Effects of salmon carcass on forest and stream ecosystems, in Hokkaido, Japan –evidence by stable isotope analysis-

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The effects of salmon carcasses on forest and stream ecosystems were determined by nitrogen stable isotope analysis in natural streams in Hokkaido, Northern Japan, where numerous chum salmon (*Oncoryhncus keta*) were migrated upstream from ocean to spawn in autumn. The leaves and soils surrounding riparian forest and stream dwelling invertebrates were collected before and after migration. The nitrogen stable isotope ratio ($\delta^{15}N$) of riparian vegetation (*Salix* spp.) were different depending on the presence of salmon and distance from the stream. The $\delta^{15}N$ of stream dwelling invertebrates were different between salmon present and absent stream. This difference was tested using the experiment channel by implanting salmon carcasses. The nitrogen stable isotope ratio of epilithic algae and leaf shredding animals were nearly 3‰ higher in the salmon implanted treatment suggesting that around 20% of salmon derived nitrogen was uptake either in algae and leaf shredding invertebrates. These results suggest that the salmon carcasses effects not only on stream primary production but also on primary consumers, which decompose leaves fertilized with nitrogen from carcasses

Key-words: Riparian forest, Stream invertebrates, Stable isotope, Salmon carcasses and Hokkaido.

1. Introduction

In some ecoregions, a massive flux of organic material and nutrients occurs annually from marine to freshwater and terrestrial ecosystem via upstream migration of anadromous fishes[3]. Recent studies in Northern America have demonstrated that salmon carcasses influence on nutrient levels of stream water[14], enhance the primary productivity[5)17], and productivity and community composition of the aquatic food web[1)2)4]. Those effects are largely due to the releases of often limiting nutrients such as phosphorus and nitrogen, and cascading effects of enhanced nutrient levels on primary producers, primary as well as secondary macroinvertebrate consumers, and predacious fish including juvenile salmonids. The effects of salmon carcasses extends beyond aquatic ecosystems as carcasses uplifted to forest by large mammals such as fox, mink and bear[8], enrich forest soil and the growth of trees in riparian zones[7].

Despite the large populations of anadoromous salmonid species such as chum (*Oncorhynchus keta*) distributed along the Pacific Coast and their socio-economic significance, there is little knowledge about the ecological roles of salmon carcasses, in critical ecosystem functioning such as organic matter processing in East Asia[15].

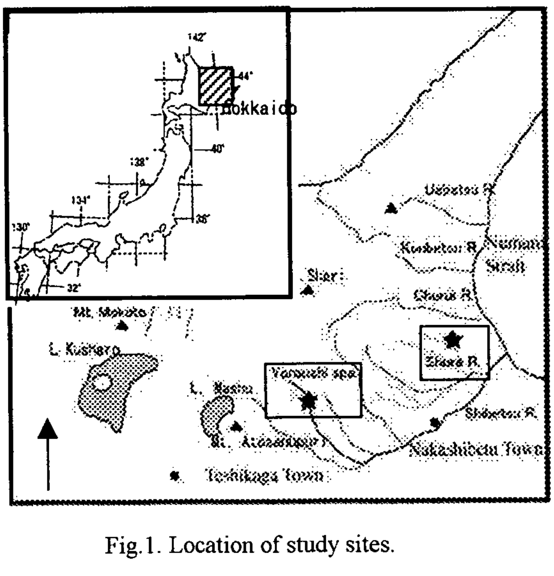

Fig.1. Location of study sites.

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The objective of this study was to examine the effects of carcass loading on riparian forest and stream ecosystems. In order to evaluate the uptake rate of Marine Derived Nutrient, we especially employed the stable isotope analysis techniques.

2. Method

2.1. Study site

We carried out field sampling at Shibetsu river, which is drained from Mashu Lake to Okhotsuku Sea and has an area of 671km^2(Fig.1). Sampling sites were selected in the two tributary with similar stream morphology but different salmon redding density: Shura and Yoroushi sites. The redding density at Shura site was 1.8/m, whereas no redding salmon was observed at Yoroushi site. These streams were ten meters wide, two percent slope having cobble substrate. The riparian vegetation was dominated by willow (*Salix* spp.), maple (*Acer mono*) and oak (*Quercus mongolica* var. *grosseserrata*). The fishes dwelling in the stream were mainly resident masu salmon (*Oncorhynchus masou*) and sculpin (*Cottus nozawae*). Anadoromous masu salmon and chum salmon (*Oncorhynchus keta*) were migrated from ocean to spawn from September to December.

The artificial channel experiment was conducted at Gokibiru brook that drains an area of 20 km^2 into the Sea of Japan, western Hokkaido. The channel was constructed adjacent natural stream, directly introduced water from the stream.

2.2. Flield sampling

The terrestrial tree leaves and soils were collected for stable isotope analysis at two riparian sites with different distances from the stream: within 5m and beyond 25m. The former site seemed to be influenced much stronger by salmon carcasses. The sampling species was willow (*Salix* spp.), which was collected from more than three different individuals with similar height and diameter. The soil samples were also collected at two different distances from stream. The surface humic soil layers (A0 layer) were collected with more than three replication.

Aquatic invertebrates were sampled before migration (August) and after migration (November) at both sites. The collected species were targeted on five species, which are the most common species such as *Jezogammarus jesoensis*, Tipulia sp., Ephemerella sp., *Epeoros* sp. and *Stenopsyche marmorata*.

2.3 Artificial Channel experiment

The effects of salmon carcasses was tested by artificial channel constructed in the natural stream. The experimental channel consisted of nine lanes, each of which sized 30cm wide, 20cm high and 10m long. The gradient of channel was adjusted to approximately 2%, allowing water to flow slowly with less than 5cm per second and approximate depth of 3cm.

Leaf bags and ceramic tiles were provided in all 9 lanes to quantify decomposition rates and primary productivity to test the effects of salmon carcasses and aquatic invertebrates. The 9 lanes were randomly assigned to two treatments and control with 3 replicates: 1) salmon and invertebrates, 2) without salmon but invertebrates, and 3) control. The arrangements of the treatments were randomly determined so as not to have same treatments in adjacent lanes. The salmon treatment was achieved by placing carcasses of chum salmon. The carcasses were consistently exposed to fresh flowing water. Three replicated samples of epilithic algae growing on the upper surface of the tile were collected every two weeks by scraping and rinsing them with distilled water into polyethylene bottles. Water samples for dissolved nutrient analyses were collected at the downstream end of the lanes with 200ml polyethylene bottle after each sampling occasion. Aquatic invertebrate inside leaf bags were also collected, and number and wet mass were measured.

2.4. Stable isotope analysis

The riparian tree leaves, soil and stream macroinvertebrates were prepared by filtration through GF/F fiber glass and grinding to fine particle after drying at 50 °C for 48 hours, respectively, and were analyzed using element analyzer (CE Instruments EA1110, Italy) and mass analyzer (Finnegan MAT, DELTA plus), which were owed by aqua-sphere ecosystem laboratory, Tohoku University. The fluctuation of stable isotope in natural environment was extremely small, therefore stable isotope composition was represented by following equation :

$$\delta X = [(R_{sample} - R_{standard}) / R_{standard}] \times 1000 \ (‰)$$

: where R_{sample} is ratio of stable isotope, $R_{standard}$ is ratio of standard sample, namely nitrogen in atmosphere.

3. Results

3.1. Stable isotope ratio for leaves and soils

The nitrogen stable isotope for willow leaves was different from the salmon presence and distance (Fig.3). The $\delta^{15}N$ at the Shura site was much higher than Yoroushi site on each riparian point. Within 5m, the Shura site showed -0.01‰, whereas that of Yoroushi was −1.8 ‰. Similar trend was observed beyond 25m. We did not observed significant difference of $\delta^{15}N$ between the two distance at Yoroushi sites ($P>0.05$). The $\delta^{15}N$ for surface soil was significantly different between Shura and Yoroushi sites($P<0.05$)(Fig.4.).

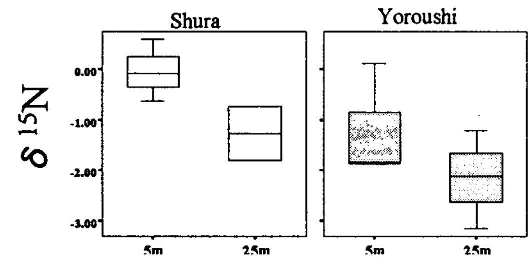

Fig.3. Mean $\delta^{15}N$ for willow leaves at different distance from stream at salmon present and absent sites.

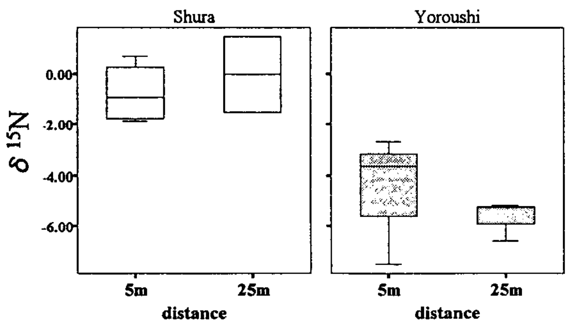

Fig.4. Mean $\delta^{15}N$ for surface soil layer at different distance from stream at salmon present and absent

The Shura site was 3 to 4‰ higher than Yoroushi. However, the significant difference on the distance at same sites was not recognized ($P<0.05$).

3.2 Stable isotope ratio for aquatic invertebrates

The $\delta^{15}N$ difference before and after salmon migration was demonstrated in Fig.5. The *Stenopyche* sp. and *Jezogammarus.*, which was classified as collector gatherer and shredder, showed 1 to 2‰ increase and the Ephemerella sp. showed 7‰ increase at Shura. The $\delta^{15}N$ of *Epeorus* sp. and *Stenopsyche* sp. at Yoroushi was changed very slight.

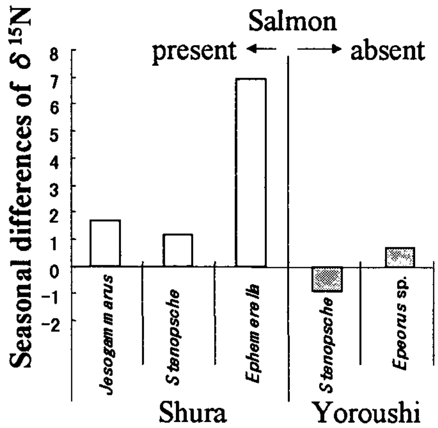

Fig.5. Seasonal change of $\delta^{15}N$ for aquatic macroinvertebrates at Shura and Yoroushi site.

3.3 channel experiment

The concentrations of dissolved nutrients during the study period were shown in Table 1. The 'salmon and invertebrates' treatment showed consistently higher concentration of NH_4^+ relative to the other. Although the 'salmon and invertebrates' treatment showed the highest mean concentration of NO_3 (2.4 $mg \cdot l^{-1}$) among three treatments, the treatment effect was unclear. The DOC values change temporally that low concentration with 1 $mg \cdot l^{-1}$ from 2weeks after rose up to 1.86 $mg \cdot l^{-1}$ at 6 weeks, however significant differences between three treatments was not observed ($P=0.78$).

We observed higher concentration of the chlorophyll in the salmon placement treatment throughout of the experiment: 'salmon and

Table 1. Mean concentrations ($mg \cdot l^{-1}$) (± 1 SE) of nutrients (NO_3^-, NH_4^+ and DOC) at each sampling occasion for the three treatments.

Nutrient		Treatment		
		Control	Invertebrate	Salmon and invertebrates
NH_4^+	2 weeks	0	0.01 (0.01)	0.04 (0.01)
	4 weeks	0	0.01 (0)	0.05 (0.01)
	6 weeks	0.01 (0.01)	0.01 (0)	0.06 (0.01)
NO_3^-	2 weeks	2.38 (0.03)	2.43 (0.03)	2.47 (0.03)
	4 weeks	2.33 (0.01)	2.48 (0.11)	2.36 (0.05)
	6 weeks	1.97 (0.02)	2.04 (0.03)	2.48 (0.11)
TOC	2 weeks	0.88 (0.12)	0.91 (0.02)	1.04 (0.04)
	4 weeks	1.37 (0.04)	1.39 (0.18)	1.21 (0.09)
	6weeks	1.46 (0.31)	1.6 (0.27)	1.86 (0.25)

invertebrates treatment' was 1 $mg \cdot l^{-1}$, while other treatments were under 0.25 $mg \cdot l^{-1}$, that was also significantly different ($P<0.01$). The concentration gradually decline at four and six weeks in the former treatment to 0.8 $mg \cdot l^{-1}$, that was relatively higher comparing to the other treatment (0.3 $mg \cdot l^{-1}$).

The mean value of stable nitrogen for leaf shredding invertebrate, *G. satoi* after four weeks was illustrated in Fig. 6. The 'salmon and invertebrates' treatment showed definitely higher isotope value than that of 'invertebrates' treatment throughout the experiment period. The means of 'salmon and invertebrates' treatment was 3‰, whereas invertebrates without salmon carcass were around 0‰. This was significantly different ($P<0.05$). The epilithic algae in salmon placement treatment also showed significant higher value than other treatment at four weeks($P<0.05$). This result means that the nitrogen derived from salmon carcasses was uptake not only by epilithic algae, even by

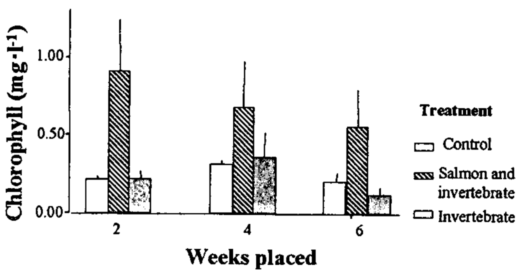

Fig. 5. Mean (±1SE) chlorophyll concentration at each sampling occasion for three treatments.

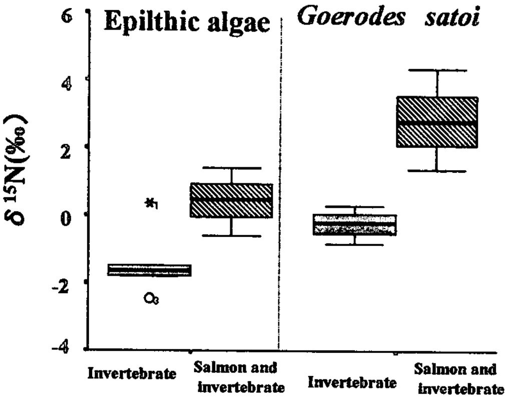

Fig 6. Stable nitrogen ($\delta^{15}N$) *in Goerodes satoi* from the treatments with and without salmon carcass at four-week sampling occasion. Error bars represent maximum and minimum value, and box indicates 75% (upper) and 25% (lower) values,

shredders. Based on the calculation of two factor mixing model, salmon contributed 15.3, 22.7% of epilithic algae and *G. satoi* production, respectively.

4. Discussion

Several studies using stable isotope analysis in North America suggested that the salmon carcasses possibly effect on the growth of riparian vegetation[1)7)]. For instance, conifer and undergrowth shrub growing riparian zone where salmon carcasses present in Washington states showed three per mil higher $\delta^{15}N$ than salmon absent stream[1)]. The willows growing Fraser river showed around 2.73‰ of $\delta^{15}N$, and higher $\delta^{15}N$ was observed in higher density of salmon carcasses[10)]. The contribution of MDN for riparian vegetation was around 22 ~24% at Alaskan stream[7)]. In this study, the $\delta^{15}N$ at salmon redded stream showed relatively higher than salmon absent stream. This is possible that salmon carcasses uplifted from spawning site influenced the riparian vegetation. Another possibility for the reason of higher $\delta^{15}N$ attributed to agricultural influence. The Shura watershed was utilized as a cattle farming, and higher nitrate released from cattle breeding facility. However, the sampling sites were located on the higher terrace and it was unlikely that the vegetation was always affected by water contaminant from agricultural facility. The MDN contribution rate was in the range of north American case.

The $\delta^{15}N$ for soil layer at Shura site was relatively higher than Yoroushi site, however, no trend was observed in terms of the distance from the stream. The $\delta^{15}N$ beyond 25m showed much higher than within 5m in both sites. The $\delta^{15}N$ for soil nitrogen tend to be affected depending on the topography, soil depth and edaphically condition[6]. The topography of sampling sites were mostly plain, but small differences for height from water level was observed. That made some differences of water condition and denitrification, which eventually may result in the difference of $\delta^{15}N$ value.

With respect to stream community, we can observe large increase of $\delta^{15}N$ after salmon migration at Shura site, whereas significant difference was not observed at Yoroushi site. There were many studies dealing with salmon effects for stream ecosystem, especially for primary production and consumers. The $\delta^{15}N$ for epilithon was +6 ‰ higher after salmon migration, which was attributed MDN released from salmon carcasses[12]. The mayfly, which scrape epilithic algae, may be indirectly influenced by salmon carcasses at Shura site. Other taxa, such as an amphipoda may also indirectly influenced by salmon, for amphipod preferred and grew larger by eating leaves fertilized by salmon carcasses[9].

The channel experiment clearly showed the indirect effect of salmon carcasses on stream water quality, primary production and leaf processing by comparing non-salmon placement treatment. We firstly observed significantly higher ammonium and chlorophyll concentration in the salmon placement channels. Previous studies have shown that the fish carcasses leach out and release nutrients into the water to stimulate growth of suspended and attached algae in spawning streams and lakes[4)5)16]. Considering these studies, salmon carcasses could enhance the ammonium concentration and stream productivity in Northern Japanese stream.

There were also some experimental studies that reported the role of shredders on leaf decomposition enriched by salmon in the laboratory. The growth rate of Limnephilus sp. (Limnephildae) was higher when both salmon and leaves were supplied and suggested that carcasses would enhance food quality of leaves[13]. We did not detected significant increase of shredder abundance in salmon placement channels, but we detected an increase in $\delta^{15}N$ on epilithic algae and shredder immediately downstream of carcasses that confirm the incorporation of carcass-derived nitrogen into biofilm and stream biota. Periphyton with about 90% marine derived nitrogen incorporated into its biomass in Alaskan stream[11]. The rate of salmon derived nitrogen for wood biofilm are ranging from 14 to 27%[5]. These values were similar to our results calculated by a double endpoint-mixing model.

Determining all functions of salmon escapements in freshwater- riparian ecosystems is crucial for understanding marine-freshwater-terrestrial linkages for ecosystem management. In Japan, most of the rivers and stream have been altered by human activity after 1960's. Dams and weirs prevent salmon migration from ocean to stream. Numerous sediment and nutrient derived from agricultural land also deteriorated water quality, which eventually destroy spawning habitat for salmonid fishes. Recently, many restoration works have carried out by near nature engineering technique, and some physical environments may be improved. However, habitat restoration should include not only measures to ensure the protection and enhancement of physical attributes of the stream but also consider the nutritional health of the system. There have been few studies dealing with the salmon carcasses effects and we could not determine the magnitude of damage for fisheries production and ecological healthiness by interception of salmon migration from ocean. More studies are needed and should focus on the influence of salmon migration not only on stream but terrestrial ecosystems.

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