Otolith Marking with Fluorescent Substances at the Eyed-egg Stage of Chum Salmon

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Otolith marks have some advantages for mass marking of salmon because of their simplicity and short marking time. Moreover we can mark otoliths at the embryonic stages. However, to find the marked fish we have to extract the otoliths from the heads and process the specimens by grinding. We have found that otolith marking with fluorescent substances at the embryonic stage may be a better method than thermal marking (Tsukamoto 1985, 1988; Tsukamoto et al. 1989). A fluorescent mark in an otolith can be found without any treatment, and we seldom mistake the mark due to the clear coloration of fluorescent mark. We used an alizarin complexone (ALC) for mass marking chum salmon, *Oncorhynchus keta*. In order to improve salmon propagation technology for the Sea of Japan stocks in Hokkaido we have examined the early coastal ecology of juvenile chum salmon by means of mass marking of otolith with ALC from 1994 to 1999.

We used eggs from the Syokanbetsu River on the northern coast of the Sea of Japan in Mashike of Hokkaido, Japan. The eggs were moved into the Mashike Branch of the Hokkaido Fish Hatchery. We marked otolith of eggs at the eyed stage with ALC. The eggs were put into two box type incubators, whose capacity was six hundred thousand eggs, and the refrigerator and pump were set in another incubator to maintain a water temperature at 7°C to 9°C. The eggs were immersed for 24 hours in a solution of ALC (200mgl-1) with 1N KOH before dilution (Tsukamoto 1988). Because the otolith continues to grow with the cumulative temperature changes, we were able to use a single or double treatments for marking. The cumulative temperatures of the embryo ranged from 242°C to 427°C in November to December. After treatment the eggs were reared in a vertical type incubator with other eggs. The juveniles with marked otolith were stocked, ranging from 795,000 fish to 2,274,000 fish, in the Syokanbetsu River in April from 1995 to 1999 (Table 1). After releasing we recaptured the marked juvenile and adult chum salmon in coastal and offshore waters, and the Syokanbetsu River from 1995 to 2000 (Kawamura et al. 1998, 2000). We observed the fluorescent marks in otoliths using a UV light microscope.

We recaptured 15 juvenile fish (1995), 397 fish (1996), 447 fish (1997), 645 fish (1998) and 1616 fish (1999) whose otoliths were marked in the inshore waters off Mashike with Sayori townet (Table 2). One otolith marked chum salmon (22.9cm, 118g) was recaptured in offshore waters off the west Kamchatka in the Sea of Okhotsk in September 1996 (Ueno et al. 1998). As a result of our observations of adult chum salmon which returned to the Syokanbetsu River from 1997 to 2000, we found some marked otoliths among adult salmon ranging from 3 to 5 years old (Table 3). The otolith marked chum salmon were as follows; 4 fish at 3 years old (1997), 21 fish at 3 years old and 70 fish at 4 years old (1998), 92 fish at 3 years old, 243 fish at 4 years old and 27 fish at 5 years old (1999), and 18 fish at 3 years old, 59 fish at 4 years old and one fish at 5 years old (2000). We could easily locate the marked otoliths among different aged fish under UV microscope.

Five otolith marked juveniles were found in the stomachs of arabesque greenling, *Pleurogrammus azonus* captured by set net located 90 km north of Mashike in 1999, and eleven otoliths with ALC were observed in the fecal materials of gulls (*Larus schistisagus, L. crassirostris*) around the mouth of the Syokanbetsu River in 1999 (Table 4).

We confirmed that a fluorescent mark in otolith with ALC was retained for more than five years. We concluded that otolith marking with ALC was beneficial to the study of chum salmon on distributions, migration and prey-predator interaction.

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Table 1. Otolith marking with ALC and releases of juvenile chum salmon.

Date	Stage	Cumulative temperatures	Treatment	No. juveniles released	Body weight (g)
1994.12.8-12.8	eyed-egg	419-427	single	795,000	1.08
1995.11.23-11.24	eyed-egg	242-249	single	1,123,000	0.95
1995.12.13-12.14	eyed-egg	391-399	double	1,138,000	1.32
1996.11.21-11.22	eyed-egg	245-253	small single	1,282,000	0.77
1996.12.10-12.11	eyed-egg	391-398	large single	1,264,000	1.09
1997.12.15-12.16	eyed-egg	405-412	single	2,274,000	0.93
1998.11.24-11.25	eyed-egg	284-301	small single	1,726,000	0.75
1998.12-7-12.8	eyed-egg	380-400	large single	1,902,000	0.85
Total				11,504,000	

Table 2. Recapture of juvenile chum salmon with marked otoliths.

Year	Location	Total of sample fish	No. marked fish	Treatment	% of marked fish
1995	coastal waters off Mashike	1,624	15	single	0.92
1996	coastal waters off Mashike		199	single	
	coastal waters off Mashike		198	double	
	Subtotal	5,446	397		7.29
	offshore waters in the Okhotsk Sea	84	1	double	1.19
1997	coastal waters off Mashike		249	small single	
	coastal waters off Mashike		198	large single	
	Subtotal	5,473	447		8.17
1998		7,723	654	single	8.47
1999	coastal waters off Mashike		698	small single	
	coastal waters off Mashike		918	large single	
	Subtotal	4,140	1,616		39.03
Total		24,490	3,130		12.78

Table 3. Recapture of adult chum salmon with marked otoliths.

Year	Location	Total of fish samples	Age	No. marked fish	Treatment	Ratio (%)
1997	Syokanbetsu River	872	3	4	single	0.46
1998	Syokanbetsu River		3	21	single or double	
	Syokanbetsu River		4	70	single	
	Sub-total	2,089		91		4.36
1999	Syokanbetsu River		3	92	small or large single	
	Syokanbetsu River		4	243	single or double	
	Syokanbetsu River		5	27	single	
	Sub-total	2,979		362		12.15
2000	Syokanbetsu River		3	18	single	
	Syokanbetsu River		4	59	small or large single	
	Syokanbetsu River		5	1	single or double	
	Sub-total	1,011		78		7.72
Total		6,951		535		7.70

Table 4. Predators feeding on juvenile chum salmon based on marked otolith with ALC.

Year	Predator	Sample	No. sample	No. marked juveniles or marked otolith
1999	fish (arabesque greenling)	stomach contents	72	5
1999	sea birds (gulls)	fecal materials	36	11

REFERENCES

Kawamura, H., M. Miyamoto, M. Nagata, and K. Hirano. 1998. Interaction between chum salmon and fat greenling juveniles in the coastal Sea of Japan off northern Hokkaido. N. Pac. Anadr. Fish Comm. Bull. 1: 412–418.

Kawamura, H., S. Kudo, M. Miyamoto, M. Nagata, and K. Hirano. 2000. Movement, food and predators of juvenile chum salmon (*Oncorhynchus keta*) entering the coastal Sea of Japan off northern Hokkaido in warm and cool years. N. Pac. Anadr. Fish Comm. Bull. 2: 33–41.

Tsukamoto, K. 1985. Mass-marking of Ayu eggs and larvae by tetracycline tagging. Bull. Japan. Soc. Sci. Fish. 51: 903–911.

Tsukamoto, K. 1988. Otolith tagging of Ayu embryo with fluorescent substances. Nippon Suisan Gakkaisi 54: 1289–1295.

Tsukamoto, K, Y. Seki, T. Oba, M. Oya, and M. Iwahashi. 1989. Application of otolith to migration study of salmonids. Physiol. Ecol. Japan, Spec. 1: 119–140.

Ueno, Y., M. Nagata, H. Kawamura, K. Suzuki, H. Mayama, J. Seki, S. Urawa, T. Ariyoshi, and N. Nakamura. 1998. The stock origins and migration routes of juvenile chum salmon distributed in the Okhotsk Sea in autumn. Salmon Rep. Ser. 46:64–92. (In Japanese with English abstract)