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The effect of water velocity on stable carbon and nitrogen isotope signatures of periphyton

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Abstract

Water movement affects carbon and nitrogen isotopic signatures of algae, possibly through effects on boundary-layer thickness. We describe a laboratory experiment carried out in artificial streams, which supports this hypothesis. Periphyton $\delta^{13}C$ and $\delta^{15}N$ signatures were significantly depleted in ^{13}C and ^{15}N when grown at higher water velocity over the range of 5–62 cm s^{-1}, the normal range found in small streams. $\delta^{13}C$ signatures ranged between −16.7‰ and −28.1‰ from the lowest to the highest water velocity, respectively. Similarly, $\delta^{15}N$ signatures ranged between 7.2‰ and 2.3‰ from the lowest to the highest water velocity, respectively. This pattern was found for algal communities growing on glass (mainly diatoms) as well as for those growing on rock (mainly filamentous green algae). For both C and N, the slopes of the responses were not different between the periphyton communities growing on each substrate, although the effect was statistically weaker for the communities on rocks. The intercept for $\delta^{13}C$ was significantly higher for the communities on rocks, but not different for $\delta^{15}N$. Thus, while both isotopes are fractionated to a greater extent as velocity increases, the diatom communities growing on glass appeared to fractionate C isotopes more than the filamentous green algae growing on rock. This relationship between the stable isotopic signatures of aquatic plants and water velocity will hopefully allow a better understanding of the differences in isotopic signatures of fish in different habitats.

Stable carbon and nitrogen isotopes ($\delta^{13}C$ and $\delta^{15}N$) are increasingly used in ecology to study food-web structure and functions. The influence of abiotic factors, such as water velocity, on the isotopic signatures of algae is of particular interest because algae are at the base of the food web, and such factors can indirectly influence the signatures of primary and secondary consumers. In order to properly interpret isotopic signatures of invertebrates and fish, it is therefore necessary to understand how physical factors affect the signatures of primary producers.

Field studies on aquatic plants have generally reported $\delta^{13}C$ values enriched in ^{13}C (lower degree of isotopic fractionation) in low-energy lentic systems than in high-energy lotic systems (Keely and Sandquist 1992; Hecky and Hesslein 1995). In low-energy environments, boundary layers of aquatic plants are thicker due to more stagnant water. This results in lower diffusion rates and reduced isotopic discrimination against the heavier ^{13}C. Moreover, phytoplankton, which normally live in open-water turbulent environments, generally exhibit $\delta^{13}C$ values depleted in ^{13}C (higher degree of isotopic fractionation) than littoral benthic algae (Hecky and Hesslein 1995). Laboratory experiments have shown that $\delta^{13}C$ values for individual algal species were decreased under conditions of high turbulence (Degens et al. 1968; France and Holmquist 1997). Osmond et al. (1981) also found that freshwater macrophytes from low-energy lakeshore sites had $\delta^{13}C$ values enriched in ^{13}C compared with the same species collected in fast-moving water. Therefore, $\delta^{13}C$ signatures of aquatic plant tissues appear to be influenced by the extent of mixing between inorganic carbon in boundary layers and that of the surrounding water.

This effect also appears to influence the $\delta^{13}C$ signatures of whole food webs. In a comparative study, Finlay et al. (1999) found a strong negative relationship between water velocity and herbivore $\delta^{13}C$ signatures, which reflect algal $\delta^{13}C$ signatures, in productive rivers. This result supports the idea that boundary-layer thickness has a strong influence on the supply rate of inorganic carbon, namely CO_2, which diffuses slowly in water, affecting the baseline signature that is passed along to consumers in the community.

Nitrogen isotopic signatures of primary producers are known to vary greatly among and within systems over time (Cabana and Rasmussen 1996). Nitrogen, like phosphorus, is recognized as a limiting factor to aquatic algal growth and its availability is positively related to algal growth rates and $\delta^{13}C$ signatures of aquatic plants (Gu et al. 1999). Unfortunately, there is much less information on nitrogen isotopic fractionation in relation to water movement. Although, if the boundary-layer/diffusion hypothesis is valid, one might predict that the effect of water velocity on $\delta^{15}N$ signatures of algae would be generally similar to that of $\delta^{13}C$ signatures.

In contrast, some field studies have yielded data that conflict with the general trend that fractionation increases with water velocity. MacLeod and Barton (1998), in a comparative study, found that isotopic fractionation of both carbon and nitrogen isotopes in periphyton was strongly influenced by light intensity and temperature, which affect the rate of metabolic activity, but detected no effect of current velocity. Also, France and Cattaneo (1998), contrary to what they expected, found a positive relationship between the $\delta^{13}C$ of benthic algae and water motion. They suggest that photoassimilation of respired carbon with low $\delta^{13}C$ value, which is

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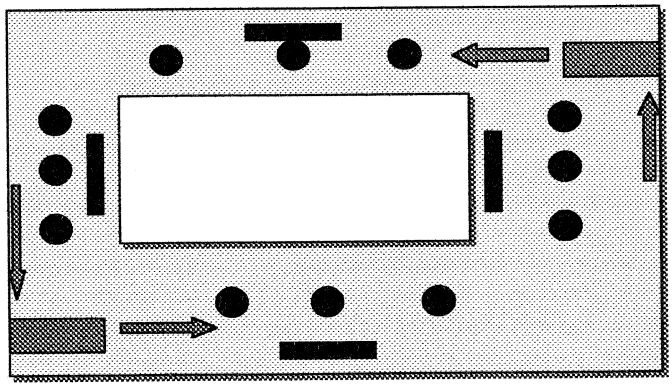

Fig. 1. Artificial stream set up. Water was circulated between the walls of a large 40-liter aquarium and a small 10-liter aquarium; the small aquarium being placed inside the large one. The two dark gray rectangles represent aquarium pumps (six artificial streams had only one pump and three had two pumps), the arrows represent the direction of flow, the circles represent rocks, and the four black rectangles each represent a set of four glass slides.

generated from microbial decomposition of mainly terrestrial organic matter, confounded the effect of water motion on boundary-layer thickness.

To date, no controlled laboratory experiment testing the effect of water velocity on $\delta^{13}C$ and $\delta^{15}N$ values of attached algae has been done. In our experiment, the primary goal was to grow attached algae under a range of current velocities similar to those found in natural streams to determine if water velocity has a significant effect on the carbon and nitrogen signatures of periphyton. We hypothesized that $\delta^{13}C$ and $\delta^{15}N$ signatures would be depleted in ^{13}C and ^{15}N, respectively, as the velocity regime that they were grown under increased. That is, at low water velocities, the algae should be less isotope selective due to nutrient depletion in the boundary layer, meaning that the enzymatic bias against heavy isotopes (^{13}C and ^{15}N) would be weaker, making $\delta^{13}C$ and $\delta^{15}N$ signatures enriched in ^{13}C and ^{15}N. Conversely, at high water velocities, we would expect the algal boundary layer to be less nutrient-depleted, permitting algae to express their enzymatic bias against heavy isotopes, leading to signatures depleted in ^{13}C and ^{15}N similarly to those normally seen in phytoplankton.

Methods

To test our hypothesis, we collected cobble-sized rocks (5–20 cm) during the winter of 2001 and allowed algal communities to develop in an artificial laboratory stream environment. The rocks were obtained from a small stream associated with the Lachine canal system (Ville LaSalle, Quebec) near the St. Lawrence River and transported back to the laboratory in a cooler of stream water, which was kept for 3 d in a 10°C cold room before the beginning of the experiment.

A total of nine artificial streams were used in the experiment (Fig. 1). Each consisted of a large aquarium (40-liter maximum capacity) wherein water was circulated using aquarium pumps (AquaClear Powerhead pumps with a maximum output capacity of 17 L min^{-1}). A small aquarium (10-liter maximum capacity) was placed in the center of each large aquarium in such a way that the pump would circulate the water around the smaller inside aquarium. Each artificial stream had four different water velocities depending on the degree of constriction of the flow (Fig. 1). Dechlorinated tap water originating from the St. Lawrence River was used for the experiment. Rocks were placed as evenly as possible in all the aquariums. A set of two neon lights was also placed about 1 foot over the top border of each aquarium to help the algae grow faster. In addition to the rocks, four sets of four frosted glass slides were placed vertically in the water of each aquarium; one set on each side of each aquarium for a total of 16 slides per aquarium. They were placed parallel to the current so that they did not obstruct the flow and the algae could attach to them easily. Because of the low temperature of the stream from which the rocks were collected (approximately 4°C) and the low light intensity at this time of the year, the periphyton growth on the rocks at the beginning of the experiment constituted no more than a thin slime layer.

For six of the artificial streams, only one pump was used per aquarium. The water velocities at different sites within these ranged from 5 cm s^{-1} to 35 cm s^{-1} depending on the degree of constriction of the current and the water level. In the other three aquariums, two pumps were used in order to achieve higher velocities. The water velocities then ranged from 12 cm s^{-1} to 62 cm s^{-1}, thus expanding the range of water velocities and increasing the chances of detecting significant effects on periphyton signature. Every 2–3 d, water was added in the aquariums to keep the water level constant (a total of about 1–2 liters of water $week^{-1}$ $aquarium^{-1}$) so that water velocity was kept constant throughout the whole experiment.

Macroscale measurements of water velocities, comparable with those carried out in field studies, were done using a Gurley Pygmy current meter (625DF8N—Wading Rod Suspended Pygmy type current meter outfit with Model 1100 digital indicator). The current meter was placed at a standard distance (3 cm) in front of the algae to be sampled. Measurements were repeated twice to ensure reliability.

Variables known to affect photosynthesis were kept constant as much as possible. Water temperature remained relatively constant at about 18.5°C throughout the whole experiment. Light intensity was also constant and the photoperiod was the same for all the aquariums as the algae were exposed to light 24 h a day throughout the whole experiment. The addition of 1–2 liters of water per week to offset evaporation also ensured a constant supply of essential nutrients, such as nitrogen and phosphorus, so that growth and photosynthetic rate were not affected.

The periphyton communities that grew on rock substrate in the laboratory consisted mainly of *Cladophora* sp. (Chlorophyta; a filamentous green algae) and some *Melosira* sp. (Bacillariophyceae; a diatom). In addition to these, although much less abundant, were a variety of pennate diatoms, *Tolypothrix* (Cyanophyta), and *Mougeotia* (Chlorophyta). The communities that grew on glass substrate contained a similar species composition as the communities growing on rocks, although they were dominated by diatoms (mainly *Melosira*)

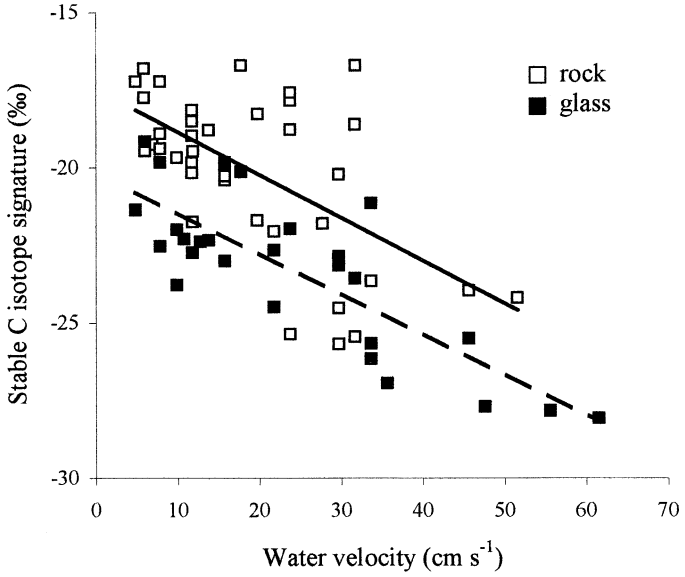

Fig. 2. The effect of water velocity on the $\delta^{13}C$ signature of periphyton growing on rock and glass substrates. The solid linear regression is for algae growing on rock substrate and the dotted one is for algae growing on glass substrate.

rather than by green algae. All these species are typically found in freshwater streams and rivers (Round 1970).

The periphyton were sampled after 6 weeks of growth. The algal communities on rocks (>90% *Cladophora*), which reached 2–3 cm in length, were trimmed with scissors and retained for analysis. In a few cases, some long and thin filaments (5 cm or more) extended downstream into a different velocity regime. When this occurred, we snipped off the ends of the long filaments and retained only the basal 2–3 cm, ensuring that the sample was associated with the corresponding current measurement. It should be noted, however, that this procedure would have had a negligible influence on the isotopic signature of these samples because the tips of the few long filaments that were excluded would have made up a very small fraction of the sample weight. The algal communities growing on glass slides (>95% *Melosira* sp.) were removed by scraping with another glass slide. The samples of algae were then partially dried with Kimwipes® and placed into microfuge tubes. The closed microfuge tubes containing the samples were then lyophilized (freeze dried). Each sample was pulverized and placed into a tin capsule (between 1.5 and 2.0 mg of sample per capsule) for analysis by mass spectrometry (Finnigan-Mat Delta-Plus continuous flow isotope-ratio mass spectrometer coupled to a Carlo-Erba elemental analyzer on line; G. G. Hatch Isotope Laboratories, University of Ottawa). The analytical precision of that instrument is typically one standard deviation for carbon and nitrogen and is in the range of 0.05–0.2‰, which is small relative to the range of values that occur in nature (Kendall and Caldwel 1998).

Results

The $\delta^{13}C$ values for the periphyton growing on rock substrate ranged from −16.7‰ to −25.7‰ and from −19.2‰ to −28.1‰ for those growing on glass substrate. The $\delta^{15}N$ values for the periphyton growing on rocks ranged from 2.4‰ to 7.1‰ and from 2.3‰ to 7.2‰ for those growing on glass. The ranges of periphyton signatures obtained are similar to those found in other unpolluted, freshwater environments ($\delta^{13}C$ signature range of −12‰ to −30‰, Boutton 1991; −15‰ to −27‰, France 1999; and $\delta^{15}N$ signature range of 1.5‰ to 7‰, MacLeod and Barton 1998). Because the nitrogen content of five samples was too low to give reliable $\delta^{15}N$ signatures, they were removed from the N stable isotope analysis to ensure that they did not bias the results.

Table 1. Statistics of the linear regressions between water velocity and the stable isotopes studied ($\delta^{13}C$ and $\delta^{15}N$) for periphyton growing on rock and glass substrates (SE, standard error).

Substrate	Isotope	Slope (±SE)	Intercept (±SE)	r^2	P	n
Rock	^{13}C	−0.14±0.03	−17.5±0.7	0.36	<0.001	39
	^{15}N	−0.055±0.015	6.4±0.3	0.28	0.001	38
Glass	^{13}C	0.13±0.02	−20.2±0.5	0.66	<0.001	27
	^{15}N	−0.066±0.014	6.7±0.4	0.53	<0.001	23

As expected, the linear regression reveals that the $\delta^{13}C$ signatures tended to decrease as water velocity increased ($P < 0.001$, Fig. 2). The strength of the relationship (r^2) between water velocity and the $\delta^{13}C$ signatures of the algae on glass substrate was almost twice that observed for the algae on rock substrate (Table 1). While the slopes of the $\delta^{13}C$ signatures versus velocity relationships were not different on rock and on glass substrate ($P = 0.85$), the intercepts were different ($P < 0.001$) with $\delta^{13}C$ signatures on rock substrate being enriched in ^{13}C by on average 2.7‰ at the same velocity compared with those on glass substrate (Table 1). In the same way, the linear regression in Fig. 3 shows that $\delta^{15}N$ signatures also tended to decrease as water velocity increased ($P < 0.001$) and the r^2 values were again two times that of the communities growing on glass substrate (Table 1). For $\delta^{15}N$, however, the slopes and intercepts did not differ between glass and rock substrates as they did for $\delta^{13}C$ ($P = 0.57$ and $P = 0.84$, respectively).

Discussion

Our results support the hypothesis that increased water velocity leads to $\delta^{13}C$ and $\delta^{15}N$ signatures depleted in ^{13}C and ^{15}N for periphyton. There is no indication of a maximum water velocity at which the algal $\delta^{13}C$ and $\delta^{15}N$ values stop decreasing. Although, if water velocities were increased beyond the range studied here, saturation might be expected because fractionation of $\delta^{13}C$ had nearly reached the maximum levels normally observed in phytoplankton and terrestrial plants.

There are at least three possible explanations for the $\delta^{13}C$ signatures enriched in ^{13}C on rock versus glass substrate. The periphyton mat that developed on rock substrate, which mainly consisted of the thick filamentous green algae *Cladophora*, was much thicker than on glass substrate, which consisted mainly of a thin layer of the diatom *Melosira*. It

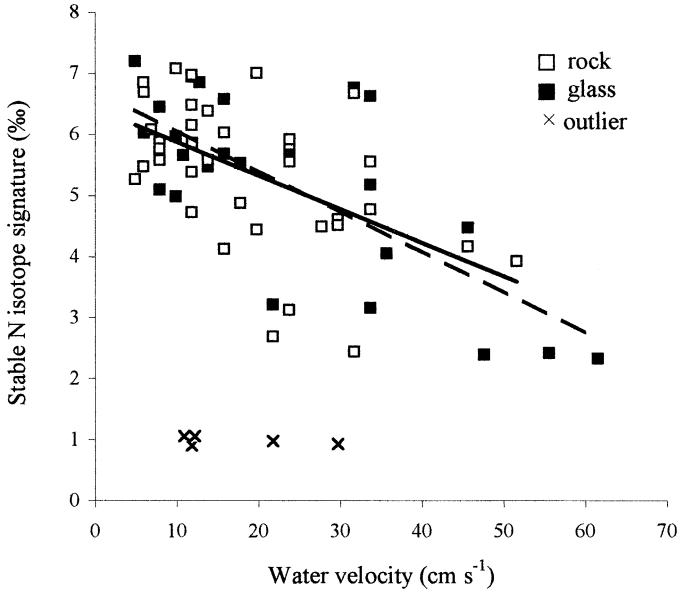

Fig. 3. The effect of water velocity on the $\delta^{15}N$ signature of periphyton growing on rock and glass substrates. The solid linear regression is for algae growing on rock substrate and the dotted one is for algae growing on glass substrate. The outliers are represented by x symbols.

would thus seem reasonable that boundary-layer depletion is more pronounced around the thicker periphyton mat growing on rocks compared with on glass, especially for the inner layers of the algae mat. This explanation is consistent with the gas exchange and isotope ratio measurements carried out on attached algae by Raven et al. (1982). The curved and irregular surface of rocks may also have contributed to the reduced fractionation by algae on rock substrate compared with on glass substrate. Only at the very top of a curved rock does the algal community experience the same water velocity that a similarly placed flat surface would experience, and surfaces facing downstream experience lower velocities.

For algae, low diffusion resistance and the use of CO_2 as an inorganic carbon source are usually associated with $\delta^{13}C$ values depleted in ^{13}C. Conversely, high diffusion resistance, CO_2 depletion, and the use of HCO_3^- are associated with $\delta^{13}C$ values enriched in ^{13}C (Raven et al. 1982). Under low turbulent conditions, boundary-layer thickness is augmented, causing greater diffusion resistance of CO_2 and HCO_3^-, hence reduction of isotopic fractionation, which leads to $\delta^{13}C$ signatures enriched in ^{13}C (Maberly et al. 1992). According to Sharkey and Berry (1985), the depletion of CO_2 results in signatures enriched in ^{13}C by reducing carbon isotope discrimination. Different algae species present in a community may also assimilate preferentially different forms of dissolved inorganic carbon (DIC) and respond differently to CO_2 depletion (Falkowski 1990). Consequently, reliance on CO_2 and/or HCO_3^- as a carbon source might explain some of the variability that we observed in the isotope signatures. HCO_3^- is known to reduce the discriminating effect of RUBISCO, which is potentially the major discriminating factor in carbon fixation. The communities of algae growing on rock substrate in this experiment may have a greater ability to use HCO_3^- as a carbon source compared with the communities growing on glass substrate. This may explain why the relationships between velocity and $\delta^{13}C$ signatures of periphyton communities on rock and glass substrates were significantly different ($P < 0.001$).

There are different possible explanations for the residual variability in the relationship between $\delta^{13}C$ and $\delta^{15}N$ signatures and macroscale water velocity measurements. Temporal and spatial variation in velocities and boundary conditions on the microscale not captured by our velocity measurements are probably the most important sources of residual variation. This is supported by the fact that the residual variability is reduced by almost half on glass substrates, which are flat and spatially homogeneous surfaces, compared with cobble-size rock substrate. This difference in residual variability was especially pronounced at midrange velocities, which suggests that the relationship between macro and microscale hydrodynamics is weakest at medium velocities for irregular substrates. It is reasonable to suggest that the filamentous algae experienced greater microscale variation in velocity compared with the diatom layer in two ways. First, the mat of filamentous algae was very thick relative to the thin layer of diatom-dominated communities. Thus, there must be greater microscale variation in velocity from the inner to the outer layers of the filamentous algae, while the diatom communities are probably not exposed to such variation. Second, the curved and irregular surface of rocks must also cause greater microscale variation in velocity around the periphyton on rock substrate compared with on glass substrate. The thickness/biomass of the algal layer might also explain some of the residual variability in the signature data through local differences in the flux (uptake) of C and N, and possibly physical properties of the boundary layer.

Microscale variation in light intensity may be another factor accounting for some of the residual variability in the data. While there were no significant differences in the light intensity measured in the different aquariums, there were probably local differences from rock to rock resulting from partial shading and differences in the angle of orientation of the light hitting the substrates. There was probably some local variation in light intensity around the glass substrates as well, however, to a lesser extent than around rocks. Thus, some of the variability of isotope signatures among sample points and between communities on rock and glass substrates could have resulted from local differences in the light regime experienced by the algae. We did not attempt to account for the angle of orientation of the algal samples taken from rocks. It is also possible that the constant light regime used in this experiment may have affected the signature patterns we observed by exacerbating nutrient depletion, thus reducing carbon isotopic fractionation (Gu et al. 1999).

MacLeod and Barton (1998) found that seasonal differences in temperature influenced $\delta^{13}C$ and $\delta^{15}N$ signatures. In the present experiment, local temperature differences, if any, were relatively very small compared with seasonal ones. France (1999) found that lake DOC (dissolved organic carbon) concentration partially explained the variability in epiphyton $\delta^{13}C$ and $\delta^{15}N$ signatures. In our experiment, we only

used well-mixed, recirculated tap water low in DOC. Thus, none or insignificant amounts of residual variability in the signatures found in our study should be attributed to spatial variation in temperature and DOC concentration.

Other experiments during the past few decades have revealed that $\delta^{13}C$ variations are also related to algal differences in photosynthesis mechanisms, rates of primary production (Wienke and Fisher 1990; Fry and Wainright 1991), species composition (Fry 1984), and possibly changes in algal cell size (Gu et al. 1999). It would be interesting to test these factors separately in a controlled fashion to determine their importance relative to the $\delta^{13}C$ and $\delta^{15}N$ signatures of aquatic plants.

The majority of field studies on algae done in Canadian well-mixed streams, rivers, and lakes show that the range of variation normally encountered is of about 10‰ for C and 5‰ for N (MacLeod and Barton 1998; France 1999). Our regressions explain a range of variation of 6–7‰ for C and 3–4‰ for N across a velocity range normally found in natural lotic environments, which is a substantial fraction of the variation normally encountered. Our experiment controlled for, however did not manipulate, other factors that have been recognized to significantly influence $\delta^{13}C$ and $\delta^{15}N$ signatures. Though it is not our intention to downplay other factors, we believe that water velocity is probably as important as other abiotic factors, such as DOC, temperature, and light intensity, in determining algal $\delta^{13}C$ and $\delta^{15}N$ signatures.

This experiment allows us to conclude that water velocity, over the range commonly encountered in rivers and streams, does in fact have an effect on carbon and nitrogen signatures of periphyton as hypothesized. In agreement with the first prediction, carbon and nitrogen signatures tend to decrease as water velocity increases. This experiment by no means explains all of the variability we observed in isotope signatures. However, it suggests that further study of periphyton signatures in flowing water may prove rewarding in many ways because these signatures seem to reflect important underlying processes and are passed on to benthic invertebrates and fish through the food web, thus providing potentially useful habitat tracers. In fact, several field studies on drift-feeding salmonids have reported that fish from fast-water habitats have $\delta^{13}C$ signatures depleted in ^{13}C compared with those from slower waters (Finlay et al. 1999; Finlay et al. 2002; Morinville and Rasmussen 2003). France (1994) argued that isotopic signatures have been used uncritically in many field studies. Thus, there is a great need to understand the factors, such as water velocity, that determine C and N isotopic signatures of attached algae and the extent to which these influences are transmitted up the food web.

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