Stable isotope analyses of benthic organisms in Lake Baikal

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Abstract

Stable isotope ratios of carbon and nitrogen were used successfully to elucidate the biogeochemical and ecological frameworks of the trophic structure of benthic organisms in Lake Baikal, Siberia. Analysis of the benthic animals showed a considerable variance in both carbon and nitrogen stable isotope ratios. Two main primary producers of benthic plants and planktonic organic matter were clearly differentiated by δ^{13}C, and thus the diets of these two primary producers' groups could be analyzed with the use of a two source mixing model. The trophic position of each benthic animal was estimated by the analysis of δ^{15}N. Contrasting characteristics between food webs in shallow and deep benthic areas were clearly observed on the δ^{13}C – δ^{15}N map. Food webs in shallow benthic areas were complex, and many primary producers and various animals were present with diverse isotope distributions. In contrast, food webs in deep benthic areas were composed of single organic matter origin exhibiting simple predator and prey relationships. Both δ^{13}C and δ^{15}N values of benthic gammarids were correlated with the sampling water depth. A trend of δ^{13}C decrease and δ^{15}N enrichment was observed with increasing water depth. The stable isotope ratios of the benthic animals indicated that the complexity of the food web structure in their ecosystem decreased as the depth of the water increased.

Introduction

Lake Baikal, in the central part of southern Siberia, is the deepest and oldest lake in the world, containing as much as 20 percent of the world's fresh water supply (23 000 km^3). The biota in the lake is highly diversified; a major part of the species present is endemic; and a wide variety of animals are living from just beneath the surface to the deepest point of the lake. The benthic area of Lake Baikal is remarkably diverse. Of the various animals of Lake Baikal, most of them are benthic, and it is the gammarids and sculpins in particular that are widely differentiated. These benthic animals can also be found from the surface level of the lake to its deepest point and from several inner bays to abyssal benthic areas. Therefore, intraspecific and interspecific differences in feeding habits as well as food web structures might show contrasting and distinctive features dependent on these differing environments.

Compared with the traditional gut content analysis, the study of stable isotopes offers some advantages in attempting to describe a food web structure of an ecosystem. Firstly, gut content analyses are usually based on observations of only a limited number of species in a community. Studies of whole ecosystem structures are laborious, and thus unusual, due to the presence of small species such as plankton – the gut contents of which are difficult to analyze. In contrast, the effectiveness and reliability of the stable isotope technique is rapidly improving due to progress in the field of mass spectrometry. This is allowing a lot of samples to be measured in a short time. Secondly, stable isotopes are universal indicators that can be used for both biological and non-biological samples. Thirdly, conventional stomach content analysis can provide only a 'snap-shot' of the feeding habits of an organism, whereas the stable isotopic composition of an organism will provide information about its feeding habits over significant periods of time, corresponding to organic carbon turnover times (Fry & Arnold, 1982). Finally, results from the conventional gut contents approach can be misleading if some of the gut contents

are not completely assimilated (Kling et al., 1992). Stable isotope analysis does not need to consider assimilation problem because stable isotope ratios are measured with assimilated tissues.

The $\delta^{13}C$ value has already been used to trace the flow of organic matter along the food chain from the primary producers (food base) to those animals at higher trophic levels (Haines, 1976; DeNiro & Epstein, 1978; Fry et al., 1978; Fry & Parker, 1979; Haines & Montague, 1979; Rau, 1980, 1981). For example, $\delta^{13}C$ enrichment of less than 1‰ was reported for whole animal bodies (DeNiro & Epstein, 1978; McConnaughey & McRoy, 1979; Rau et al., 1983). In fact, since the $\delta^{13}C$ value of organisms is close to that of the food bases in their food chain, it has been possible to estimate the food source of each species. In addition, previous isotopic studies have determined that phytoplankton and benthic plants are differentiated by carbon stable isotope ratios (e.g. Bootsma et al. 1996). Therefore, $\delta^{13}C$ can be used to determine the food source of animals if there are only a few primary producers and if they exhibit distinct $\delta^{13}C$ ratios. Because lipid fractions of organisms show low $\delta^{13}C$ values relative to whole organisms and other protein-rich fractions (Abelson & Hoering, 1961; Park & Epstein, 1961; Parker, 1964; Degens et al., 1968; Gormly & Sackett, 1977; Fry et al., 1978), a step-wise enrichment of $\delta^{13}C$ throughout the food web was often small and variable. This was because of differing lipid content amongst the organisms (Wada et al., 1987, Fry 1988).

The $\delta^{15}N$ value of animals also reflects their diets. Enrichment of $\delta^{15}N$ along the trophic network is widely recognized amongst most animals, including the vertebrate and the invertebrate (DeNiro & Epstein, 1981; Minagawa & Wada, 1984). DeNiro & Epstein (1981) and Minagawa & Wada (1984) reported that $\delta^{15}N$ enrichment during single feeding processes (diet /whole body relationships) was 3.0 ± 2.6 and 3.4 ± 1.1‰, respectively. Wada et al. (1987) observed a constant $\delta^{15}N$ trophic effect of 3.3‰ in an Antarctic marine ecosystem. Because there was clear enrichment of $\delta^{15}N$ throughout the food chain, this was used to estimate the trophic position in each species. The $\delta^{13}C$ and $\delta^{15}N$ data of aquatic organisms can, therefore, provide useful information about food sources and trophic level. Isotopic ecological structures have recently been determined in several aquatic ecosystems (e.g. Wada et al., 1987; Hobson & Welch, 1992 for marine ecosystem, Yoshioka et al., 1994 for lake ecosystem); however, only a prelimin-

ary study has been carried out in the benthic region of Lake Baikal. Kiyashko et al. (1991) reported the $\delta^{13}C$ value of plankton, benthic algae, and allochthonous organic matter in Lake Baikal (about −30, −5 to −12 and −27‰, respectively). The stable isotope analysis method can determine the food sources of animals only when there are a few isotopically distinctive primary producers and the result of Kiyashko et al. (1991) suggested that the benthic region of Lake Baikal satisfied this condition.

In this study, therefore, carbon and nitrogen isotope ratios of various benthic animals were analyzed to determine the overall food web characteristics of each geographical region and the feeding behavior of each species. The aim of this study is to provide new information regarding the food web structure in the benthic regions of Lake Baikal, where many endemic animals exist in various benthic environments.

Materials and methods

Lake Baikal is located in the central part of southern Siberia (52°–56° N, 104°–110° E) at an altitude of 455.6 m above sea level. The lake is 635 km long and has a breadth of 80 km across at its widest point, covering an area of 31 500 km². Lake Baikal is unique in several ways. Firstly, it is the deepest lake in the world, with a maximum depth of 1637 m (Stewart, 1990), and with more than 80% of its area deeper than 250 m (Kozhov, 1963). In order to separate the benthic areas into two parts, I adopted the 'shallow benthic area' and 'deep benthic area'. A shallow benthic area indicates a benthic area in a coastal belt with a water depth of less than 200 m, where both phytoplankton and benthic plants are available to the benthic animals. A deep benthic area denotes a benthic area of open water of no less than 100 m depth, where few benthic plants are consumed by benthic animals. Secondly, the lake is the oldest in the world (20–25 million years) and, as a result, Afanasyev (1960) reported that the residence time of its water was approximately 330 years. Lake Baikal is dimictic, with the surface water column turning over twice a year (Votintsev 1985), and the surface layer above 400 m mixed well in May and October. According to the vertical distribution of Chlorofluorocarbons (Weiss et al., 1991), the renewal time of the bottom water is approximately eight years. Thirdly, the lake has high levels of dissolved oxygen in its bottom waters (Maddox, 1989; Weiss et al., 1991). This contrasts with the oxygen content of the large and ancient Lake

Tanganyika, where an anoxic layer develops below 200 m. High levels of dissolved oxygen from the surface to the bottom might well have contributed to the evolution of a diverse biota. Finally, there are more than 2000 species of animals in Lake Baikal, and two thirds of them are endemic (Kozhov, 1963).

A variety of organisms were collected from various parts of Lake Baikal during the research expeditions undertaken with R. V. Obruchev (40 tonnage) in the June – August periods of 1992, 1993 and 1994 (Figure 1). Sampling sites were chosen so as to collect as many species as possible, and these sites included inner bays (Maloye More, Chivyrkuy bay and Barguzin bay) and open waters (Southern, Central and Northern Basin). The depth of the sites varied from surface to 745 m, and the samples included organisms of every trophic level: from plants to fish such as macrophytes, Nostoc, benthic gammarids, oligochaetes, Mollusk, Trichoptera, Planaria, Spongia and benthic sculpins. Phytoplankton was collected by 10–50 m vertical tows with a 40 μm mesh plankton net, with zooplankton removed by filtration with a 100 μm mesh net. Fish samples were collected during the expedition, some of which came from Southern Baikal near Listvyanka and were obtained from fishermen. For fish samples, only muscle tissue was analyzed but, because of their small size, entire body parts of other organisms were used for analysis. All the samples were oven dried and crushed into powder.

In order to remove the effect of lipid – which has low δ^{13}C level compared to other tissue – the lipid fraction of the powdered animal samples was extracted and removed by filtration after leaving the 30 mg samples in 10 ml chloroform:methanol (2:1) solution for about 24 h. These samples (2–8 mg) were transformed into CO_2 and N_2 gases via the sealed tube combustion method (Minagawa et al., 1984). The gases produced were separated and purified cryogenically using dry ice-ethanol trap and liquid nitrogen traps. The nitrogen and carbon dioxide gases were collected into a glass tube until isotope analysis could be undertaken.

A mass spectrometer (Delta-S or Delta-V, Finnigan-mat) was used to analyze carbon and nitrogen stable isotope ratios. Isotope ratios are expressed in terms of permil deviation from standard (Pee Dee belemnite (PDB) for carbon, and atmospheric nitrogen gas for nitrogen):

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

where $R = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$.

The laboratory standard of DL-Alanine (δ^{13}C= −23.5‰, δ^{15}N = −1.6‰) was used as a running standard for isotopic measurements. By replicate measurements of this running standard, the level of analytical precision was determined to be less than ±0.2‰ for nitrogen and ±0.1‰ for carbon.

Results and discussion

In the pelagic ecosystems of Lake Baikal, small stepwise enrichment of δ^{13}C (1.2‰) was clearly confirmed with the use of lipid-free samples (Yoshii et al., 1999). This fact indicates that δ^{13}C is applicable to the indicator of food source in Lake Baikal. In the case of δ^{15}N, enrichment of 3.3‰ per trophic level was clearly observed in pelagic food webs (Yoshii et al., 1999). Therefore, the δ^{15}N enrichment factor of 3.3‰, as well as the δ^{13}C enrichment of 1.2‰, was applied to the organisms in the benthic areas in this study, so as to analyze the trophic positions and food sources of each benthic organism.

Considerable variation in both the δ^{13}C and δ^{15}N values were observed. These ranged from −29.0 for phytoplankton (*Aulacoseira baicalensis*) to −5.0‰ for benthic algae for δ^{13}C, and from 1.6 for Nostoc to 15.6‰ for benthic sculpin (*Batrachocottus multiradiatus*) for δ^{15}N. The δ^{13}C – δ^{15}N map of all the samples collected in this study appears in Figure 2. The range of the δ^{13}C values became smaller as the trophic level increased.

Stable isotope ratios of organisms provide an averaged 'picture' of feeding behavior over significant periods of time corresponding to organic carbon or nitrogen turnover times (Fry & Arnold, 1982). Therefore, organisms of upper trophic level can be further averaged due to their longer lifetime, thus causing a smaller range of δ^{13}C values to appear as the trophic level increases. Whilst benthic invertebrates and benthic fish could not be statistically differentiated, the tendency of δ^{15}N to increase in order of primary producers (δ^{13}C = −18.7 ± 9.9‰, δ^{15}N = 3.3 ± 1.1‰), benthic invertebrates (δ^{13}C = −19.9 ± 5.3‰, δ^{15}N = 8.2 ± 2.4‰), and benthic fish (δ^{13}C = −17.4 ± 2.9‰, δ^{15}N = 11.1 ± 1.0‰) was observed (Figure 2).

Benthic plants such as macrophyte and Nostoc showed extremely high δ^{13}C values compared to that of other organisms in this study (Figure 2). Such a high δ^{13}C level of benthic plants has been reported before (Doohan & Newcomb, 1976; McMillan et al.,
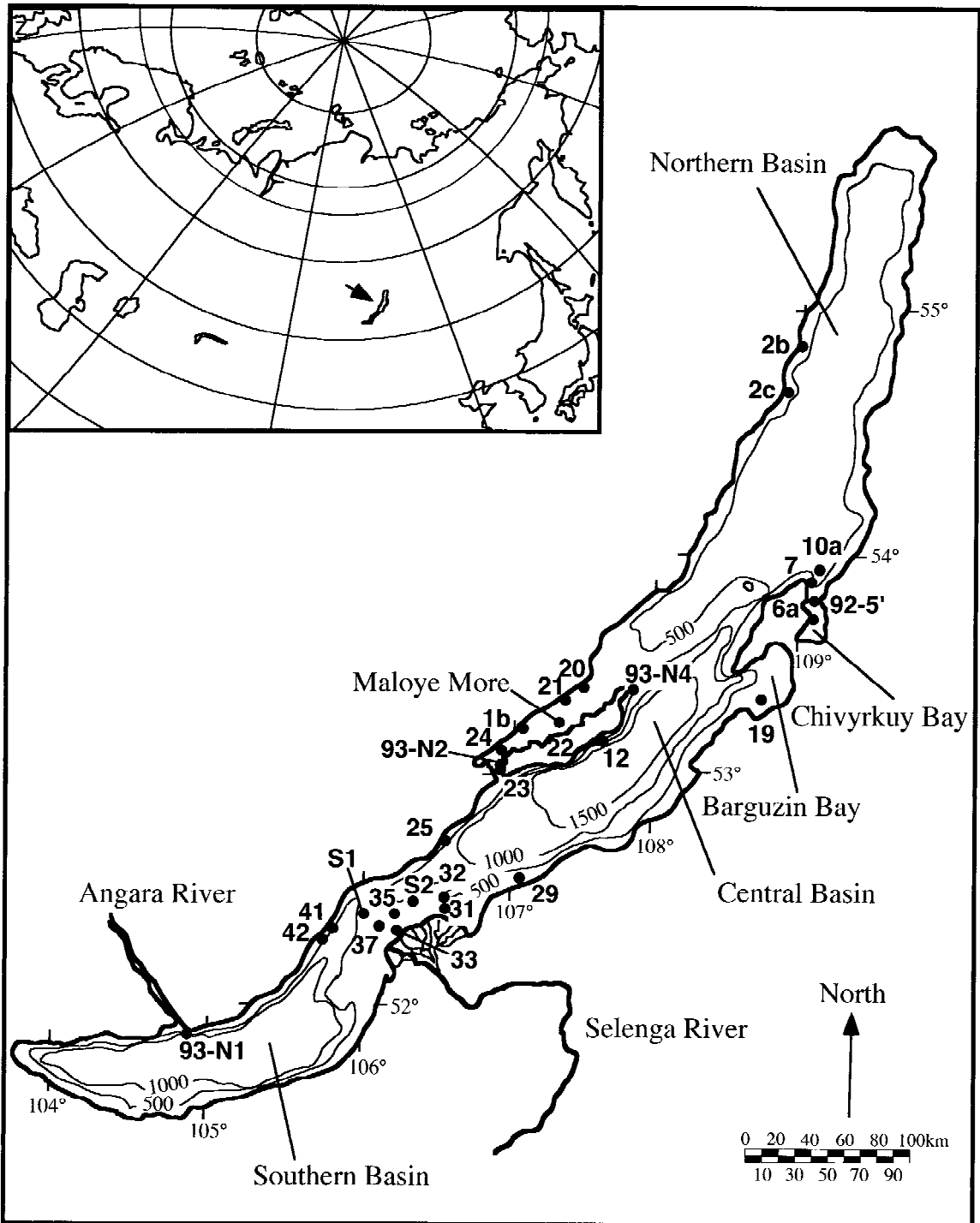
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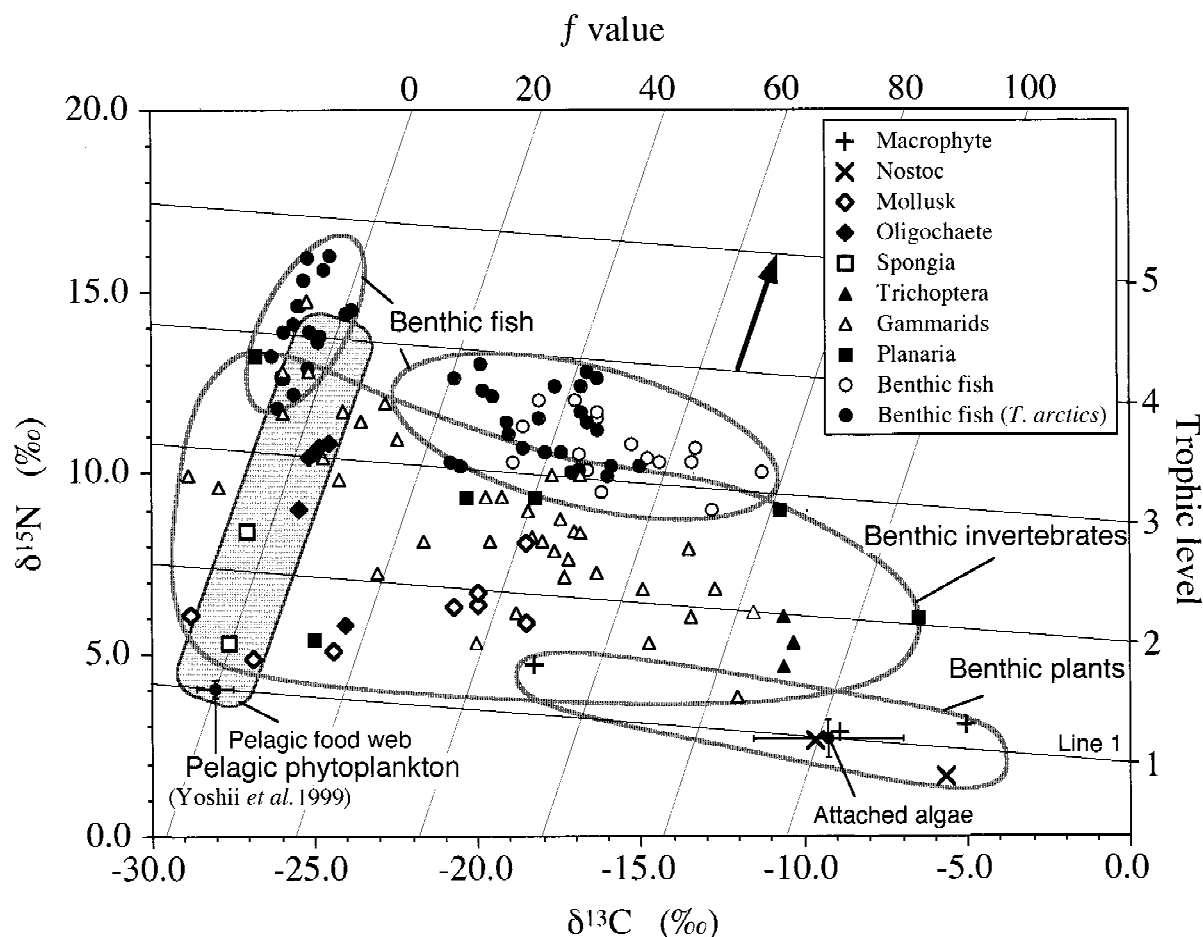*Figure 1.* Location and sampling stations of Lake Baikal.

Figure 2. $\delta^{13}C-\delta^{15}N$ map of all the benthic animals and benthic plants collected in various sampling sites in Lake Baikal. Each symbol of benthic fish denotes individual, and that of other organisms denotes some individuals. Trophic levels and f value were determined by the same way as Appendix. Horizontal and vertical bars in symbols of pelagic phytoplankton and attached algae are standard deviations.

1980; McMillan & Smith, 1982; Fry et al., 1982; Nichols et al., 1985). According to Descolas-Glos & Fontugne (1990), this phenomenon may be associated with the small diffusion coefficient for CO_2 relative to photosynthetic CO_2 fixation in water environments, which suppresses the occurrence of carbon isotopic discrimination.

There are a variety of organisms including sculpins, gammarids, oligochaeta, mollusks, planarians, sponges, caddis fly, benthic plants in the benthic area of Lake Baikal. In present study areas, there exist two major primary producers: benthic plants and phytoplankton. These two sources exhibited different $\delta^{13}C$ values ($-28.0 \pm 1.1‰$ for phytoplankton and $-9.5 \pm 5.3‰$ for benthic plants). Therefore, on the assumption that the contribution of other sources such

as terrestrial plants is negligible, $\delta^{13}C$ could indicate which food sources each benthic animal consumes.

In order to estimate the food source of each animal quantitatively, the average data shown above regarding phytoplankton and benthic plants were used for each food source. $\delta^{13}C$ and $\delta^{15}N$ enrichment factors evaluated in pelagic food webs of the lake (1.2‰ and 3.3‰, respectively) were applied in this calculation. Because $\delta^{13}C$ and $\delta^{15}N$ enrichment factors are 1.2 and 3.3‰, the $\delta^{13}C$ and $\delta^{15}N$ are expected to rise by the arrow in Figure 2 (slope is 3.3/1.2) by the increase of trophic level. Therefore, animals of higher trophic level have higher $\delta^{13}C$ values, even if the same proportion of two food sources has been consumed. The f value – the proportion of benthic plants that was consumed by animals – was calculated by applying

the two source mixing model, after adjusting the effect of δ^{13}C enrichment caused by the increase of trophic level. The same assumption was also used to estimate the trophic level of the animal. On the line 1 of Figure 2, trophic level was determined to be 1 because it was drawn so as to go through both primary producers of phytoplankton and benthic plants. The effect of different δ^{15}N levels amongst two primary producers was considered to calculate the precise trophic level of each animal. The f value and trophic levels of each animal are shown in the Appendix. Although the f value of benthic animals changes depending on which benthic plants they consumed because the δ^{13}C of benthic plants is dispersed, f value can still be a useful indicator to estimate the dietary differences between two primary producer groups. The trophic levels in the following discussions were based on the data that resulted from this calculation.

By the calculation of f value, the food source of Trichoptera was indicated to be predominantly benthic plants ($f = 90 \pm 2\%$), and that of oligochaeta to be planktonic organic matter ($f = 7 \pm 3\%$). The f values of mollusks, planarian and gammarids were widely distributed, and these organisms were characterized as feeding behavior consuming both primary producers (phytoplankton and benthic plants) in different rates (Figure 2).

Spatial variations in δ^{15}N values of primary producers and primary consumers within the lake have been observed in a few freshwater studies (Estep & Vigg, 1985; Angradi, 1994). Vander Zanden & Rasmussen (personal communication) reported significant differences in primary consumer δ^{15}N levels amongst littoral ($x = 1.58‰$), pelagic ($x = 3.05‰$) and profundal ($x = 5.17‰$) habitats. Stable isotope ratios of geographical distribution were examined in Figure 3. Food webs of three areas – pelagic, shallow benthic (Maloye More) and deep benthic (Central basin) – were compared in δ^{13}C–δ^{15}N maps. Maloye More is located in the middle part of the lake and is a typical shallow littoral area (Figure 1). The organisms in each area differ significantly. The deep benthic area is mainly inhabited by benthic gammarids, benthic fish (mostly benthic sculpins) and oligochaetes, with endemic species predominant. In the shallow benthic area, organisms such as benthic gammarids, mollusk, oligochaeta and sponge can be found (Kozhov, 1963). In contrast, however, the range of species present in the pelagic food web is comparatively small and composed of mainly phytoplankton (*Aulacoseira baicalensis*), mesozooplankton (*Epischura baicalensis*), mac-

rozooplankton amphipod (*Macrohectopus branickii*), Pisces (*Coregonus autumnalis migratorius*), four species of cottoid fish, and the seal (*Phoca sibirica*). (Kozhov, 1963). The δ^{13}C–δ^{15}N maps of these areas also contrasted sharply. Pelagic animals have distinctive δ^{13}C and δ^{15}N levels in each species and showed clear step-wise enrichment with 1.2‰ for δ^{13}C and 3.3‰ for δ^{15}N, starting from phytoplankton (Yoshii et al., 1999). This indicates that pelagic animals consume only planktonic organic matter and the prey – predator relationship is simple enough to analyze the diets of each species quantitatively. A similar trend regarding δ^{13}C and δ^{15}N was observed in the animals of the deep benthic area in the Central basin. The food base would appear to be dominantly planktonic organic matter because the δ^{13}C values ($-25.0 \pm 1.3‰$) of the animal is close to that of phytoplankton. The trophic effect of δ^{15}N (about 3–4‰) and similar δ^{13}C levels between benthic gammarids and benthic fish suggested a clear prey – predator relationship. Therefore, the simple ecosystem that is associated with a clear food chain was also observed in the food web of the deep benthic area. In contrast to the pelagic and deep benthic areas, however, the δ^{13}C values of animals in the shallow benthic area (Maloye More) ranged widely ($-17.7 \pm 5.1‰$, Figure 3). This could be explained by the various feeding behaviors of animals, by the multiple food bases of benthic algae and planktonic organic matter, and by the complex trophic relationships that exist amongst the organisms. The δ^{15}N values indicated that approximately three trophic levels seemed to be present in Maloye More: primary producers; primary consumers, such as benthic gammarids and Trichoptera; Pisces and carnivorous benthic animals.

An exceedingly diverse gammarids (amphipod) characterizes Lake Baikal. As many as 256 species were identified (Kamaltynov, 1992) and new species has been found year after year. They exist in various environments from the shallow to the deep area of the lake (Bekman, 1984). The δ^{13}C and δ^{15}N values of various benthic gammarids were correlated with the depth of sampling sites (Figure 4). The rise of δ^{15}N values as the depth of the water increased might have been caused by two factors. First, carnivorous benthic gammarids having high δ^{15}N are generally dominant in deep benthic areas, and many herbivorous ones in the lower trophic level are living in shallow waters (Kozhov, 1963). Second, the δ^{13}C data showed that the gammarids in deep benthic areas consume predominantly planktonic organic matter which have comparatively higher δ^{15}N values ($4.2 \pm 0.6‰$) than
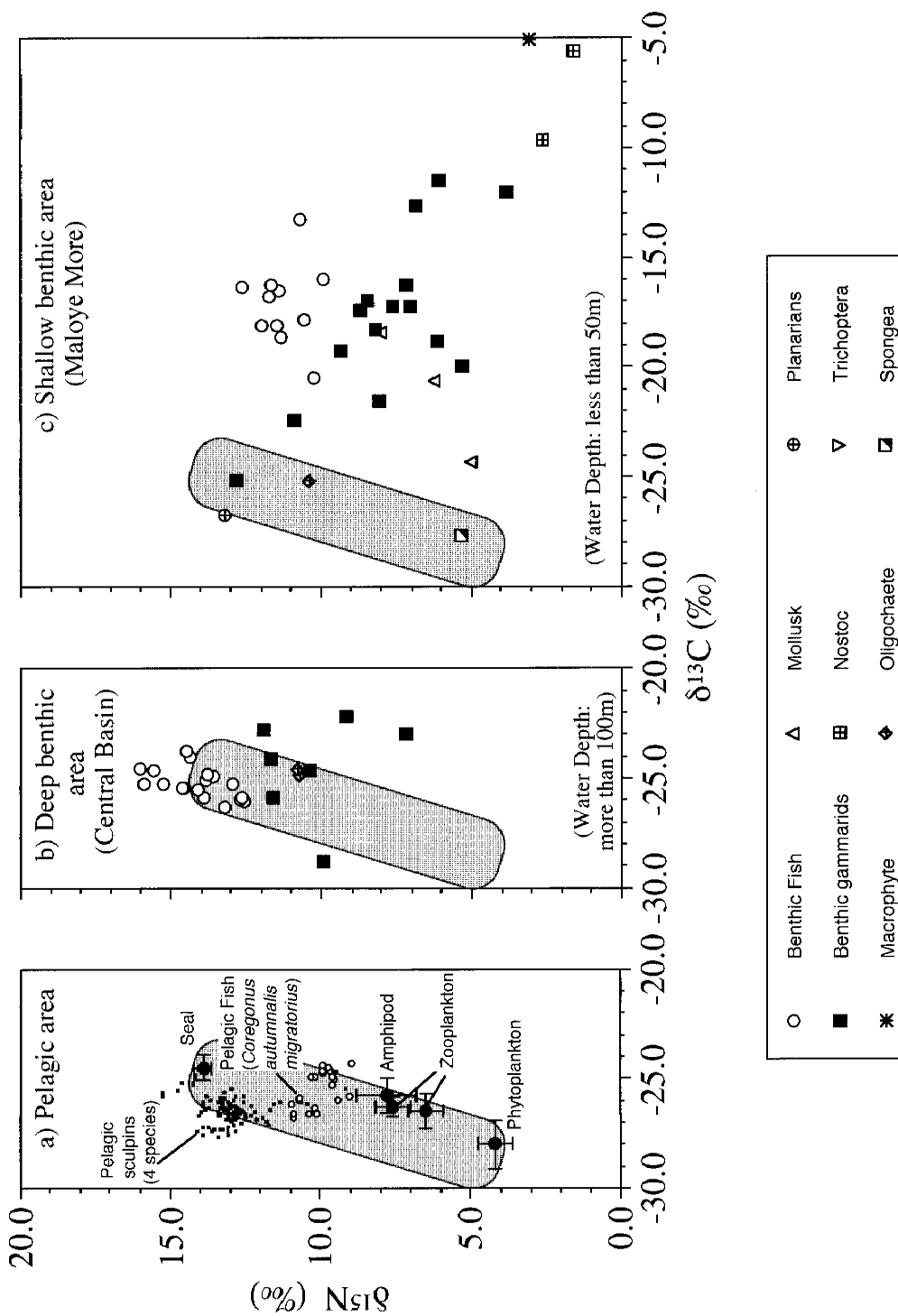
Figure 3. $\delta^{13}C$–$\delta^{15}N$ map of food web in (a) pelagic, (b) shallow benthic (Maloye More, water depth less than 50 m), and (c) deep benthic (water depth more than 100 m) areas.
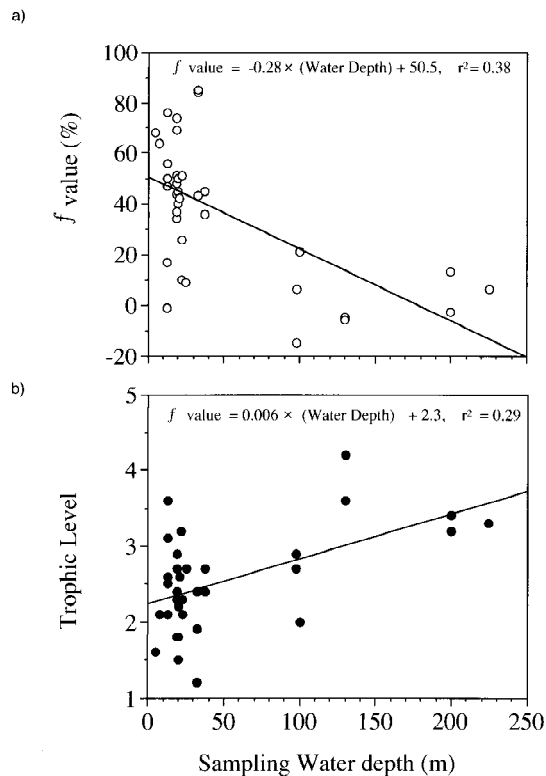
a)

b)

f value $= 0.006 \times$ (Water Depth) $+ 2.3$, $r^2 = 0.29$

Figure 4. Relationships between sampling water depth and (a) f value and (b) trophic level of benthic gammarids. Trophic levels and f values were determined by the same way as Appendix. Each open circle denotes several individuals. Solid straight line denotes linear regression.

those of benthic plants such as macrophyte (2.9 $\pm$ 1.1‰) and attached algae (3.2 $\pm$ 0.6‰). Therefore, animals in deep benthic areas could have higher $\delta^{15}N$ than those inhabiting shallow benthic area even if the trophic level is the same. The decrease in $\delta^{13}C$ was generally accompanied by the increase in the $\delta^{15}N$ throughout the indicated water depth (Figure 4). The $\delta^{13}C$ level of benthic gammarids collected above a depth of 100 m showed wide-ranging f values of -1 to 85% (43 $\pm$ 24%, $n=32$), though those below 100 m were low and varied little (4 $\pm$ 11%, $n=6$). This data suggests that both planktonic organic matter and benthic plants are used for food by gammarids living less than 100 m below the surface. In general, many gammarids in deep layers are carnivorous, and those in shallow water are omnivorous (Bazikalova, 1945). This tendency could be confirmed isotopically in our present $\delta^{15}N$ data, because benthic animals in deep benthic areas showed higher $\delta^{15}N$ than those in shallow benthic areas.

Next, I examined the food source of each gammarid. The $\delta^{13}C$ and $\delta^{15}N$ values of benthic gammarids fluctuated widely between species (Figure 5), and this indicated that each species has its own diverse feeding behavior. The f value indicates their food sources: organic matter of phytoplankton origin or benthic plants. Isotopically, benthic plants could be considered as the main food source of *Acanthogammarus victorii maculosus*, *Brandtia latissima*, *Eulimnogammarus czerskii* and *Pallasea cancellus*. The food sources of species inhabiting the deep benthic areas could be largely organic matter of phytoplankton origin because of the low f value. However, on the other hand, $\delta^{15}N$ can suggest the trophic level of each gammarid. Those gammarids showing a trophic level of more than three are likely to be carnivorous, whilst a level of two suggests a herbivorous species. *Ommatogammarus*, which is known to be a typical carnivorous species (Kozhov, 1963), exhibited one of the highest $\delta^{15}N$ values amongst benthic gammarids. Therefore, a reasonable illustration of feeding habit might be isotopically possible for other species. Benthic gammarids of high $\delta^{15}N$ such as *Acanthogammarus brevispinus*, *Acanthogammarus grewingki*, *Garjajewia cabanisi*, *Ceratogammarus cornutus* and *Pachyschesis bazikalowae* might therefore be carnivorous.

Benthic fish samples included various endemic sculpins and many other species. The $\delta^{13}C$ of these fish ranged widely between $-26.3‰$ and $-11.3‰$ ($-19.8 \pm 4.3‰$, Figure 6). Only benthic sculpin species were found at the site with a water depth of more than 700 m, and they were characterized by a low f value (Cottoidei *Abyssocottus korotneffi*, *A. platycephalus*, *Batrachocottus nikolskii*, *B. multiradiatus*, *Cottinella boulengeri*, *Limnocottus bergianus*, *L. godlewskii*, *L. griseus*, and *L. pallidus*). Benthic sculpins are typical fish that exist at various depths in the coastal belt (Kozhov, 1963). Sideleva & Mechanikova (1990) analyzed the frequency of each organism observed in the gut of some benthic cottoid fish (*B. multiradiatus*, *L. bergianus* and *A. korotneffi*) found between 300 and 500 m, and found that more than 70 percent of the diet of the cottoid fish was benthic gammarids, and less than 15 percent was benthic fish. Eggs of fish, detritus, oligochaetes and planarians were not observed. Both $\delta^{13}C$ and $\delta^{15}N$ ratios could support this result. In addition, a wide range of trophic levels from 3.5 to 4.5 indicates that each species was consuming diets of different trophic levels. A number of different fish groups were found in shallow benthic
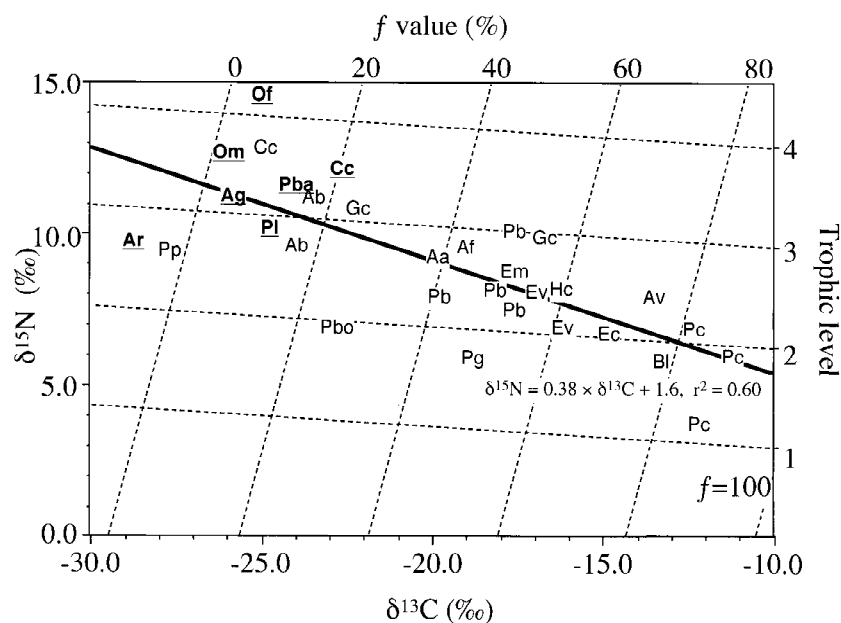
Figure 5. δ^{13}C–δ^{15}N map of benthic gammarids. Each open circle denotes several individuals. Bold and underlined symbol denotes samples collected in the water depth more than 100 m. Trophic levels and f values were determined by the same way as Appendix. Solid straight line denotes linear regression. Aa; *Acanthogammarus albus,* Ab; *Acanthogammarus brevispinus,* Af; *Acanthogammarus flavus,* Ag; *Acanthogammarus grewingki,* Ar; *Acanthogammarus reicherti,* Av; *Acanthogammarus victorii maculosus,* Bl; *Brandtia latissima,* Cc; *Ceratogammarus cornutus,* Ec; *Eulimnogammarus czerskii,* Em; *Eulimnogammarus maacki,* Ev; *Eulimnogammarus verrucosus,* Gc; *Garjajewia cabanisi,* Hc; *Hyalellopsis carpenteri,* Hp; *Hyalellopsis potanini,* Oa; *Ommatogammarus albinus,* Of; *Ommatogammarus flavus,* Pba; *Pachyschesis bazikalowae,* Pb; *Pallasea brandti,* Pc; *Pallasea cancellus,* Pg; *Pallasea grubei,* Pp; *Paragarjajewia petersi,* Pbo; *Parapallasea borowskii,* Pl; *Parapallasea lagowskii.*

area (Cottoidei *B. multiradiatus, Cottus kessleri, Paracottus kneri, Procottus jeittelesi, Pr. major,* Esocidae *Esox lucius,* Cyprinidae *Leuciscus leuciscus baicalensis* and *Rutilus rutilus lacustris,* Gadidae *Lota lota,* Percidae *Perca fluviatilis* and Thymallidae *Thymallus arcticus*), and these include both sculpin species and other fish, and showed a more marked variation in δ^{13}C than those in the deep benthic areas. The δ^{15}N values of benthic fish collected in the shallow benthic areas above a depth of 200 m (11.1 ± 1.0‰) proved lower than that of the fish collected in deep benthic areas below this depth (14.0 ± 1.1‰). This tendency of δ^{13}C and δ^{15}N in benthic fish is related to the distribution of benthic gammarids, and thus these might well be the main diet of benthic fish. On the other hand, each species occupied its own area in δ^{13}C – δ^{15}N map, especially as regards sculpin species (Figure 6). For example, amongst benthic fish in deep benthic areas, *B. multiradiatus* (15.8 ± 0.2‰) and *A. platycephalus* (12.6 ± 0.1‰) hadhighly different δ^{15}N values, but intraspecific differences were small. This may be because, whilst feeding behavior differs between species, it is similar amongst single species

inhabiting the same layer. Sideleva et al. (1992) reported that each sculpin has its own habitat differentiated by water depth (*Cottus kessleri* inhabits shallow water from 0 to 5 m, *Procottus jeittelesi*: 5–100 m, *A. platycephalus*: 100–300 m, *B. multiradiatus, Batrachocottus nikolskii*: 300–500 m,*Abyssocottus* sp. and *B. multiradiatus*: more than 500 m). Feeding behavior might well, therefore, be correlated with habitat. Thus stable isotope ratios have the potential to assist in the analysis of the diet compositions of each sculpin species and, since the levels of δ^{13}C and δ^{15}N in their diet (gammarids) are clearly related to their sampling depth, they can provide information concerning their various layers as well.

Furthermore, δ^{13}C of *T. arcticus,* which is one of the major species present in the shallow water, was particularly variable (−15.6 ± 2.2‰), and their individual feeding behavior may differ because of these considerable changes in δ^{13}C levels. In order to confirm the diversity of individual feeding behavior, I examined the prey-predator relationship of the δ^{13}C and δ^{15}N values between individual *T. arcticus* and its gut contents (Figure 7). *T. arcticus* is typical of
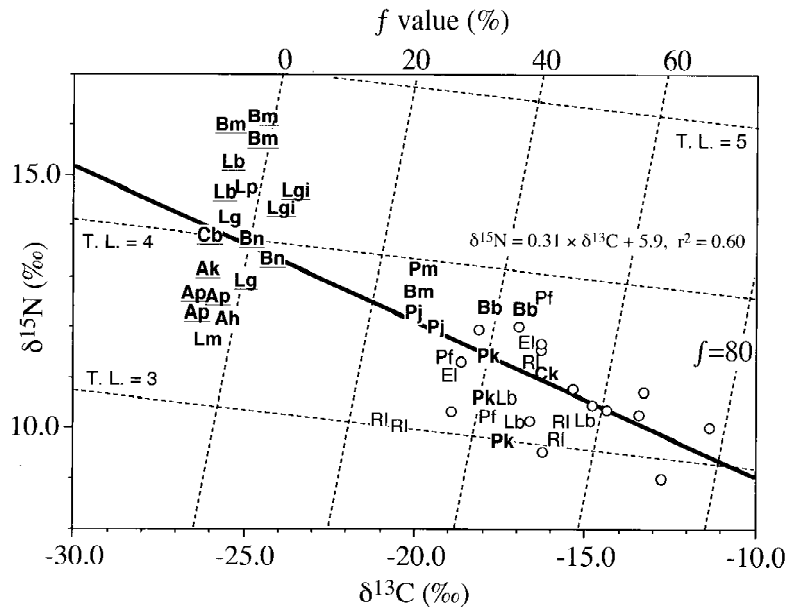
Figure 6. δ^{15}N–δ^{13}C map of benthic fish. Each symbol denotes individual. Underlined symbol denotes that the water depth of the sampling site is more than 200 m. Trophic levels and f values were determined by the same way as Appendix. Solid straight line denotes linear regression. **Ab**; *Abyssocottus korotneffi,* **Ah**; *Asprocottus herzensteini,* **Ap**; *Asprocottus platycephalus,* **Bb**; *Batrachocottus baicalensis,* **Bm**; *Batrachocottus multiradiatus,* **Bn**; *Batrachocottus nikolskii,* **Cb**; *Cottinella boulengeri,* **Ck**; *Cottus kessleri,* **Lb**; *Limnocottus bergianus,* **Lg**; *Limnocottus godlewskii,* **Lgi**; *Limnocottus griseus,* **Lm**; *Limnocottus megalops,* **Lp**; *Limnocottus pallidus,* **Pk**; *Paracottus kneri,* **Pj**; *Procottus jeittelesi,* **Pm**; *Procottus major,* Lb; *Leuciscus leuciscus baicalensis,* Rl; *Rutilus rutilus lacstrius,* El; *Esox lucius,* Ll; *Lota lota,* Pf; *Perca fluviatilis,* E; *Thymallus arcticus.*

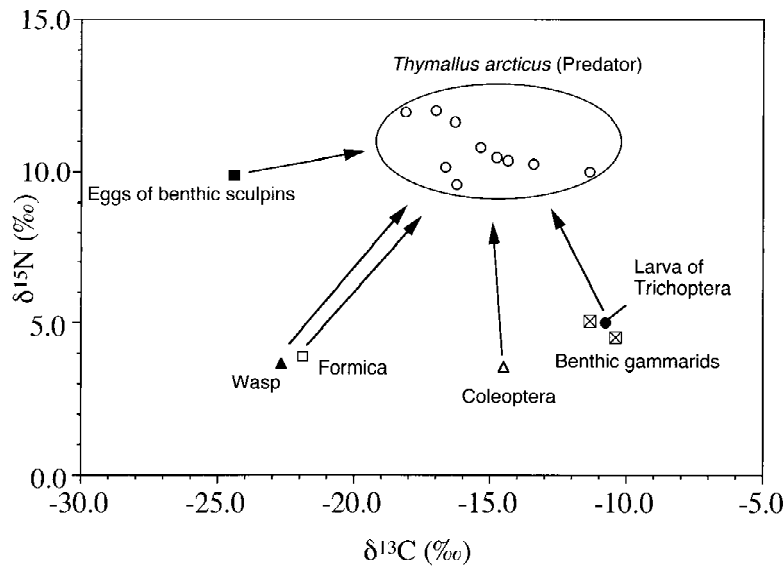*Figure 7.* Relationship of *T. arcticus* and its gut contents in δ^{13}C–δ^{15}N map. Each symbol except larva of Trichoptera denotes individuals.
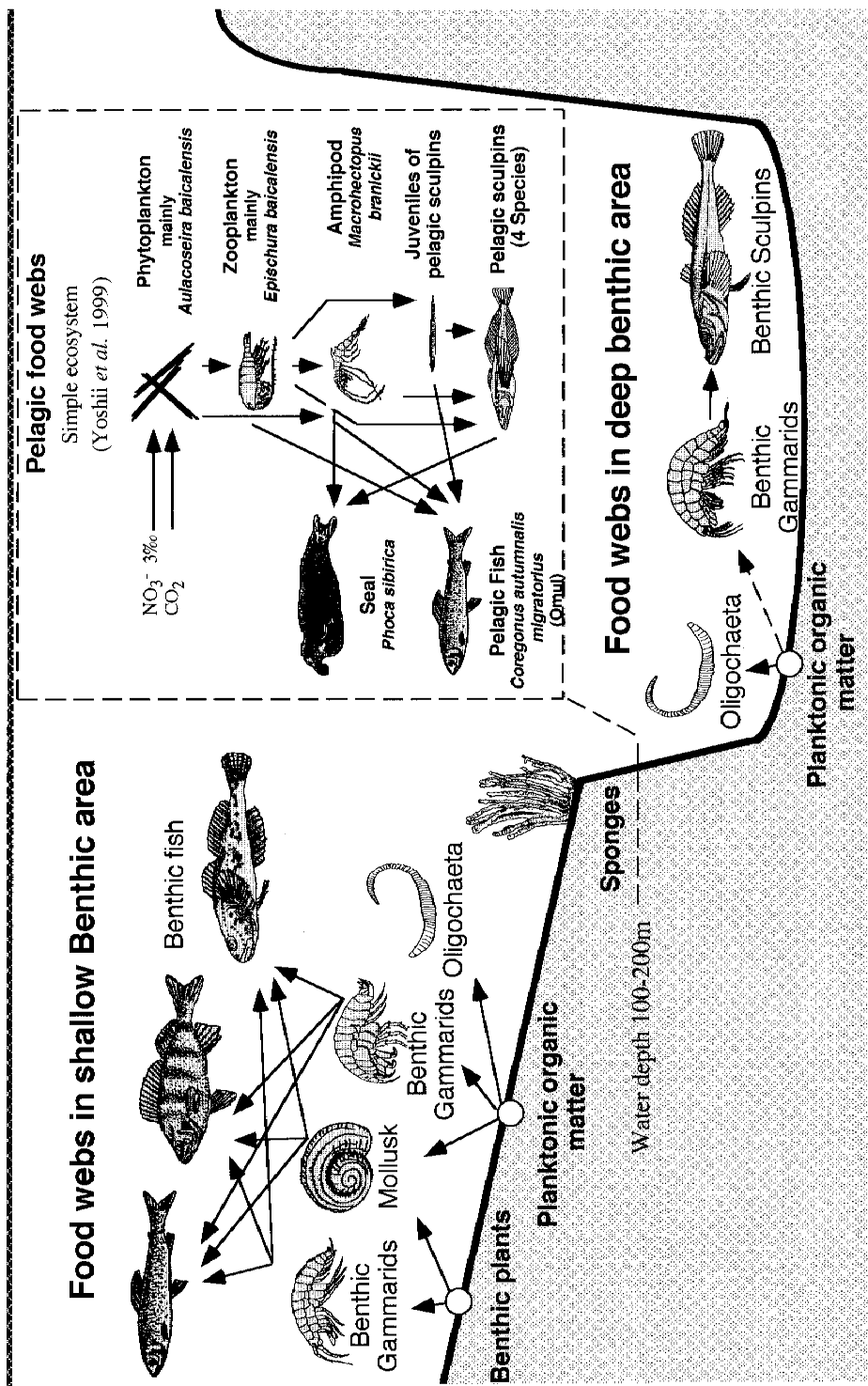
Figure 8. Schematic illustration of ecosystem in Lake Baikal.

the fish that inhabit shallow coastal areas, and many kinds of organisms, even including those of terrestrial origin (eggs of benthic sculpins, Trichoptera, benthic gammarids, mollusks, terrestrial insects such as wasp, bug, formica and beetle) were found in the gut. These various diets exhibited a variety of isotope ratios that reflected their different food sources and trophic levels, whilst Figure 6 indicates that the wide range of $\delta^{13}C$ of *T. arcticus* is caused by differing dietary distributions between individuals. In contrast to $\delta^{13}C$, the $\delta^{15}N$ levels of *T. arcticus* were fairly constant among individuals ($10.7 \pm 0.8‰$). By taking both $\delta^{13}C$ and $\delta^{15}N$ into consideration, among various possible diets, invertebrates such as benthic gammarids could well be the main diet of *T. arcticus*. Therefore, wide intraspecific $\delta^{13}C$ variations in *T. arcticus* might be partly caused by invertebrates such as benthic gammarids with various isotope signatures.

Conclusion

The stable isotope ratios of benthic organisms were analyzed to establish an isotopic food web structure of benthic ecosystems in Lake Baikal. $\delta^{13}C$ value could clearly differentiate between two primary producers of benthic plants and phytoplankton, and could also indicate that various benthic animals consumed these food sources at different rate. Clear contrasts between the food webs of shallow and deep benthic areas were identified isotopically. Animals existing in shallow benthic areas were diverse organisms, exhibiting various feeding habits between animals consuming both food bases of benthic plants and planktonic organic matter at various rates and, judging from the f value and trophic level calculated by using $\delta^{13}C$ and $\delta^{15}N$, a complex trophic structure. The organisms found at a depth of over 200 m had a more constant, and lower, $\delta^{13}C$ value than those found in shallower waters. Relatively clear prey–predator relationships and single food bases of planktonic organic matter could be indicated in the area. The feeding habits of the many species of benthic gammarids present in Lake Baikal consuming organic matter originate from both primary producer groups, and the existence of both carnivorous to herbivorous species resulted in widely dispersed stable isotope distribution. The stable isotope data of benthic gammarids correlated with the depth of water sampled. It was estimated that carnivorous species predominated beneath 100 m, and that both carnivorous and herbivorous species inhabited the first 50 m of water.

Ecosystem in the shallow benthic areas of Lake Baikal turned out to be characterized by interspecific diversity of feeding behavior that includes the consumption of both planktonic organic matter and benthic plants at various rates. In contrast, benthic animals in deep benthic areas were found to consume predominantly planktonic organic matter. Therefore, in closing, this study strongly suggests that stable isotope analyses of ecosystem can be a useful indicator in estimating the level of complexity and diversity of food web structures in specific areas. Since the stable isotope ratios of organisms reflect their feeding behavior, the present natural abundance method can be a powerful tool in the process of examining both interspecific and intraspecific differences between possible food sources in Lake Baikal

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Appendix 1. Stable isotope compositon of benthic organisms and terrestrial plant. *f* value denotes the estimated percentage of benthic plants as food sources. T. L. denotes trophic level. Trophic levels and *f* values were calculated with average δ^{13}C and δ^{15}N data on the assumption of two source mixing model (see text in detail).

No.	Biota	Station number	Body length (mm)	Water depth (m)	δ^{13}C (‰)	δ^{15}N(‰)	T. L.	*f* (%)
	Primary Producers							
1	*Allium victorialis* (Terrrestrial plant)	2c			−28.0	−0.6		
2	Attached algae	41,93-N1	0.1–1		−9.44 ± 2.0 (3)	3.0 ± 0.6 (3)		
3	Macrophyte	6a, 20, 41	0.1		−10.7 ± 6.8 (3)	3.5 ± 1.0 (3)		
4	Nostoc	10a, 21	0.1		−7.6 ± 2.8 (2)	2.1 ± 0.7 (2)		
5	Spongia	1b, 92-5′	20		−26.4 ± 1.6 (3)	6.8 ± 2.2 (3)	1.8	3
6	*Benedictia limnaeoides* (Mollusk)	1b	20		−20.6	6.2	1.7	35
7	*Megalovalvata demersa* (Mollusk)	1b	20		−18.4	8.0	2.3	44
8	*Baicalia carinata* (Mollusk)	1b	20		−24.3	5.0	1.3	18
9	*Benedictia baicalensis* (Mollusk)	7	17–25		−19.9 ± 0.1 (2)	6.5 ± 0.2 (2)	1.8	38
10	Oligochaete	19, 24, 32, 27	13–200		−24.8 ± 0.6 (5)	9.3 ± 2.1 (5)	2.6	7
11	Tricoptera	41	5–20		−10.3	5.3	1.7	91
12	*Baicalina reducta* (Trichoptera	2c	0–10		−10.6	6.0	1.9	88
13	*Thomastes dipterus* (Trichoptera)	2c	0–10		−10.6	4.6	1.5	91
14	*Baicalobia guttata* (Turbellaria)	6a			−18.2	9.3	2.7	42
15	*Geocentrophora wagini* (Turbellaria)	7	17–25		−20.3	9.3	2.7	31
16	*Baikalobia variegata* (Turbellaria)	19	20–22		−6.5	6.0	2.0	110
17	*Bdellocephala angarensis* (Turbellaria)	25	5		−10.7	9.0	2.8	82
18	*Rimacepharus arcepta* (Turbellaria)	31	13		−26.8	13.2	3.7	−11
	Benthic Gammarids							
19	*Acanthogammarus albus*	2b	19		−19.7	9.3	2.7	34
20	*Acanthogammarus brevispinus*	29,32	22–30		−23.9 ± 0.5 (2)	10.6 ± 1.1 (2)	3.0	9
21	*Acanthogammarus flavus*	23	37		−19.2	9.3	2.7	36
22	*Acanthogammarus grewingki*	35	200		−25.9	11.6	3.2	−3
23	*Acanthogammarus reicherti*	31	100		−28.8	9.9	2.7	−15
24	*Acanthogammarus victorii maculosus*	2b	19		−13.5	7.9	2.4	69
25	*Acanthogammarus* sp.	1b, 7, 21	17–45		−18.6 ± 2.1 (4)	7.9 ± 0.8 (4)	2.3	42
26	*Brandtia (Spinacanthus) parasitica*	1b	20		−20.0	5.3	1.5	40
27	*Brandtia lattissima*	42	19		−13.4	6.0	1.8	74
28	*Cerratogammarus cornutus*	24, 35	13–200		−22.8	11.9	3.4	13
29	*Eulimnogammarus czerskii*	12	8		−14.9	6.8	2.1	64
30	*Eulimnogammarus maackii*	24	13		−17.4	8.7	2.6	47
31	*Eulimnogammarus verrucosus*	24	13		−16.6 ± 0.5 (2)	7.8 ± 0.9 (2)	2.3	53
32	*Eulimnogammarus* sp.	2c	0–10		−14.7	5.3	1.6	68
33	*Garjajewia cabanisi*	24	13		−22.4	10.9	3.1	17
34	*Hyalellopsis carpenteri*	41, 42	19		−16.8 ± 0.1 (2)	9.1 ± 1.1 (2)	2.7	50
35	*Hyalellopsis potanini*		30			10.1		
36	*Ommatogammarus albinus*		130		−25.9	12.8	3.6	−5
37	*Ommatogammarus flavus*		130		−25.2	14.7	4.2	−6
38	*Pachyschesis bazikalowae*		200–250		−24.1	11.7	3.3	6
39	*Pallasea brandtii*	23, 42	19–37		−18.2 ± 0.9 (4)	8.5± 1.0 (4)	2.5	43
40	*Pallasea cancellus*	21, 24	13–32		−12.1± 0.6 (3)	5.6± 1.6 (3)	1.7	81
41	*Pallasea gribei*	1b, 7	20		−18.2± 0.9 (2)	7.0± 1.2 (2)	2.0	46

Appendix 1. contd.

No.	Biota	Station number	Body length (mm)	Water depth (m)	δ¹³C (‰)	δ¹⁵N(‰)	T. L.	f (%)
42	*Paragarjajewia petersi*				−27.9	9.6	2.6	−10
43	*Parapallasea bolowskii*	31,37		100	−23.0	7.2	2.0	21
44	*Parapallasea lagowskii*	31		100	−24.7	10.4	2.9	6
45	*Poekilogammarus pictus*	21		32	−18.3	8.2	2.4	43
	Pisces							
46	*Abysocottus korotneffi*	S1	102	745	−26.3	13.2	3.7	−8
47	*Asprocottus herzensteini*	S2	70	60	−25.6	12.2	3.4	−3
48	*Asprocottus platycephalus*	S1	80–97	80–97	−26.0 ± 0.1 (3)	12.6 ± 0.1 (3)	3.5	−6
49	*Batrachocottus baicalensis*	93-N1	79–148	120–200	−17.2 ± 0.5 (2)	12.4 ± 0.1 (2)	3.6	41
50	*Batrachocottus milturadiatus*	S1	97–140	120–700	−23.8 ± 2.1 (4)	15.0 ± 1.6 (4)	4.3	1
51	*Batrachocottus nikolskii*	S1	140–160	700	−25.0 ± 0.1 (2)	13.7 ± 0.2 (2)	3.9	−2
52	*Cottinella boulangeri*	S1	80	700	−25.9	13.9	3.9	−7
53	*Cottus kessleri*	93-N1	92	120–200	−16.3	11.2	3.3	48
54	*Esox lusius*	92-5′, 93-N2	405	1.5–10	−17.9 ± 1.5 (2)	11.4 ± 0.4 (2)	3.3	39
55	*Leuciscus leuciscus baicalensis*	93-N1, 93-N4	157–165	1.5–200	−16.4 ± 1.2 (3)	10.3 ± 0.3 (3)	3.0	49
56	*Limnocottus bergianus*	S1	140–185	745	−25.4 ± 0.1 (2)	14.9 ± 0.5 (2)	4.2	−7
57	*Limnocottus godlewskii*	S1	109–112	700	−25.4 ± 0.2 (2)	13.5 ± 0.6 (2)	3.8	−4
58	*Limnocottus griseus*	S1	115–141	700	−24.2 ± 0.6 (3)	14.2 ± 0.4 (3)	4.0	1
59	*Limnocottus megalops*	S2	115	60	−26.1	11.8	3.3	−5
60	*Limnocottus pallidus*	S1	135	745	−25.4	14.6	4.1	−6
61	*Lota lota*	2c	451	1–10	−16.6	12.8	3.8	44
62	*Paracottus kneri*	93-N1, 24, 42	23–93	20–200	−17.9 ± 0.7 (3)	10.7 ± 0.7 (3)	3.1	41
63	*Perca fluviatilis*	92-5′, 93-N2	140–291	1.5–10	−17.8 ± 1.4 (3)	11.5 ± 1.0 (3)	3.4	40
64	*Procottus jeittelese*	93-N1	89–116	120–200	19.6 ± 0.2 (2)	12.2 ± 0.1 (2)	3.5	29
65	*Procottus major*	93-N1	96	120–200	−19.9	13.0	3.8	26
66	*Rutirus rutirus lacstrius*	10a, 93-N1, 93-N2	151–247	1.5–200	−17.9 ± 2.5 (5)	10.4 ± 0.6 (5)	3.0	41
67	*Thymallus arcticus*	2c, 93-N1, 93-N4	166–355	1.5–10	−15.6 ± 2.2 (16)	10.7 ± 0.8 (16)	3.2	53