Short Research Note

Spatial changes in carbon and nitrogen stable isotopes of the plankton food web in a saline lake ecosystem

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Abstract

We investigated spatial changes in the isotope ratios of the plankton food web in Lake Chany, Siberia, Russia, especially at an estuarine transition zone of the lake. The δ^{13} C values of particulate organic matter (POM) varied among the sampling sites, and increased with increasing pH of the lake water. This may reflect a shift by phytoplankton from using CO₂ to using bicarbonate for photosynthesis with increasing pH. The δ^{13} C values of zooplankton community also changed at each site along with those of the POM. This was indicative of carbon isotope changes of plankton food webs between the stations along an environmental gradient.

Introduction

Carbon and nitrogen stable isotope ratios have been increasingly used to analyze food web structures in lake ecosystems, with most studies focusing on a single water body as a whole-lake (e.g., Yoshioka et al., 1994; Zohary et al., 1994). Recently, stable isotope analyses have demonstrated that the food web structure in a lake can be separated both horizontally and vertically (e.g., Bootsma et al., 1996; Harvey & Kitchell, 2000; Vadeboncoeur et al., 2003).

Carbon isotope ratios of the producers vary widely in freshwater ecosystems along environmental and physiological gradients (e.g., Zohary et al., 1994; Fry, 1996; Grey et al., 2000; Doi et al., 2003). In large shallow lakes, the base resources of food webs such as phytoplankton are predicted to be spatially different even in each region, due to limitations of water movement and geomorphological complexity. An understanding of stable isotope baseline variations is very important for food web studies. However, spatial variations in isotopic signatures of producers and consumers in lake systems have not been adequately demonstrated.

Lake Chany is a large and shallow inland, moderately saline system. Lake Chany is an enclosed water body, in which several environmental gradients, such as salinity, change horizontally between the inflowing river and the end of the lake due to no-outflowing structure of the lake and the evaporation of the lake water (Doi et al., 2004). In the estuarine zone of the shallow lake, we hypothesized that the stable isotope values of the producers and consumers may be spatially different due to environmental gradients and limitations of water movement. In the present study, carbon and nitrogen isotope ratios in particulate organic matter (POM; predominantly phytoplankton) and zooplankton were measured from the inflowing river to the end of the lake in order to demonstrate spatial differences in isotope baseline of the planktonic food web in the estuarine part of the saline lake along an environmental gradient. We analysis the trophic relationships between zooplankton using the isotope enrichment factors for carbon and nitrogen isotopes (DeNiro & Epstein, 1978; Minagawa & Wada, 1984). In addition, we measured the isotope signature of plankton at the far end of Lake Chany as an environmental extreme relative to the estuary.

Methods

Lake Chany is in the Novosibirsk region in the Barabinskaya lowland of Western Siberia ($54^{\circ} 30'$ $-55^{\circ} 09'$ N, $76^{\circ} 48'-78^{\circ} 12'$ E) at an altitude of 106 m above sea level. This lake is a shallow, inland, and saline system (average depth, 2.2 m; maximum depth, 8.5 m) characteristic of the Western Siberian forest-steppe (Aladin & Plotni-kov, 1993; Doi et al., 2004). Lake Chany is an enclosed water body comprised of three lakes,

Bolshye Chany, Malye Chany, and Yarkul, which are connected by small channels (Fig. 1). In 2002, we selected five study sites: the riverine part of the Kargat River (Station 1), the upper estuarine part of the Kargat River (Station 2), the lower estuarine part of the Kargat River (Station 3), Lake Malye Chany (Station 4), and the far end of Lake Bolshye Chany (Yarkov Pool; Station 5) (Fig. 1).

Water temperature (WT), pH, electrical conductivity (EC), and dissolved oxygen (DO) of the surface water were measured at the study sites in 9-13 August 2002 using a multiple water quality sensor (U-22, Horiba Co.). Chlorophyll *a* concentrations in the surface lake water were measured in five replicates using a fluorescence chlorophyll *a* meter (Aqua-flow, Turner Designs Co.).

All sampling was conducted in 9–13 August 2002. Particulate organic matter (POM) samples at the surface water of each site were obtained by filtering surface lake water through Whatman GF/F glass fiber filters (precombusted at 500 °C for 2 h) with 3 replicates. POM samples were treated with 1 mol 1⁻¹ HCl to remove bicarbonate prior to isotope measurements. Zooplankton was collected with 250 μ m mesh plankton nets at the surface water of each site with 3 replicates, sorted under a stereomicroscope, and their lipids were extracted



Figure 1. Map of the Lake Chany complex.

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using a 2:1 (wt%) chloroform:methanol mixture. Terrestrial plants (Betula platyphylla) and marsh plants (Phragmites australis) were collected near Station 1. All samples were dried in an oven at 60 °C and then kept frozen until the isotope analyses were conducted.

The isotope ratios of zooplankton were measured using whole individuals. Carbon and nitrogen isotope ratios of the samples were measured with a mass spectrometer (DELTA plus, Finnigan Mat) connected to an elemental analyzer (NA-2500, CE Instruments). The results are reported in the delta notation as below:

$$\delta^{13}$$
C or δ^{15} N = $(R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000(\%)$

where *R* is the ${}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$ ratio for $\delta^{13}C$ or δ^{15} N, respectively. PeeDee Belemnite was used as a standard for δ^{13} C, and air N₂ was the standard for δ^{15} N. The analysis errors were within $\pm 0.2\%$ for both δ^{13} C and δ^{15} N.

Results

1.5

Water temperature and dissolved oxygen showed no outstanding trends from the river (Station 1) to the far end of Lake Bolshye Chany (Station 5) in Table 1. However, pH tended to increase from Station 1 (pH = 7.1) to Station 5 (pH = 8.7). Of all the sites, Station 5 had the highest values of electrical conductivity (EC) with 1.15 mS m^{-1} . EC at Stations 1-4 had low values ranging from 0.11 to 0.14 mS m^{-1} . The difference between Station 5 and Stations 1-4 reflects the evaporation of water and an accumulation of salt (Doi et al., 2004). Chlorophyll *a* showed higher values at the low EC stations (Stations 1-4).

For POM, the δ^{13} C values at Stations 1–3 ranged from -32.2 to -28.6% (Fig. 2), and decreased significantly from Station 1 to Station 3 (multiple comparison Holm test, p < 0.01, n = 3). δ^{15} N values of POM at Stations 1-4 ranged from 0.8 to 3.5%, and tended to decrease from Station 1 to Station 4. At Lake Bolshye Chany (Station 5), the δ^{13} C value of POM (-23.7 ± 0.2%) was significantly higher by 4.7-8.5% than at the other four stations (Fig. 2), and the $\delta^{15}N$ value of POM $(7.4 \pm 1.1\%)$ was significantly higher by 3.5–6.8‰ (Holm test, p < 0.01, n = 3). Plants were also collected to show the isotopic signatures of terrestrial and marsh biota, and fell within the general range of C₃ plants (δ^{13} C: -30 ~ -26%; France, 1995).

For zooplankton species, at Stations 1-4, the δ^{13} C values of herbivores *Ceriodaphnia* spp., *Sida* crystallina crystallina and Daphnia longispina were similar to those of POM (Fig. 2). The isotope map showed the δ^{13} C values of herbivorous and carnivorous zooplankton paralleling those of their respective POM (Fig. 2). At Station 2, The δ^{15} N value of *Leptodora kindtii* $(7.3 \pm 0.1\%)$ was higher than that of *D. longispina* $(4.3 \pm 1.0\%)$; Holm test, p < 0.05, n = 3). At Station 3, The δ^{15} N values of L. kindtii $(7.2 \pm 1.4\%)$ and Eudiaptomus graciloides $(6.1 \pm 0.2\%)$ were higher than those of S. crystallina crystallina and D. longispina $(4.4 \sim 4.6\%)$; Holm test, p < 0.05, n = 3). At Station 4, the δ^{15} N values of L. kindtii (7.3 ± 1.0%), *E.* graciloides $(7.3 \pm 0.4\%)$, and *Bythotrephes longimanus* (8.0 \pm 0.3%) were higher than those of D. longispina $(4.4 \pm 0.6\%)$; Holm test, p < 0.01, n = 3). At Station 5, the δ^{15} N values of Cerio*daphnia* sp2 $(9.2 \pm 0.5\%)$, mean ± 1 SD, n = 3) were lower than *Diaptomus salinus* (13.7 ± 0.9) ; Holm test, p < 0.05, n = 3). Daphnia magna had an intermediate δ^{15} N value (11.5 ± 0.5%).

Discussion

The δ^{13} C values of herbivorous and carnivorous zooplankton paralleling those of their respective

Table 1. Water temperature (WT), pH, electrical conductivity (EC), dissolved oxygen (DO) and Chlorophyll a (Chl a) at five stations in Lake Chany. Chl *a* is reported as mean ± 1 SD (n = 5)

Station	WT (°C)	pH	EC (mS m^{-1})	DO (mg l^{-1})	Chl <i>a</i> (μ g l ⁻¹)
1	18.4	7.1	0.12	4.7	13.7 ± 0.2
2	20.6	7.6	0.11	8.4	26.2 ± 0.5
3	20.6	8.0	0.12	4.9	13.4 ± 0.4
4	21.5	8.3	0.14	11.0	17.9 ± 0.6
5	19.0	8.7	1.15	8.4	1.6 ± 0.1



Figure 2. Carbon and nitrogen isotope plots of samples from Lake Chany. Each symbol represents a mean value and error bars are ± 1 SD (n = 3).

POM, and the $\delta^{15}N$ values of these two zooplankton were higher by 1.1-1.3% than those of the POM, indicating that these species were primary consumers that fed on POM. The $\delta^{15}N$ values of Leptodora kindtii and Eudiaptomus graciloides were 1.5-2.7% higher than those of S. crystallina crystallina and D. longispina. Therefore, L. kindtii and E. graciloides were considered secondary consumers. In general, E. graciloides is considered to filter feeder, and based on our data, E. graciloides may be a secondary consumer that filter-feeds on small primary consumers such as protozoa and their detritus. At Station 5, the $\delta^{15}N$ values of POM and zooplankton species increased in the following POM < *Ceriodaphnia* order: sp2 < Daphnia magna < Diaptomus salinus. Thus, it is clear that Ceriodaphnia sp2 is a primary consumer, and D. salinus is a secondary consumer that

filter-feeds on small primary consumers and their detritus. *D. magna*, which had an intermediate δ^{15} N value among the three zooplankton species, may be an omnivore that feeds on both POM and small primary consumers.

The δ^{13} C values of herbivores were 2–4‰ lower than those of the POM at each station. The δ^{13} C of animals directly reflects their diet with only slight enrichment (<1‰) during the feeding process (DeNiro & Epstein, 1978). However, the ¹³C-depletion (0 to -4‰) of grazer zooplankton relative to POM has also been reported in many recent studies on the pelagic food webs of freshwater lakes, because of their selective feeding and assimilation of POM (e.g., Zohary et al., 1994; Grey & Jones, 1999; Grey et al., 2000). Thus, the ¹³C-depletion of the herbivores may be due to their selective feeding and assimilation as the previous studies.

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In general, when the phytoplankton biomass increases or the supply of dissolved inorganic carbon (DIC) is limited, consequently, the δ^{13} C values of phytoplankton increase due to the reduced isotope fractionation during photosynthesis (Fry, 1996). The pH of the lake water varied from 7.1 (Station 1) to 8.7 (Station 5; Table 1), and we found a significant positive correlation between δ^{13} C values of POM and pH of the lake water, except at Station 1 $(r^2 = 0.885, p < 0.001,$ n = 12, Fig. 3). In this pH range, the relative abundance of each molecular and ionic state of carbon dioxide changes drastically. At pH of 7, 8, and 9, the proportion of dissolved CO_2 gas is 20.8, 2.5, 0.3%, respectively, while the proportion of bicarbonate increases over this pH range (Wetzel, 2001).

A difference in DIC species generally produces considerable variation in the fractionation factor



Figure 3. Relationships between carbon isotope values of plankton and pH of the lake water at each site. POM is represented by open squares. Consumer zooplankton (black circles) are Daphnia longispina, Sida crystallina crystallina, Simocephalus vetulus, Ceriodaphnia sp1, Ceriodaphnia sp2, and Daphnia magna; second consumer (open triangles) are Leptodora kindtii, Eudiaptomus graciloides, Bythotrephes longimanus, and Diaptomus salinus (based on our results). Each symbol represents a mean value and error bars are ± 1 SD (n = 3).

of phytoplankton in a lake (Yoshioka, 1997). In general, phytoplankton use only CO2 gas, although at low CO₂ concentrations, phytoplankton can take up bicarbonate (HCO_3) and convert it into CO₂ by intracellular carbonic anhydrase (CA; Lucas & Berry, 1985). Algal use of bicarbonate as a carbon substrate results in enriched δ^{13} C values, because the δ^{13} C values of bicarbonate are isotopically heavier than those of CO₂, and the isotopic fractionation of CA is negative (Yoshioka, 1997). Thus, with low pH (high CO₂ concentration), the δ^{13} C values of POM in Lake Chany were low because the phytoplankton was using CO₂ gas. On the other hand, at high pH (low CO_2 concentration) the $\delta^{13}C$ values of POM were high because the phytoplankton used mainly bicarbonate instead of CO₂ gas.

The δ^{13} C values of herbivorous and carnivorous zooplankton also changed along with the values of POM at each site ($r^2 = 0.912$, p < 0.001, n = 24, Fig. 3), although the δ^{13} C values of herbivorous zooplankton were a little lower than those of the POM. This indicated spatial differences in stable isotope ratios of phytoplankton and those consumers at the different sites, even though Stations 1–4 were very close spatially. Thus, our results showed spatial changing of isotope baseline of plankton food webs in Lake Chany along an environmental gradient.

The δ^{13} C values of producers and consumers varied across different lakes types (Vander Zanden & Rasmussen, 1999; Grey et al., 2000). On the other hand, our results showed that the variations of the δ^{13} C values of producers and consumers also were in the one water body. In large lakes like Lake Chany, the base resources of food webs such as spatially changed due to spatial differences in the environmental factors. Thus, the isotope baseline in lake ecosystems might vary among each habitat of the lake. For lake food web analyses, special attention should be paid to the spatial changes in the isotope baseline with environmental changes, especially the estuarine parts of lakes with environmental gradients.

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References

- Aladin, N. V. & I. S. Plotnikov, 1993. Large saline lakes of former USSR: a summary review. Hydrobiologia 267: 1–12.
- Bootsma, H. A., R. E. Hecky, R. H. Hesslein & G. F. Turner, 1996. Food partitioning among lake Malawi nearshore fishes as revealed by stable isotope analysis. Ecology 77: 1286–1290.
- DeNiro, M. J. & S. Epstein, 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 48: 1135–1140.
- Doi, H., E. Kikuchi, S. Hino, T. Itoh, S. Takagi & S. Shikano, 2003. Seasonal dynamics of carbon stable isotope ratios of particulate organic matter and benthic diatoms in strongly acidic Lake Katanuma. Aquatic Microbial Ecology 33: 87–94.
- Doi, H., E. Kikuchi, C. Mizota, N. Satoh, S. Shikano, N. Yurlova, E. Zuykova & E. Yadrenkina, 2004. Carbon, nitrogen, and sulfur isotope changes and hydro-geological processes in a saline lake chain. Hydrobiologia 529: 225–235.
- France, R. L., 1995. Critical examination of stable isotope analysis as a means for tracing carbon pathways in stream ecosystems. Canadian Journal of Fisheries and Aquatic Sciences 52: 651–656.

- Fry, B., 1996. ¹³C/¹²C fractionation by marine diatoms. Marine Ecology Progress Series 134: 283–294.
- Grey, J., R. I. Jones & D. Sleep, 2000. Stable isotope analysis of the origins of zooplankton carbon in lakes of differing trophic state. Oecologia 123: 232–240.
- Grey, J. & R. I. Jones, 1999. Carbon stable isotopes reveal complex trophic interactions in lake plankton. Rapid Communication in Mass Spectrometry 13: 1311–1314.
- Harvey, C. J. & J. F. Kitchell, 2000. A stable isotope evaluation of the structure and spatial heterogeneity of a Lake Superior food web. Canadian Journal of Fisheries and Aquatic Sciences 57: 1395–1403.
- Lucas, W. J. & J. A. Berry, 1985. Inorganic carbon transport in aquatic photosynthetic organisms. Physiologia Plantarum 65: 539–543.
- Minagawa, M. & E. Wada, 1984. Stepwise enrichment of ¹⁵N along food chains: further evidence and the relation between δ^{15} N and animal age. Geochimica et Cosmochimica Acta 48: 1135–1140.
- Vadeboncoeur, Y., E. Jeppesen, J. M. Vander Zanden, H. Schierup, K. Christoffersen & D. M. Lodge, 2003. From Greenland to green lakes: cultural eutrophication and the loss of benthic pathways in lakes. Limnology and Oceanography 48: 1408–1418.
- Vander Zanden, J. M. & J. B. Rasmussen, 1999. Primary consumer δ^{13} C and δ^{15} N the trophic position of aquatic consumers. Ecology 80: 1395–1404.
- Wetzel, R. G., 2001. Limnology Lake and River Ecosystems (3rd edn.). Academic Press, San Diego, California.
- Yoshioka, T., 1997. Phytoplanktonic carbon isotope fractionation: equations accounting for CO₂-concentrating mechanisms. Journal of Plankton Research 19: 1455–1476.
- Yoshioka, T., E. Wada & H. Hayashi, 1994. A stable isotope study on seasonal food web dynamics in a eutrophic lake. Ecology 75: 835–846.
- Zohary, T., J. Erez, M. Gophen, I. Berman-Frank & M. Stiller, 1994. Seasonality of stable carbon isotopes within the pelagic food web of Lake Kinneret. Limnology and Oceanography 39: 1030–1043.