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Infection of *Anisakis simplex* (Nematoda: Anisakidae) larvae in chum salmon (*Oncorhynchus keta*) in the North Pacific Ocean, Bering Sea, and a river of Hokkaido

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ABSTRACT

In summer season, chum salmon (*Oncorhynchus keta*) in the Bering Sea had higher prevalence and abundance of the nematode *Anisakis simplex* larvae than those in the Gulf of Alaska. The infection level of *A. simplex* larvae was much higher in adult chum salmon, which returned to the Chitose River, Hokkaido. There were no difference in the abundance of parasite larvae among ocean age groups of adult chum salmon in the same return year, while significant differences were detected among ocean age groups of the same brood year class. These results indicates that sibling of Chitose River chum salmon population was not infected simultaneously but received the sympatric infection with *A. simplex* larvae in the ocean. Japanese chum salmon may be highly infected with *A. simplex* larvae during the homing migration from the Bering Sea to the Hokkaido coast.

KEY WORDS: *Anisakis simplex*, chum salmon, Hokkaido, North Pacific Ocean, Bering Sea

INTRODUCTION

The nematode *Anisakis simplex* larvae have been found in various species of marine fish and squids (Kagei 1970), including masu (*Oncorhynchus masou*) and chum salmon (*O. keta*) in Japan (Urawa 1986). The adult form of *A. simplex* lives in the stomach of marine mammals, especially cetaceans. Minke whale (*Balaenoptera acutorostrata*) is considered as a major final host of *A. simplex* in the western North Pacific Ocean, because of abundant infection with adult worms in their stomach (Kuramochi et al. 1996). The eggs are released from the digestive tract of final hosts into seawater, and after hatching in the sea the larvae infect small crustaceans, especially euphausiids such as *Euphausia pacifica* and *Thysanoessa longiceps* (Shimazu and Oshima 1972). The larvae transit to fish, squid or whales, which feed the infected crustaceans. The nematodes remain in larval stage in fish or squids which serve as paratenic hosts, while they develop to adult stage in cetaceans, which consume the infected crustaceans, fish or squids.

The infection level of *A. simplex* larvae in fish differs among sampling sites and changes in season (Yamaguchi et al. 1968; Saito et al. 1970; Oshima 1972; Konisi and Sakurai 2002). Uzmann (1957) reported that the infection level of *A. simplex* larvae in chum salmon was higher in the western North Pacific Ocean than in the eastern water.

Anisakis simplex larvae infect several tissues of fishes. In walleye pollock (*Theragra* *chalcogramma*) and common mackerel (*Scomber japonicus*), the larvae mainly infect the visceral organs (Itagaki and Ishimaru 1967; Otsuru 1968 a, b; Saito et al. 1970). In Pacific herring (*Clupea pallasii*), the parasite was more abundant in the visceral organs than in the muscle (Otsuru 1968a). In chum salmon, however, *A. simplex* larvae appeared mainly in the muscle (Uzmann and Lander 1958). Kuiper et al. (1960) and Van Thiel et al. (1960) assumed that *A. simplex* larvae moved from visceral organs to muscle after the death of hosts, because of the shift of host's physiological condition. However, Oshima (1972) observed *A. simplex* larvae in the muscle of salmon immediately after their death. Urawa (1986) also reported that *A. simplex* larvae markedly appeared in the muscle of adult chum salmon returning to the Chitose River for spawning. It has been unknown why *A. simplex* larvae prefer the muscle as the infection site in salmon.

In general, the relationship between abundance of *A. simplex* larvae and body size or ocean age of host fishes indicates the positive correlation (e.g. walleye pollock (Konisi and Sakurai 2002), common mackerel (Otsuru 1968a)). On the other hand, Urawa (1986) found that adult chum salmon which returned to the Chitose River in Hokkaido did not show increase in number of *A. simplex* larvae with the ocean age of host fish. He suspected that the infection period of *A. simplex* was limited for Japanese chum salmon. However, few ecological surveys of anisakid larvae in Pacific salmon have been carried out for more than two decades since his study.

The aim of the present study was to clarify infection level of *A. simplex* larvae in chum salmon captured in the North Pacific Ocean, Bering Sea, and Chitose River in Hokkaido, and to discuss on ocean distribution of *A. simplex* larvae based on the life history strategy between chum salmon and *A. simplex* larvae.

MATERIALS AND METHODS

Fish samples

Chum salmon (n=193) were collected by surface-trawl net operation aboard R/V *Kaiyo maru* (Fisheries Agency of Japan) at three locations (WB, CB, and EB) in the Bering Sea and a location (WGA) in the Gulf of Alaska, and by gillnet operation abroad T/V *Osyoro maru* (Hokkaido University) at a location (EGA) in the Gulf of Alaska in 2002 and 2003 (Fig. 1, Table 1). Fish were measured for fork length and body weight, and sexed aboard the ships. The fish samples were immediately frozen after the measurement, and kept at the National Salmon Resource Center until the parasite surveys.

We collected adult chum salmon (n=650) returning to the Chitose River, which is a tributary of the Ishikari River System in the Japan Sea coast of Hokkaido (Fig. 1, Table 1). We measured FL and BW, sexed, and collected scales in the INPFC-preferred area of the body for each chum salmon. Subsequently, the muscle and organs were separately frozen at the National Salmon Resource Center. By the scale readings, we decided the age of salmon at maturity using the scale image processor (Ratoc System Engineering Co.).

Parasite survey

We examined the muscle for *A. simplex* larvae using candle and slice methods (Nakajima and Egusa 1976). In addition, pyloric caeca, stomach, intestine, liver, and celom were examined under the microscope or the naked eyes.

Date Analyses

The level of parasitic infection was evaluated by three measures (Margolis et al. 1982).

Prevalence: percentage of infested hosts in a sample

Mean intensity: mean number of parasites per infected host

Abundance: mean number of parasites per host examined

Because number of *A. simplex* larvae in chum salmon indicated the non-parametric distribution, non-parametric analyses such as the Mann-Whitney's U-test, the Kruskal-Wallis test, and the Spearman's correlation coefficient were used to compare the infection levels among populations, and relationship between body characters (body size and CF) and number of parasites, respectively.

RESULTS

Infections in chum salmon in the ocean

The abundance of *A. simplex* larvae was significantly higher in the Bering Sea than in the Gulf of Alaska ($Z=-7.5$ and $P<0.01$ in 2002 year, $Z=8.1$ and $P<0.01$ in 2003 year; Fig. 2). The prevalence differed between two sampling stations in the Gulf of Alaska ($Z=-3.6$, $P<0.01$), although it indicated no difference among three sampling stations in the Bering Sea ($H=5.42$, $P=0.07$; Table 2).

Infections in adult chum salmon in the Chitose River

In adult chum salmon returning to the Chitose River between 2001 and 2003, prevalence of *A. simplex* larvae was almost 100%. The mean abundance of *A. simplex* larvae indicated annual changes: 19.9 individuals in 2001, 9.5 individuals in 2002, and 30.3 individuals in 2003 ($H=119$, $P<0.05$; Table 2, Fig. 3). There were no significant differences in abundance of *A. simplex* larvae among ocean age groups of adult chum salmon returning in 2001 ($H=0.04$, $P>0.05$), 2002 ($H=1.75$, $P>0.05$), nor 2003 ($H=4.9$, $P>0.05$)(Fig. 4). Within the brood-year class, however, they indicated significant differences in abundance of *A. simplex* larvae by ocean age ($H=4.2$, $P<0.01$ in 1998, $H=45$, $P<0.01$ in 1999, and $H=6.1$, $P>0.01$ in 2000 brood year class)(Fig. 5).

There were no apparent correlations between body size of chum salmon and number of *A. simplex* larvae ($r<0.2$, $Z<1.42$, $P>0.05$; Fig. 6). There was also no sexual difference in abundance of *A. simplex* larvae in chum salmon ($Z<2$, $P>0.05$).

Infection sites

Anisakis simplex larvae were more abundant in the muscle than in other organs (Table 3). Especially, the parasites were found in the muscle around body cavity (Fig. 7).

DISCUSSION

Adult chum salmon returning to the Chitose River in the same year showed no significant difference in abundance of *A. simplex* larvae among the ocean age groups. Urawa (1986) also reported the similar results. Within the brood-year class, however, there were significant differences in abundance of *A. simplex* larvae among age groups. Sibling of chum salmon population may be not infected simultaneously, but might receive the sympatric infection with *A. simplex* larvae in the ocean.

The infection level of *A. simplex* larvae in chum salmon differed among sampling stations in the Bering Sea and the Gulf of Alaska. Uzmann et al. (1958) reported that the infection level of *A. simplex* in chum salmon was higher in the western water than in the eastern water of the North Pacific Ocean. They also confirmed the abundance of *A. simplex* larvae in chum salmon returning the river was higher in Hokkaido than in North America. In summer season, immature chum salmon originated from Japan, Russia, and West Alaska are distributed mainly in the Bering Sea, while chum salmon from southeast Alaska, B.C., Washington, and Oregon migrate mainly in the Gulf of Alaska (Salo 1991; Urawa et al. 2000). Thus the low infection of *A. simplex* in chum salmon caught in the Gulf of Alaska may reflect the origin of sample fish.

Based on the migration model of Japanese chum salmon (Urawa 2000), chum salmon juveniles migrate to the Okhotsk Sea from coast of Hokkaido in the late spring, staying there by the late autumn. Subsequently, they migrate to the northwest North Pacific Ocean for the first wintering. In the next late spring, they migrate to the Bering Sea, where they feed by the late autumn. They spend in the Gulf of Alaska in winters after the second year. At maturing, chum salmon return to Japanese coast through the Being Sea off the eastern coast of Kamchatka and along the Kuril Islands. Andrievskaya (1957) reported that the stomach contents of chum salmon caught in the western North Pacific contained 16% of euphausiids such as *Thysanoessa longipes*, *T. irermis*, *T. raschii*, and *Euphausia pacifica*, all which are known as the intermediate host of *A. simplex*. As assumed by Urawa (1986), chum salmon may be markedly infected with *A. simplex* larvae when they feed euphausiids during the spawning migration from the Bering Sea to the Japanese coast.

The present study confirmed that *A. simplex* larvae are most abundant in the muscles around the body cavity even when chum salmon migrate in the ocean or return to the freshwater, although in other marine fishes the parasites infect mainly the body cavity and visceral organs. The parasite concentration in the muscle of salmon may not be due to physiological changes in host fish nor environmental changes, but reflect some benefit for the survival of parasites. Further studies are required to clarify why *A. simplex* larvae concentrate in the muscle of salmon.

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Table 1. Sampling location, period, and number of chum salmon examined for *A. simplex*.

Location		Month & Year	Number of samples
Bering Sea	EB (55°N, 177°W)	Sep. 2002	43
	WB (52°N, 177°E)	Sep. 2002	50
	CB (57°N, 179°E)	Jul. 2003	100
Gulf of Alaska	EGA (50-56°N, 145W)	Jul. 2003	30
	WGA (53°N, 165°W)	Aug. 2003	50
Chitose River		Sep. 2001	30
		Oct. 2001	82
		Nov. 2001	74
		Dec. 2001	50
		Sep. 2002	97
		Oct. 2002	106
		Nov. 2002	91
		Dec. 2002	40
		Oct. 2003	80

Table 2. Prevalence, abundance, and mean intensity of *A. simplex* larvae in chum salmon.

Location		Year	Number of samples	Prevalence (%)	Abundance	Mean intensity
Bering Sea	EB	2002	43	98.0	16.5	16.9
	WB	2002	50	92.9	7.9	8.7
	CB	2003	100	100	12.6	12.6
Gulf of Alaska	EGA	2002	30	16.7	0.4	2.4
	WGA	2003	50	64.0	1.8	2.9
Chitose River		2001	236	99.6	19.9	19.9
		2002	334	97.6	9.5	9.8
		2003	80	100	30.3	30.3

Table 3. Abundance of *A. simplex* larvae in chum salmon by muscle and organs.

Location		Year	Pyloric caeca	Stomach	Intestine	Gonad	Liver	Spleen	Celom	Muscle
Bering Sea	WB	2002	0	0	0	0	9.5	0	2.4	7.8
	EB	2002	0	0	0	0	0	0	0	16.5
	CB	2003	-	-	-	-	-	-	0	12.6
Gulf of Alaska	EGA	2002	0	0	0	0	0	0	0	2.4
	WGA	2003	0.1	0	0.1	0	0.1	0	0	1.6
Chitose River		2001	0.1	0	0	0	0.1	0	0.1	19.5
		2002	0.1	0	0	0	0.1	0	0	9.3
		2003	0.1	0	0	0	0.2	0	0.1	29.9

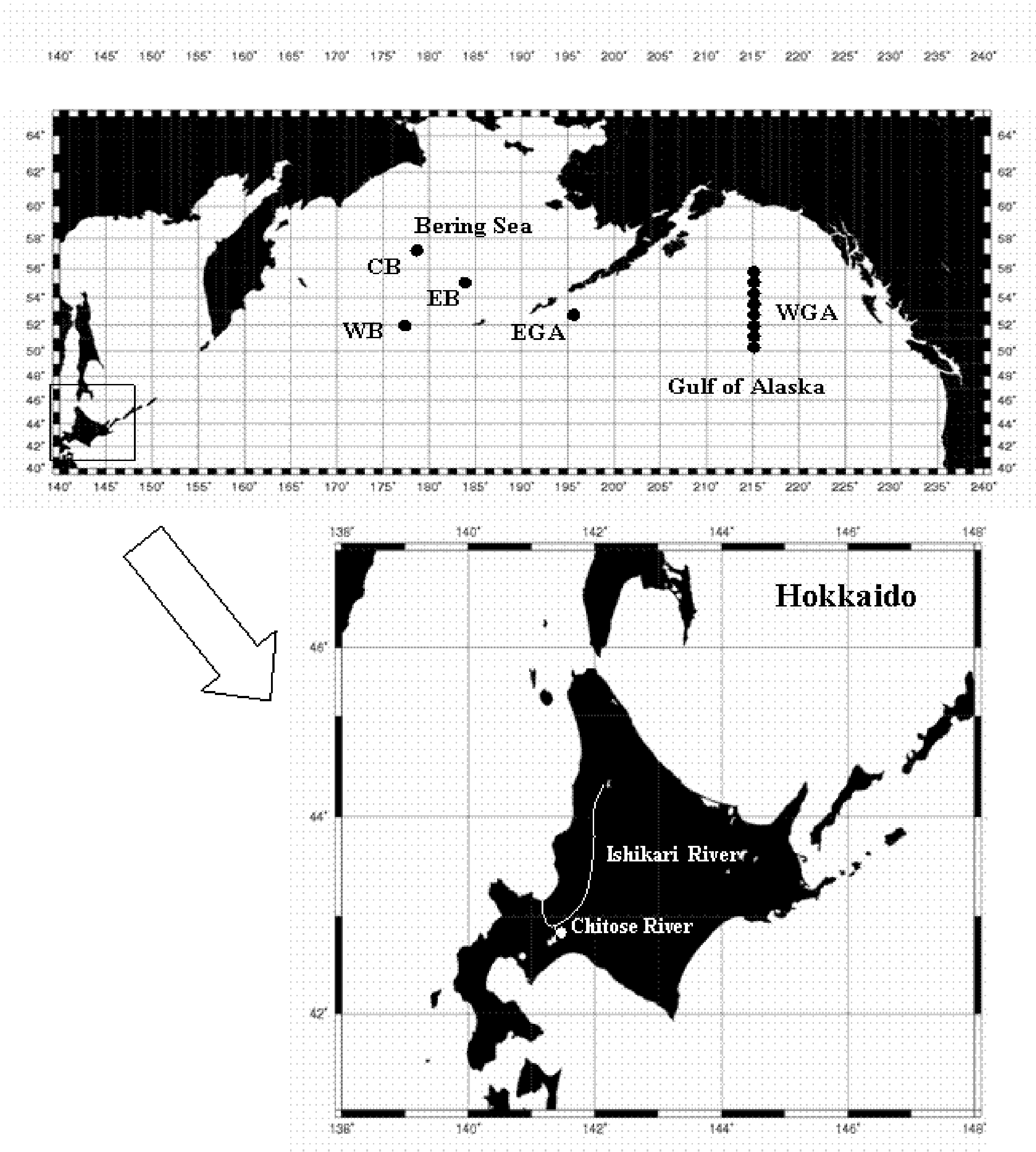

Fig. 1. Maps of sampling locations: the Chitose River of the Ishikari River System in Hokkaido, the Bering Sea (WB: 52°N, 177°E; CB: 57°N, 179°E; EB: 55°N, 177°W), and the Gulf of Alaska (WGA: 53°N, 165°W; EGA: 50-56°N, 145°W).

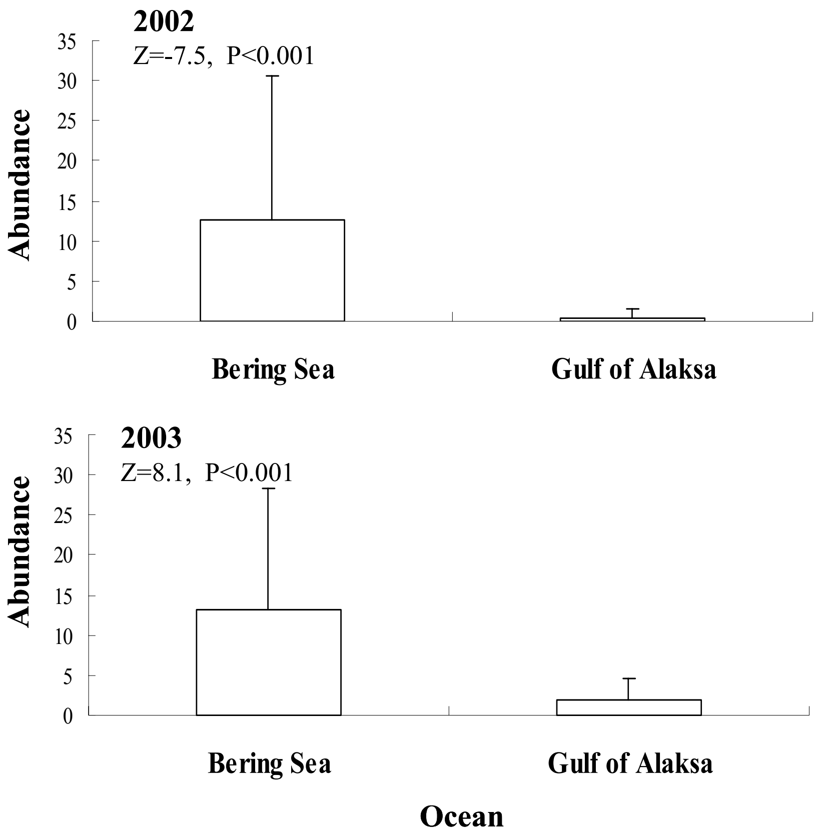

Fig. 2. Abundance of *A. simplex* larvae in immature chum salmon captured in the Bering Sea and the Gulf of Alaska, 2002-2003. Bars indicate SD. U-test.

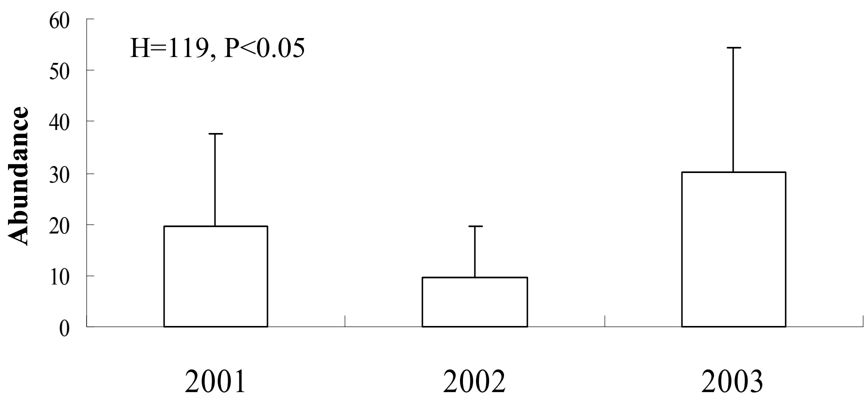

Fig. 3. Annual changes in the mean abundance of *A. simplex* larvae in adult chum salmon in the Chitose River, Hokkaido. Bars indicate SD. Kruskal-Wallis test.

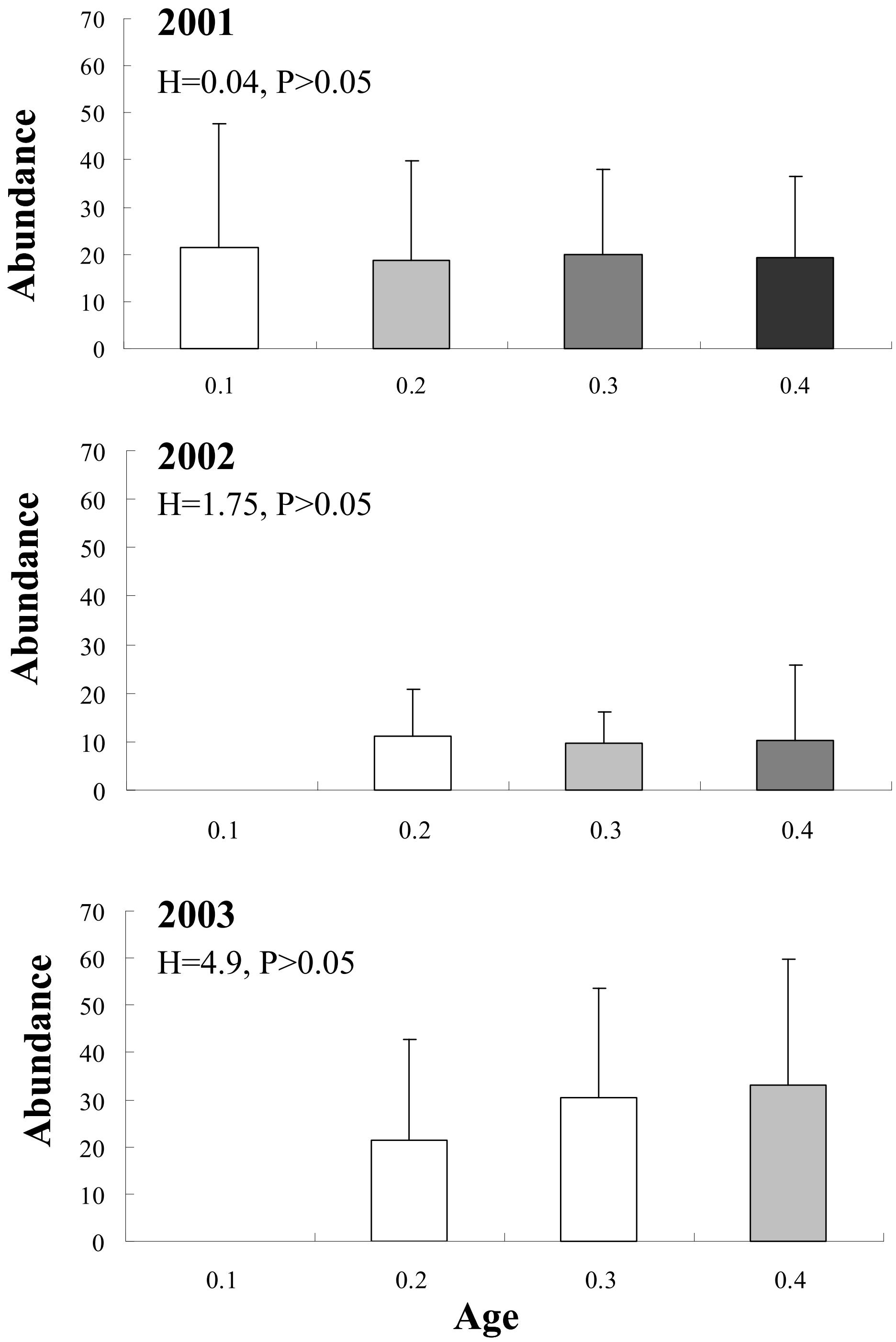

Fig. 4. The abundance of *A. simplex* larvae in adult chum salmon returning to the Chitose River in the falls of 2001-2003 by the age. Bars indicate SD. Kruskal-Wallis test.

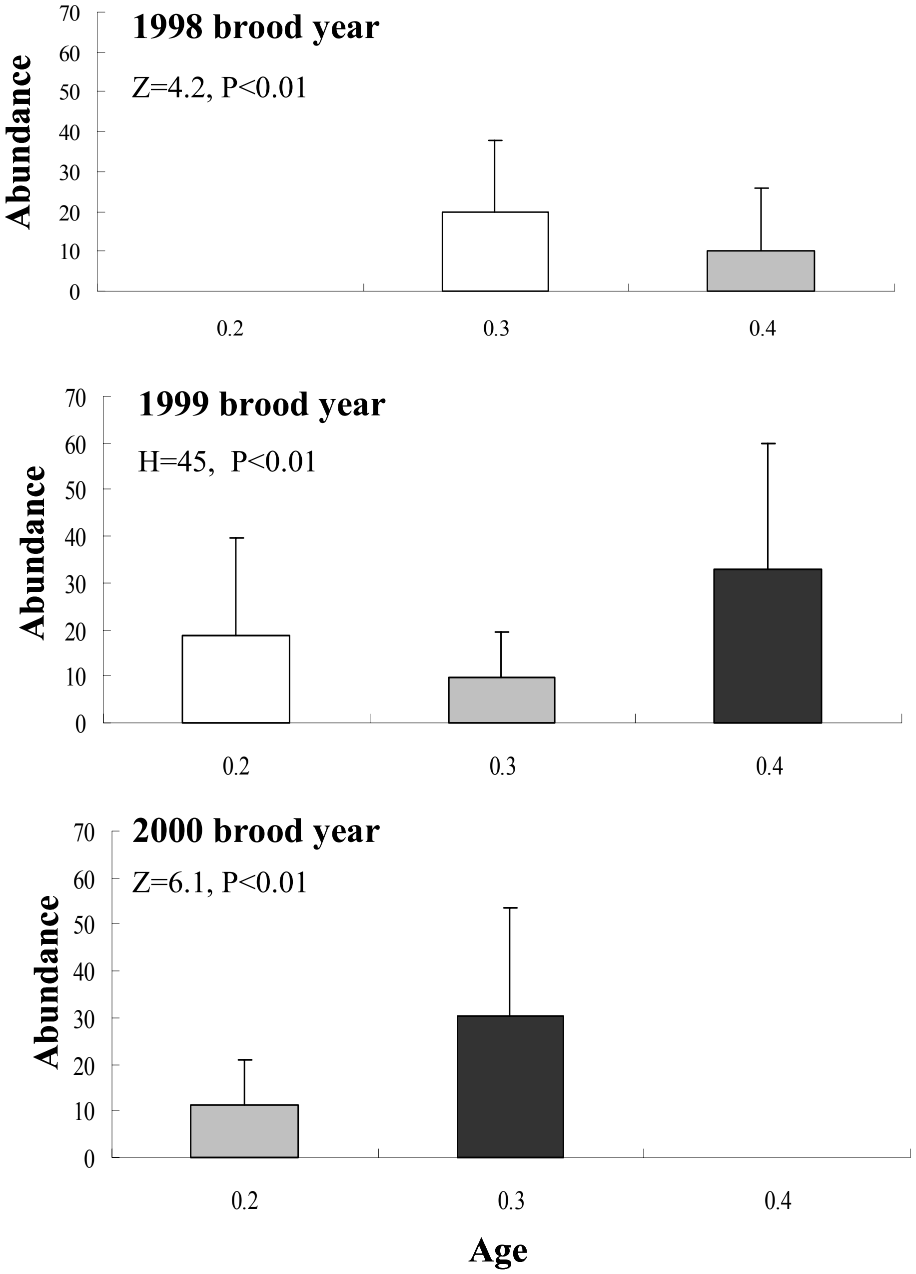

Fig. 5. The abundance of *A. simplex* larvae in 1998, 1999, and 2000 brood years of adult chum salmon returning to the Chitose River in the falls of 2001-2003 by the age. Bars indicate SD. U-test and Kruskal-Wallis test.

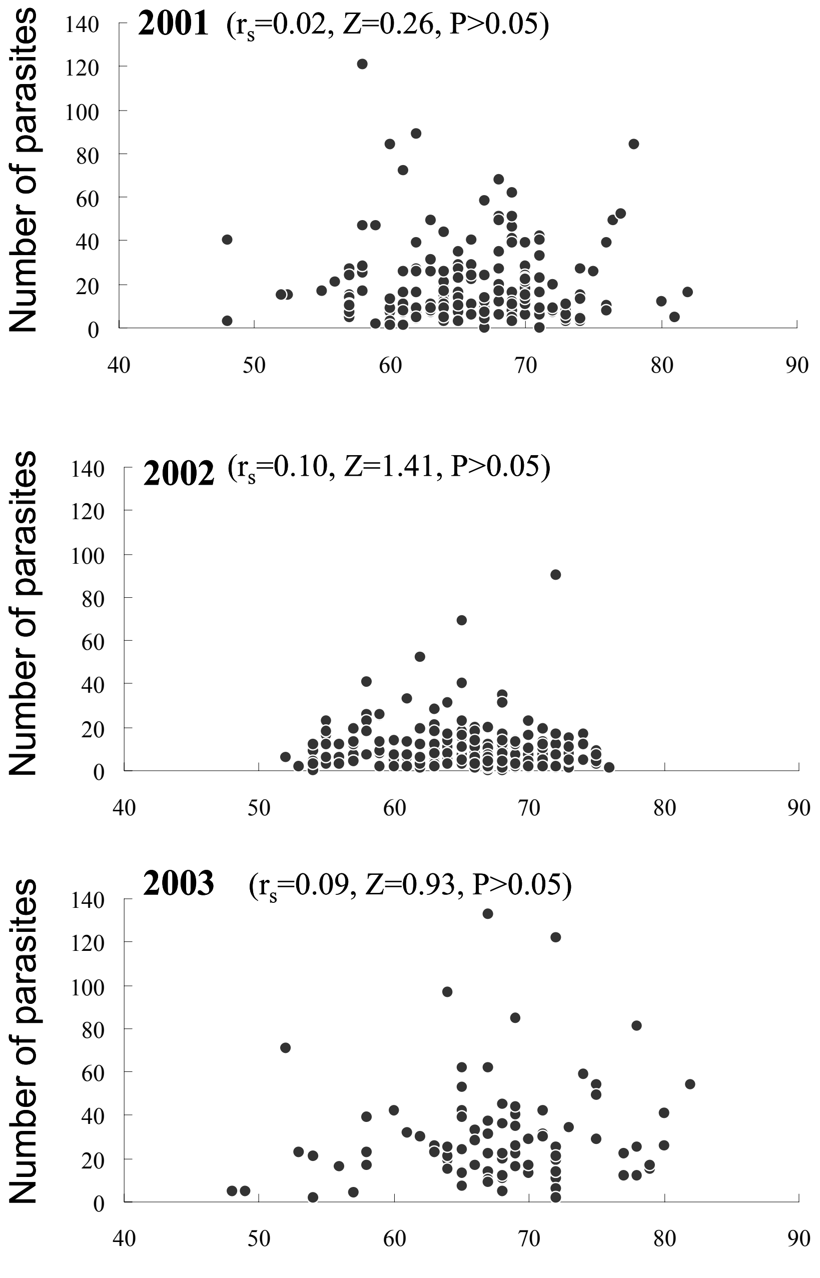

Fig. 6. Relationship between number of *A. simplex* larvae and fork length in adult chum salmon returning to the Chitose River in the falls of 2001-2003. Spearman's correlation coefficient.

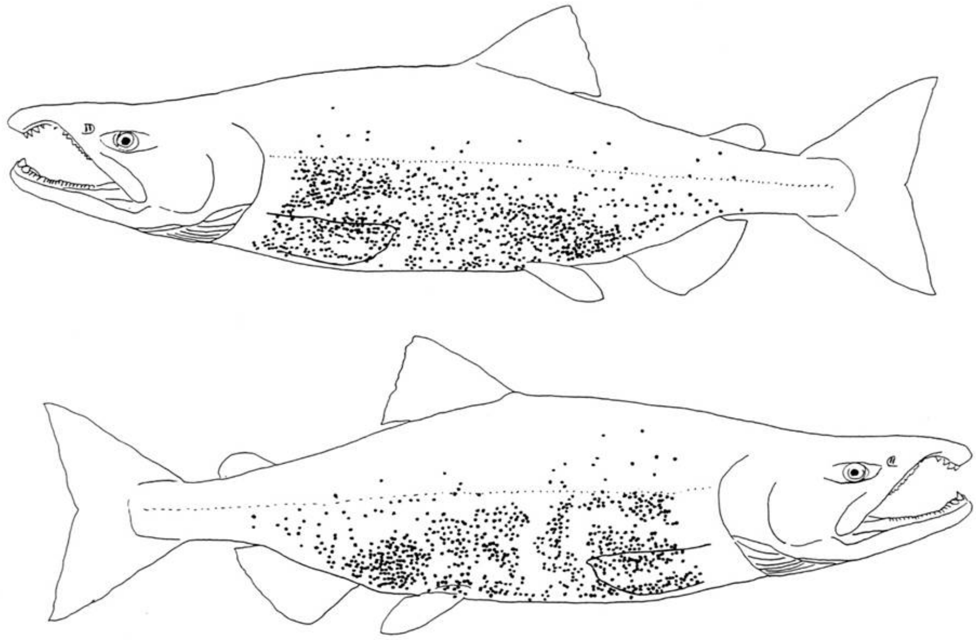

Fig. 7. Spatial distribution of *A. simplex* larvae in the muscle of adult chum salmon (n=82) returning to the Chitose River in October 2001.