



## **Evidence for methane-subsidised secondary production in a groundwater-fed lowland river.**

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We are probably familiar with the chemosynthetic ecosystems of the deep Pacific, where life in the dark is coupled to the oxidation of sulphur from 'black smokers' rather than the sun, but few, if any, would suspect such a mode of life in the classic chalk rivers of southern England. We measured the  $\delta^{13}\text{C}$  values of dominant primary consumers and their potential food sources in a groundwater-fed lowland river. The  $\delta^{13}\text{C}$  of most consumers, such as Gammarus and Simulium, reflected that of the dominant forms of photosynthetic production, whereas the cased larvae of two caddis flies (Agapetus and Silo) were consistently  $^{13}\text{C}$ -depleted throughout the year. The river water was supersaturated (50-60 times atmospheric) with methane, reflecting both supersaturation in the groundwater and local production in fine sediments. We measured significant rates of methane oxidation, which generates  $^{13}\text{C}$ -depleted organic carbon, in the biofilms on gravel, on the caddis fly cases, and on the bottom of larger rocks. In addition, there was a marked difference in the ratio of methane oxidising potential to chlorophyll a across those substrata. This ratio was below detection in the biofilm (i.e. no methane oxidation) on the tops of rocks, greater on the bottom of rocks, and maximal for the gravels and the caddis cases. If the caddis larvae acquire most of their carbon by grazing the tops of such rocks (where they are normally found), then they must acquire their depleted  $\delta^{13}\text{C}$  values by occasionally grazing biofilm where the ratio of methane oxidation to chlorophyll was much greater, and the most likely candidate is from their own cases. Grazing methane oxidising bacteria could provide the caddis larvae with up to 30 % of their carbon, which could represent a true subsidy from an ancient groundwater source.