

## Manipulating the Timing of a Chum Salmon (*Oncorhynchus keta*) Run Using Preserved Sperm

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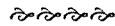
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**Abstract:** Run timing for two experimental groups of adult returns to a natal river and adjacent areas was compared for 1991 and 1993 broods of chum salmon (*Oncorhynchus keta*). In the experimental group of the 1991 brood, eggs and sperm were collected from fish returning early in December, and eggs were inseminated immediately. For the second experimental group, eggs were taken from fish returning in early December and inseminated with sperm collected in early November that had been preserved in artificial seminal plasma and antibiotics at 0°C for one month. Adults returning in each group were identified from fin-clips and scale analysis. The fresh sperm group returned mainly in early December over four years from 1994 to 1997; over four years there was a single abundance peak of returns to the natal river and adjacent areas. The preserved sperm group showed two peaks, one in late October and the other in early December. Comparable results were also observed for similar experiments conducted with 1993 brood fish. These results suggest that the return timing to a natal river is influenced by genetic factors that can be manipulated using preserved sperm.

### INTRODUCTION

It is known that the timing of upstream migration and spawning in salmonid fish is different for each river, and that there may be several spawning runs in a river (Mayama 1986). One explanation for these phenomena is that the physical conditions that influence spawning and survival are different for each river. If adults return too early or too late, the river will often not be suitable for spawning or development of their embryos (Smoker et al. in press). Thus, run timing is clearly important to the persistence of populations, and we suspect that run timing is affected genetically (Mayama 1986, Smoker et al. 1998). Mayama (1986) inferred that maturation time of salmonid fish is influenced by the spawning time of the parents. Pink salmon (*Oncorhynchus gorbuscha*) tend to return to spawn at the same time in a run that their parents did (McGregor et al. 1998). However, these reports were based on ordinary propagation; the fish were mated with fish that had matured at the same time.

In our experiment, we focused on the return timing of chum salmon to a natal river, comparing

broods raised from eggs fertilized with preserved sperm and with fresh sperm. The purposes of this experiment were to investigate whether there is any genetic control over season of run timing by using preserved sperm.

### MATERIALS AND METHODS

#### 1991 Brood

In 1991, eggs and sperm were obtained from mature chum salmon (*O. keta*) from the Akka River of Iwate prefecture, Japan. There were two different groups in this experiment. In the first group, eggs and sperm were collected from 86 females and 4 males that returned to the river on 4 December; half the eggs from the 86 females were inseminated immediately with fresh sperm (Table 1). For the second group, sperm for insemination was stripped from 4 males that were in the river on 6 November. The sperm was preserved by mixing it with artificial seminal plasma containing antibiotics (streptomycin, penicillin, fungizone). The sperm was stored at about 0°C until 4 December. The other half of the

**Table 1.** Dates and locations where eggs and sperm were collected.

Brood year	River of origin	Date sperm collected	Date eggs collected	Date of fertilization
1991	Akka R.	Dec. 4, 1991	Dec. 4, 1991	Dec. 4, 1991
	Akka R.	Nov. 6, 1991	Dec. 4, 1991	Dec. 4, 1991
1993	Tsugaruishi R.	Nov. 26, 1993	Nov. 26, 1993	Nov. 26, 1993
	Tsugaruishi R.	Oct. 22, 1993	Nov. 26, 1993	Nov. 26, 1993

eggs collected on 4 December (86 females) were inseminated immediately with the preserved sperm.

The preserved sperm was stored in two kinds of seminal plasmas with different components. The chemical components of one were 130 mM NaCl, 40 mM KCl, 2.5 mM CaCl<sub>2</sub>, 1.5 mM MgCl<sub>2</sub> and 2.5 mM NaHCO<sub>3</sub> in water for cell culture. The other consisted of 80 mM NaCl, 50 mM KCl, 2.5 mM CaCl<sub>2</sub>, 1.5 mM MgCl<sub>2</sub> and 50 mM NaHCO<sub>3</sub>. The chum salmon fry obtained from these experiments were all released at the same location and at the same time into the Osawa River on 13 April 1992 (Table 2). There were 77,700 marked fry from the fresh-sperm group and 45,300 from the preserved-sperm group. Each group was identified by fin clipping and scale analysis. The fry from the fresh-sperm group were marked by clipping both the adipose fin and the left ventral fin. The preserved-sperm group was marked by clipping both the adipose fin and the right ventral fin. From 1993 to 1997, we looked for marked fish from 1991-brood releases in the river and along the coast in Iwate prefecture, Japan.

For the purposes of run timing, we have defined the natal river and adjacent areas as the Osawa River and the Yamada fish market. The Yamada fish

market sells salmon that are caught in set nets from near the mouth of Osawa River.

### 1993 Brood

In 1993, the eggs and sperm of the fresh-sperm group were obtained on 26 November from 58 females and 3 males captured in Tsugaruishi River, Iwate prefecture, Japan. Half the eggs from 58 females were inseminated immediately with fresh sperm (Table 1).

For the preserved-sperm group, the sperm had been stripped from 4 males captured in the Tsugaruishi River on 22 October. Sperm was preserved until 26 November. The other half of the eggs collected from 58 females captured in the river on 26 November were inseminated immediately with preserved sperm. There were 36,400 marked chum salmon fry from the fresh-sperm group, and 51,200 from the preserved sperm group (Table 2). Fry were released into the Osawa River on April 20, 1994. From 1995 to 1998 we looked for marked adults returning from the 1993-releases.

### RESULTS

For the 1991-brood experiment a total of 57 marked fish from the fresh-sperm group and 46 marked fish from the preserved-sperm group were recovered (Table 3). Recovery rates were 0.07% and 0.10% respectively, but were not significantly different ( $\chi^2, p > 0.05$ ). Most recoveries in both groups were age 3+ and 4+, and were not statistically different ( $\chi^2, p > 0.05$ ) (Table 3).

**Table 2.** Summary of release information on marked chum salmon fry.

Date of release	No. released	River of release	Fork length mean±SD(cm)	Body weight mean±SD(g)	Marked fin	Group <sup>1</sup>
Apr. 13, 1992	77,700	Osawa R.	6.1±0.41	1.62±0.32	Adipose+Left ventral	F
Apr. 13, 1992	45,300	Osawa R.	6.2±0.32	1.74±0.26	Adipose+Right ventral	P
Apr. 20, 1994	36,400	Osawa R.	6.7±0.35	2.54±0.38	Adipose+Left ventral	F
Apr. 20, 1994	51,200	Osawa R.	6.2±0.31	2.16±0.33	Adipose+Right ventral	P

<sup>1</sup>F, fresh sperm; P, preserved sperm

**Table 3.** Age, sex, and numbers of marked chum salmon that were recovered in rivers and along the coast of Iwate Prefecture, Japan.

Brood year	Marked fin	Group <sup>1</sup>	Number of fish	Age				Male	Female
				2+	3+	4+	5+		
1991	Adipose+Left ventral	F	57	2	24	31	0	20	37
	Adipose+Right ventral	P	46	3	22	20	1	12	34
1993	Adipose+Left ventral	F	33	3	17	13	-	7	26
	Adipose+Right ventral	P	37	2	27	8	-	15	22

<sup>1</sup>F, fresh sperm; P, preserved sperm

For the 1993-brood experiment, 33 fish were recovered from the fresh-sperm group and 37 from the preserved-sperm group (Table 3). Recovery rates were 0.09% and 0.07%, and again were not significantly different ( $\chi^2, p > 0.05$ ). Most fish in both groups were aged 3+ and 4+, and age compositions were not statistically different ( $\chi^2, p > 0.05$ ).

**Timing of Returns**

In 1991-brood experiments, the fish that recovered from the Osawa River and Yamada fish market were 33 (fresh-sperm group) and 21 (preserved-sperm group) (Table 4). The adults returned to the area from mid September to mid December; the range was the same for both groups. However, the fresh-sperm group returned mainly in early December and had a single peak; the preserved-sperm group showed two peaks, one in late October and the other in early December. In the fresh-sperm group, about 42% of the recovered fish returned during the peak. In the preserved sperm group, about 24% returned during the first peak and 33% returned during the second. Neither distribution (run timing) was significant (Kolmogorov-Smirnov test,  $p > 0.05$ ) (Fig. 1a).

Similar results were observed in the experiment initiated in 1993. All of the recovered fish (31 from the fresh-sperm group and 32 from the preserved-sperm group) were recovered in the Osawa River and Yamada fish market (Table 4). A single peak in the fresh-sperm group was observed in early December, and the percentage of fish in the return peak was about 29%. There were two peaks in the preserved-sperm group, one in late October and the other in mid November; percentages were about 16% and 38%, respectively (Fig. 1b). Both distributions (run timing) were significant (Kolmogorov-Smirnov test,  $p < 0.01$ ).

**Location of Recoveries**

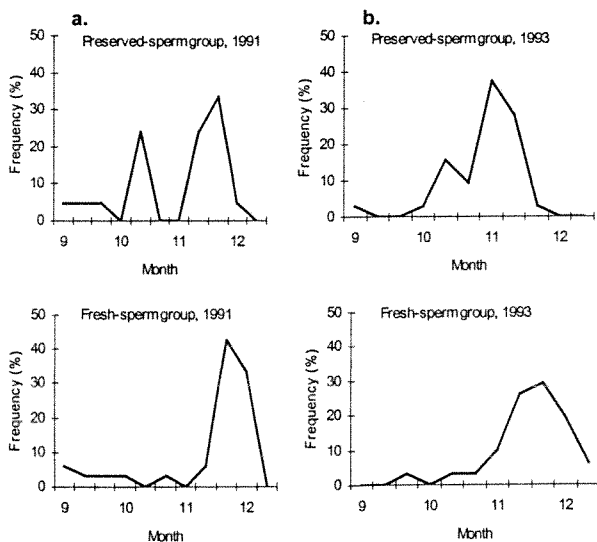
Among the fresh-sperm group in the 1991-brood experiment, 27 adults were recovered from the Osawa River, and 6 adults from Yamada fish market (24 adults were from other areas)(Table 4). The periods of recovery were different in the two areas (Fig. 2a). Most of the 27 fish recovered in the Osawa River were found in the second half of the observation period. By contrast, the 6 fish from adjacent areas were recovered in the first half. Similar results were observed for the preserved-sperm group, i.e., most of

**Table 4.** Summary of marked chum salmon recovered in the Osawa River and in Yamada fish market.

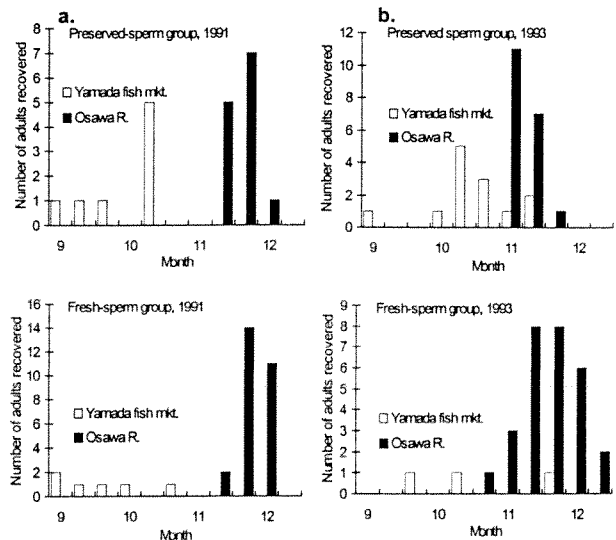
Brood year	Marked fin	Group <sup>1</sup>	Total number of fish	Number of fish			Male	Female
				Osawa	Yamada	others		
1991	Adipose+Left ventral	F	33	27	6	24	10	23
	Adipose+Right ventral	P	21	13	8	25	7	14
1993	Adipose+Left ventral	F	31	28	3	2	6	25
	Adipose+Right ventral	P	32	19	13	5	14	18

<sup>1</sup> F, fresh sperm; P, preserved sperm

**Fig. 1.** Number of marked fish recovered in the Osawa River (the river of release) and Yamada fish market (near Osawa River), Iwate Prefecture, Japan, 1994–1998.



**Fig. 2.** Number of marked adult chum salmon recovered Sept.–Dec. from the Osawa River and from the Yamada fish market, 1994–1998.



the 13 fish that were recovered in the Osawa River were found in the second half of the observation period. The 8 fish that were recovered in coastal areas near the river were found in the first half of the observation period. In these results, few fish were recovered in the Osawa River late in October.

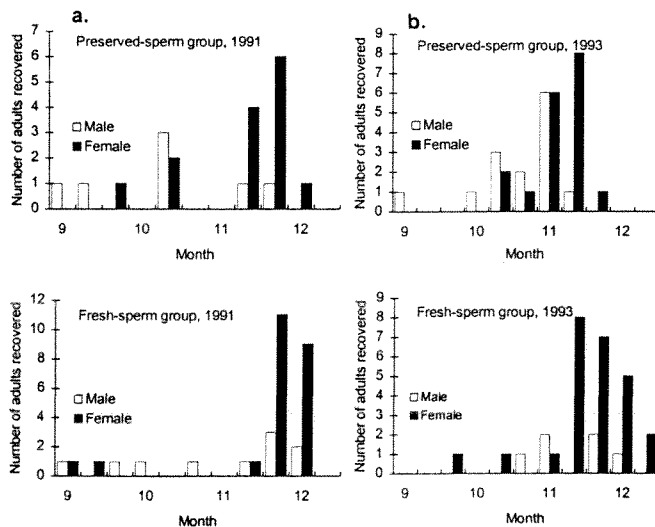
Results were similar in the 1993-brood experiments where few fish were recovered from the Osawa River in late October for either the fresh-sperm group or the preserved-sperm group (Table 4 and Fig. 2b).

### Sex Composition

Sex composition of marked fish from the 1991-brood experiments recovered in the Osawa River and Yamada fish market consisted of 7 males and 14 females for the preserved-sperm group (Table 4). For this group the peak numbers of recovered males was in late October, and for females in early December (Fig. 3a). For the fresh-sperm group, there were 10 males and 23 females from the same areas. The peak in the number of females occurred in early December, but males did not produce an obvious peak.

For the 1993-brood experiments sex composition of adults recovered in the preserved-sperm group was 14 males and 18 females (Table 4). In the case of the males, there were two abundance peaks, one in late October and the other in mid-November. Female abundance peaks appeared in late October and again in late November (Fig. 3b). In the fresh-sperm group, 6 male and 25 female fish were recovered from the same area. Though a single peak for the females occurred in late November, no peak was observed for the males.

Fig. 3. Number of male and female marked adult chum salmon recovered Sept.–Dec., 1994–1998.



### DISCUSSION

It has been known empirically that chum salmon propagated on the same day in the usual way form a unimodal distribution when they return (Kobayashi 1985; Mayama 1986). Moreover, the center of the frequency distribution is the day the parents were spawned; the range is three weeks before and after this date (Kobayashi 1985; Mayama 1986).

In our experiments, we propagated fish from pairs that differed in maturation times by using preserved sperm, and the results show an interesting pattern. When we used preserved sperm we obtained a bimodal distribution, unlike the pattern obtained with fresh sperm. That is, dates of peak returns of chum salmon that were propagated using preserved sperm coincided with the sperm-stripping date and the date eggs were collected and fertilized. However, there was no significant difference in run timing of each group in the 1991 brood experiment. However, the important point is that the date of the return peaks for chum salmon corresponded with the dates of return of their parents.

Miyagi Prefecture (1999) conducted similar experiments using sperm after cryopreservation. However, their results did not agree with ours with regard to the characteristics of the returns (especially run timing). They reported that the date when the peak occurred fell between the sperm stripping date and the date of insemination. In either case, preserved sperm groups were somewhat different from run timing in the fresh sperm group; in other words, it is likely that run timing is under genetic influence.

Mayama (1986), McGregor et al. (1998) and Smoker et al. (1998) reported that the spawning time of salmonids has a genetic component. Moreover, it has been reported for chum salmon that spawning time is closely related to the date the fish return to their natal river. Seki and Shimizu (1996), suggested that the date of return to the natal river for chum salmon is influenced by parental genetic factors. Judging from this and our experiments, run timing is under genetic influence.

Another characteristic that was observed when using preserved sperm was that some fish returned early (similar in time to when the male parent returned). These fish were recovered near the natal river, but had not entered the river. We do not understand the reason for this, but there may have been a difference in the stage of maturity. We did not observe any special trends related to sex composition.

Experimental releases were conducted by transplantation in our experiments. However, transplantation did not seriously influence the run timing of our experiments. This is because the timing of upstream migrations is not easily changed by environmental differences (Okazaki 1982a, b).

In our experiments, the number of recovered fish was not large, and we cannot be certain that the results would apply to the whole population. Therefore, further experiments will be required to clarify the relationship between run timing and genetic factors.

There have been numerous studies on techniques for preserving sperm for use in artificial propagation. Salmonid fish have frequently been used in these studies (Smith and Quistorff 1943; Barrett 1951; Forester 1965; Withler and Humphreys 1967; Hoyle and Idler 1968; Truscott et al. 1968; Withler and Morley 1968; Ott and Hortor 1971; Hiroi 1973). However, the main objectives were to determine storage methods, fertilization techniques, motility and the fertility rates of the sperm. Therefore, the biological characteristics of the adult salmon that contributed the sperm were not described in detail. Our experiments suggest that run timing can be manipulated using preserved sperm. We also suggest that it can be a useful technique for maintaining balanced stocks during artificial propagation, because there are many more male chum salmon than females during the first run. Also, sperm preservation could maintain chum salmon diversity in a river without having to transplant rare stocks of early run fish. Naturally we should be careful using preserved sperm until all features of its use are clear.

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#### REFERENCES

- Barrett, I. 1951. Fertility of salmonid eggs and sperm after storage. *J. Fish. Res. Board Can.* 8: 125-133.
- Forester, R.F. 1965. Effect of retention of spermatozoa and ova of sockeye salmon (*Oncorhynchus nerka*) in water and without addition of water, on fertility. *J. Fish. Res. Board Can.* 25: 2695-2699.
- Hiroi, O., M. Yasukawa, and T. Suetake. 1973. Studies on the retention of gametes in salmonid fishes. II—on the storage of chum salmon (*O. keta*) sperm. *Sci. Rep. Hokkaido Salmon Hatchery* 27: 39-44.
- Hoyle, R.J., and D.R. Idler. 1968. Preliminary results on the fertilization of eggs with frozen sperm of Atlantic salmon (*Salmo salar*). *J. Fish. Res. Board Can.* 25: 1295-1297.
- Kobayashi, T. 1985. The survey of fall chum salmon in Japan. *Edited by K. Zama, and H. Takahasi. Fisheries series No.55. (In Japanese)*
- Mayama, H. 1986. Abstract of the symposium on migration and physiology of salmon and trout. *Otsuchi Mar. Res. Center Rep. No. 12: 119-121. (In Japanese)*
- McGregor A.J., S. Lane, M.A. Thomason, L.A. Zhivotovsky, W.W. Smoker, and A.J. Gharrett. 1998. Migration timing, a life history trait important in the genetic structure of pink salmon. *N. Pac. Anadr. Fish Comm. Bull. No. 1: 262-273.*
- Miyagi Prefecture. 1999. Report of management and promotion in salmon and trout resources. pp. 26-32. (In Japanese)
- Okazaki, T. 1982a. Population structure in chum salmon. *J. Hered.* 36: 61-70. (In Japanese)
- Okazaki, T. 1982b. Genetic study on population structure in chum salmon (*Oncorhynchus keta*). *Bull. Far Seas Fish. Res. Lab.* 17: 25-116.
- Ott, A.G., and H.F. Hortor. 1971. Fertilization of chinook and coho salmon eggs with cryo-preserved sperm. *J. Fish. Res. Board Can.* 28: 745-748.
- Seki, J. and I. Shimizu. 1996. Effect of time of larval release on return rate in chum salmon (*Oncorhynchus keta*) in the Hiroo River, Hokkaido, Japan. *Bull. Japan. Soc. Fish.* 60: 339-347.
- Smith, R.T., and E. Quistorff. 1943. Experiments with the spermatozoa of the steelhead trout, *Salmo gairdnerii gairdnerii*, and the chinook salmon, *Oncorhynchus tshawytscha*. *Copeia* 1943: 164-167.
- Smoker, W.W., A.J. Gharrett, and M.S. Stekoll. 1998. Genetic variation of return date in a population of pink salmon: a consequence of fluctuating environment and dispersive selection? *Alaska Fish. Res. Bull.* 5: 46-54.
- Smoker, W.W., A.J. Gharrett, and M.S. Stekoll. In press. Genetic variation in seasonal timing of anadromous migration in a population of pink salmon. *Can. Sp. Pub. Fish. Aquat. Sci.*
- Truscott, B., D.R. Idler, R.J. Hoyle, and H.C. Freeman. 1968. Sub-zero preservation of Atlantic salmon sperm. *J. Fish. Res. Board Can.* 25: 363-372.
- Withler, F.C., and R.M. Humphreys. 1967. Duration of fertility of ova and sperm of sockeye salmon (*Oncorhynchus nerka*) and pink (*Oncorhynchus gorbuscha*) salmon. *J. Fish. Res. Board Can.* 24: 1573-1578.
- Withler, F.C., and R.B. Morley. 1968. Effects of chilled storage on viability of stored ova and sperm of sockeye and pink salmon. *J. Fish. Res. Board Can.* 25: 2695-2699.

