mechanisms for the strong, consistent, and
531 widespread relationship between chironomid $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, more research is required
532 to characterise the stable isotope values of potential nitrogen sources, to measure potential N-
533 fractionation by MOB, and to use complementary methods such as molecular biomarker
534 profiling (PLFAs and 16S rRNA genes) of chironomid gut contents.

535 While $\delta^{13}\text{C}$ values for dissolved CH₄ are relatively easily measured in the lab as well as in the
536 field nowadays, and hence are available from a wide range of aquatic environments, more
537 robust end-member values for MOB are required if we are to improve estimates for the
538 quantitative contribution of CH₄-carbon to total carbon budgets and production figures for
539 different ecosystems. To date, such estimates have relied on some of the earliest simple two-
540 source mixing models (i.e. only using one stable isotope: carbon) by applying a range of
541 trophic fractionation factors for MOB (reported from a very small number of laboratory
542 experiments) to values of CH₄ gas to derive one end-member. Direct measures of MOB $\delta^{13}\text{C}$
543 from aquatic environments are badly needed. Currently, it is possible to measure the $\delta^{13}\text{C}$ of
544 MOB-specific PLFAs extracted from aquatic sediments, but how these relate to the values
545 from whole MOB cells still needs to be established. More laboratory studies of how carbon
546 isotope fractionation between CH₄ and MOB may vary with different environmental and cell
547 growth conditions would be extremely useful, acknowledging that ‘controlling’ every
548 parameter even in the lab can be extremely difficult (e.g. Perga & Grey, 2010).

549 The geographic range of studies of MDC in food webs is still rather limited. Within
550 freshwaters, Jones et al. (2008) is the only paper to synthesise data from across a wide
551 latitudinal gradient and a distinct knowledge gap exists for the lower latitudes. Tropical
552 regions are responsible for approximately half of the estimated CH₄ emissions from
553 freshwater ecosystems to the atmosphere, although they have been consistently under-
554 sampled (Bastviken *et al.*, 2011). Indeed, the permanently stratified (meromictic) Lake
555 Kivu, within the western branch of the East African Rift, is one of the largest freshwater
556 reservoirs of dissolved methane (CH₄) on Earth. Given the relatively high magnitude of
557 MOB production integrated over the entire water column reported by (Morana et al.,
558 2015) (equivalent to 16–60% of the average photosynthetic primary production), and the
559 substantial contribution of MDC to the overall biomass in the oxycline, suggest that MOB
560 could potentially sustain a significant fraction of the pelagic food web in this lake. With
561 few exceptions (like Lake Kivu), it should also be noted that the majority of studies have
562 focussed upon relatively small stratifying stillwaters with strong oxygen gradients. The use of
563 MDC in river food webs – substantial quantities of CH₄ are oxidised in large riverine
564 systems, including the Amazon and the Hudson River (de Angelis and Scranton, 1993,
565 Melack et al., 2004) – may prove to be a more widespread and significant ecosystem process
566 than given credit at present (Trimmer et al., 2012). Whilst acknowledging that other
567 chemosynthetic processes tend to dominate in marine systems, the use of MDC at pelagic
568 boundaries, such as above the oxygen minimum zones of the various oceans, might well be
569 locally important (but over vast areas) to zooplankton as it is in stratifying lakes subject to
570 similar chemical gradients. There is very recent evidence for substantial oxidation of CH₄
571 within the water column above seeps off Svalbard, and carbon isotopic evidence that
572 atmospheric methane above those seeps is not influenced by contributions from the seafloor
573 source (Graves et al., 2015). Clearly then there must be MOB biomass accruing between the
574 sediment and the surface that could be incorporated into food webs, a pathway that is only
575 likely to increase in importance if gas hydrate destabilisation is promoted by warming of
576 bottom waters.
577

578 Analyses of long-term data series from lakes demonstrate that many are subject to increasing
579 average water temperature (Hampton et al., 2008, Schindler et al., 1990). While temperature
580 exerts a strong control on CH₄ efflux via the physiological stimulation of microbial
581 metabolism (Gedney et al., 2004, Yvon-Durocher et al., 2011), increasingly warm summer
582 surface water temperatures may also increase the duration of stratification, Schmidt stability
583 and hypolimnetic oxygen depletion (e.g. (Jankowski et al., 2006)), all of which will have
584 ramifications for CH₄ dynamics and the routing of MDC into biomass (Jones and Grey,
585 2011). Some limited yet tantalising empirical evidence for this arose from the physical
586 manipulation of the depth of the thermocline in a lake (compared to a nearby reference lake)
587 by installation of an impellor system (Forsius et al., 2010). As a consequence of deepening
588 the thermocline, the dominant fish species, perch (*Perca fluviatilis*) were observed to become
589 more ¹³C-depleted; a function of increased surface area of sediment adjacent to oxygenated
590 water ideal for chironomid uptake of MOB (route 3 in Figure 1), and the oxygenated water
591 allowing perch to forage on the benthos (Rask et al., 2010). Further manifestations of climate
592 change, such as an increase in both the frequency and severity of storms, could affect both the
593 strength and duration of stratification in lakes, and increase the flux of carbon from the
594 catchment. Not only might erosion from the terrestrial ecosystem provide the substrate for
595 methanogenesis in aquatic ecosystems (e.g. Sanders et al. 2007), but increased concentration
596 and use of dissolved organic and inorganic carbon in lakes and rivers (Schindler et al., 1997,
597 Evans et al., 2005, Jones et al., 2001, Worrall et al., 2004) will shift the balance toward

598 heterotrophic rather than autotrophic functioning. Stable isotope tracers will remain key to
599 unravelling the extent of MDC use in such food webs in future research.

600

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606

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967 Figure legends

968 Figure 1. Methane produced in anoxic sediments may be routed through plants (1) or lost
969 from the sediments to the atmosphere via ebullition or micro-bubbles (2). If it reaches a
970 boundary with oxygen at the sediment-water interface (under mixed or weakly stratified
971 conditions), MOB oxidise it and create biomass which routes via benthic macroinvertebrates
972 into benthic, pelagic, and terrestrial predators (3). Under strongly or permanently stratified
973 conditions, methane will diffuse upwards through the water-column, and oxygen (and MOB)
974 might first be encountered at the metalimnion, where zooplankton link MDC into higher
975 predators (4). An indirect route for MDC could be via CO₂ derived from the oxidation of CH₄
976 might then be cycled through phytoplankton, and hence on to zooplankton (5), or indeed via
977 sedimentation back down to benthic macroinvertebrates.

978
979 Figure 2. Stable isotope bi-plots of food webs across a spectrum of point to diffuse sources of
980 methane with corresponding decrease in strength of $\delta^{13}\text{C}$ value as a tracer of methane-derived
981 carbon (MDC); blue boxes indicate components of the food web with small / negligible
982 influence of MDC. (a) Gulf of Alaska (redrawn with permission from Levin and Michener,
983 2002): solid symbols – pogonophoran field infauna; open symbols – clam field infauna. (b)
984 Plußsee (strongly stratifying small lake, data from Harrod and Grey, 2006): open circles –
985 fish; solid circles – macroinvertebrates; open squares – chironomid larvae. (c) Loch Ness
986 (weakly stratifying large lake, data from Jones and Grey 2011): open circles – fish; solid
987 circles – invertebrate & basal resources; open squares – chironomid larvae.

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990 Figure 3. Stable carbon and nitrogen isotope ratios of benthic chironomid larvae collected
991 from stratifying lakes in Germany, England and Finland (data derived from Grey *et al.*
992 (2004a&b), Deines *et al.* (2007a), Ravinet *et al.* (2010). Individuals were collected from a
993 specific depth in each lake and on one date (except for Holzsee where the data are compiled
994 from 12 sampling events in one year). Species are *Chironomus plumosus* (filled black
995 markers, solid line), *Chironomus anthracinus* (filled grey markers, dashed line),
996 *Prosilocerus jacuticus* (Jyväsjärvi only; open triangle, dashed line) and *Chironomus*
997 *teniustylus* (Halsjärvi only; open marker, dashed line). Lines are least squares regressions for
998 illustrative purposes only.

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