Evidence for temperature-dependent sex determination in sockeye salmon (*Oncorhynchus nerka*)

J. Kevin Craig, Chris J. Foote, and Chris C. Wood

Abstract: Temperature-dependent sex determination has been demonstrated in several animals, but in salmonids sex is generally believed to be under strict genetic control. We observed distorted sex ratios, attributable to a temperature manipulation during embryonic development, in experiments conducted during 1991 with the anadromous and nonanadromous (kokanee) forms of sockeye salmon (*Oncorhynchus nerka*) from Takla Lake, British Columbia. Sex ratios ranged from 62 to 84% female, and the biased ratio could not be accounted for by differential mortality by sex. The effect was observed independently in sockeye and kokanee crosses, as well as in the reciprocal hybrids. Similar crosses from Takla Lake in a previous year (1989) in which incubation temperature was not manipulated resulted in normal (1:1) sex ratios. Other populations of sockeye and kokanee from the same year (1991) but undergoing no temperature manipulation maintained normal sex ratios, as did populations from several disparate locations and years (1986–1994). The parsimonious conclusion is that the temperature manipulation during development was responsible for the biased sex ratio through a direct influence on sex differentiation. Hence, the possibility that temperature-dependent sex determination occurs in *O. nerka*, and perhaps other salmonids, deserves rigorous testing.

Résumé : Chez plusieurs animaux, on a montré que la détermination du sexe peut être dépendante de la température, mais on considérait généralement que, chez les salmonidés, elle était régie par un strict contrôle génétique. Nous avons toutefois observé des distorsions du rapport des sexes, attribuables à une manipulation de la température pendant le développement embryonnaire, dans des expériences menées en 1991 sur les formes anadromes et non anadromes (kokani) de saumon rouge (*Oncorhynchus nerka*) du lac Takla, en Colombie-Britannique. Le rapport des sexes était de 62 à 84% en faveur des femelles, et cette distorsion ne pouvait pas s'expliquer par des différences dans la mortalité selon le sexe. L'effet a été observé de façon indépendante chez des croisements de saumon rouge et de kokani, ainsi que chez des hybrides réciproques. Des croisements semblables du lac Takla d'une année antérieure (1989) pendant laquelle la température d'incubation n'avait pas été manipulée a donné des rapports des sexes normaux (1:1). Chez d'autres populations de saumon rouge et de kokani de la même année (1991), mais n'ayant pas subi de manipulation thermique, les rapports des sexes étaient normaux, tout comme chez des populations provenant d'endroits divers et d'années différentes (1986–1994). Nous pouvons conclure avec circonspection que la manipulation de la température pendant le développement est responsable de la distorsion du rapport des sexes par influence directe sur la différenciation sexuelle. La possibilité que la détermination du sexe soit dépendante de la température chez *O. nerka*, et peut-être chez d'autres salmonidés, mérite donc une vérification rigoureuse.
[Traduit par la Rédaction]

Introduction

Environmental sex determination (ESD) is said to occur when gender is irreversibly influenced by the environment during development. ESD has been demonstrated in several invertebrates (Charnov and Bull 1977; Charnov et al. 1978, 1981; Naylor 1988), reptiles (reviewed in Deeming and Ferguson 1988), and a few fish species (Harrington 1968; Conover and Kynard 1981; Rubin 1985; Sullivan and Schultz 1986). However, ESD may be a more widespread mode of sex

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J.K. Craig[1] and C.J. Foote. Fisheries Research Institute, University of Washington, Seattle, WA 98195, U.S.A.
C.C. Wood. Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, BC V9R 5K6, Canada.

[1] Author to whom all correspondence should be sent at the following address: Duke University, Marine Laboratory, 135 Duke Marine Lab Road, Beaufort, NC 28516-9721, U.S.A.

determination than previously thought (Conover et al. 1992) given that an adaptive function of ESD has been proposed (Charnov and Bull 1977) and elucidated in at least one fish (Atlantic silverside, *Menidia menidia*; Conover 1984). The most commonly identified environmental variable influencing sex is temperature (Bull 1983), though effects of pH (Rubin 1985), artificial treatment with hormones (Yamamoto 1969; Hunter and Donaldson 1983), and pollutants (Torblaa and Westman 1980) on sex ratio have been reported.

Phenotypic sex in salmonids (family: Salmonidae) is generally believed to be strictly under genetic control, though the actual mechanism by which sex is determined has not been adequately investigated. Some populations of sockeye salmon, *Oncorhynchus nerka*, have morphologically distinct sex chromosomes (Thorgaard 1978; Ueda and Ojima 1984), as do some populations of rainbow trout, *Oncorhynchus mykiss* (Thorgaard 1977, 1983). However, observations of individuals of atypical karyotype or phenotypic sex have been attributed to possible environmental influences and (or) the effects of autosomal genes (Thorgaard 1977, 1983; Scheerer et al. 1991). The induction of sex reversal via hormone treatment during early developmental stages in most species of Pacific salmon, *Oncorhynchus* spp., suggests that sex is labile during this period (Hunter and Donaldson 1983), but the influence of external factors such as temperature on sex determination has not been rigorously investigated. Sex appears to be fixed after some early point in development as intersexes have not been reported in nature.

Sockeye salmon and kokanee are the anadromous and nonanadromous forms of *O. nerka*, respectively. They occur allopatrically and sympatrically, and even where sympatric they are genetically distinct populations. Genetic distinction has been demonstrated through a series of controlled breeding experiments on populations from British Columbia (Wood and Foote 1990, 1995; Taylor and Foote 1991; Foote et al. 1992) and biochemical genetic surveys throughout North America and Asia (Foote et al. 1989; Wood and Foote 1995; Taylor et al. 1995).

Throughout the prior breeding studies cited above, sex ratios were consistently 1:1. However, during breeding experiments in 1991 the sex ratios of some crosses shifted drastically, and this shift was associated with a change in incubation temperature during embryonic development. In this paper we describe the sex ratios of sockeye salmon and kokanee crosses from five disparate locations and three separate freshwater systems, and conclude that sex is thermolabile in *O. nerka*.

Methods

1991 Takla Lake population

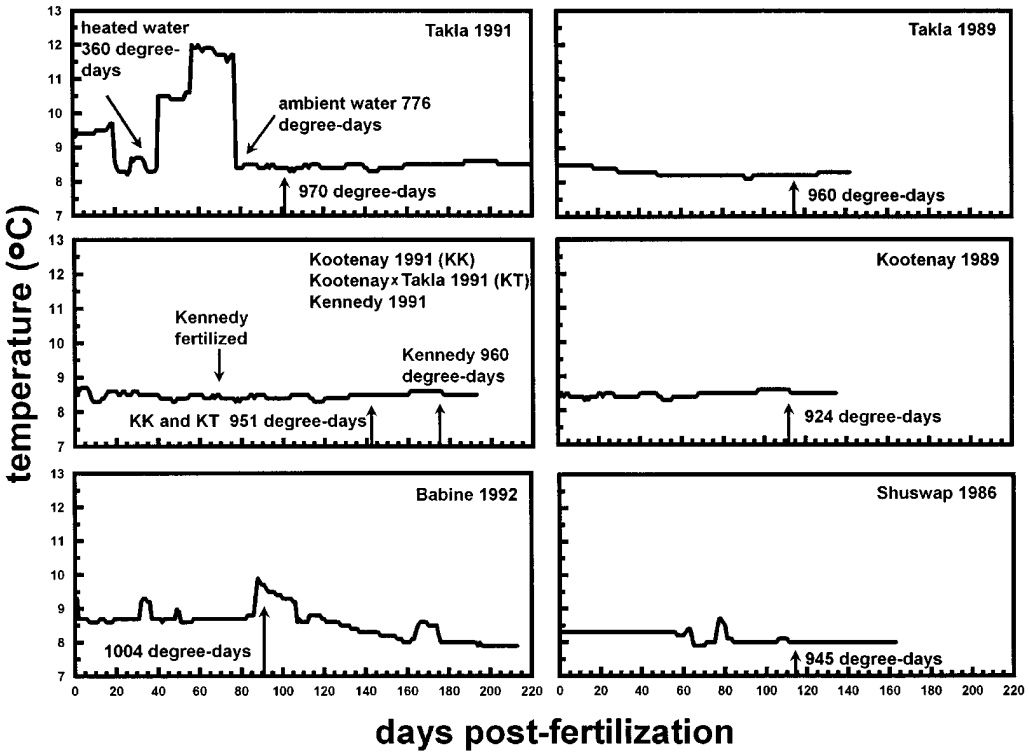
On 16 August 1991 random samples of ripe sockeye and kokanee were collected from Narrows Creek, Takla Lake, British Columbia, by beach seine. The eggs of each female were stripped into 1-L jars and fertilized. The jars were filled with chilled water and transported to a hatchery in coolers chilled to approximately 2–4°C. The genetic design consisted of a mixture of 43 pure and half sib families of pure sockeye (PS, 13 families), hybrid sockeye (HS; sockeye female × kokanee male,14 families), pure kokanee (PK, 9 families), and hybrid kokanee (HK; kokanee female × sockeye male, 7 families). A total of 43 males (20 sockeye and 23 kokanee) and 29 females (13 sockeye and 16 kokanee) were used to form the crosses.

Within 42–45 h of fertilization, the eggs were transferred to Heath tray baskets, surface disinfected with iodine solution, and incubated in 9.0 ± 0.8°C (mean ± SD) well water. Mortality was recorded at the eyed egg stage (18 September), dead eggs were removed, and the remaining live eggs were grouped in trays at a maximum density of 200 eggs. Temperature was recorded twice daily with an internal temperature probe. On 25 September (360 degree-days) the incubation temperature was deliberately manipulated in an effort to increase development rate and reduce incubation time (Fig. 1). The trays were moved from stacks supplied with ambient well water (8.3–9.7°C) to those supplied with heated well water (10.4–12.0°C) while covered to maintain light levels. On 2 November (776 degree-days) the trays were moved back to the ambient well water (8.3–8.5°C) in a similar manner. On 22 November (970 degree-days) all families were transferred as alevins ("ponded") into separate 40-L Calaprice tanks supplied with ambient well water at a density of approximately 200 fry/tank (range 74–214).

Manipulations after ponding reflect the requirements of various experiments and space limitations and probably did not affect sex ratios. On 23 January 1992 each family was randomly culled to a standard density (60 fish/tank). On 18 April alternate families were marked by clipping adipose fins so that consecutive clipped and unclipped families could be pooled into 480-L tanks (each tank contained two families of 57 fish each). On 27 July approximately 800 fish were individually PIT tagged (passive integrator transponder, Prentice et al. 1987) and moved to four 1500-L tanks. Each tank was identical in family composition, and each family was represented by an equal number of fish (203 fish/tank). The remaining fish were fin clipped to identify cross type, coded wire tagged (CWT, Bilton et al. 1982) to identify family, and reared in 480-L tanks at a density of 109 fish/tank. On 10 November all CWT fish were moved outdoors to two concrete channels (17 000 L) and one 2800-L tank. The channels contained fish of each of the four cross types, with families of each type split equally between the two channels. No HK were placed in the 2800-L tank because of their low numbers. The fish in the 2800-L tank were sampled monthly from January to July 1993. PIT-tagged fish were reared in indoor tanks for the duration of the experiment.

All fish were reared on a natural photoperiod for latitude 49°N. Dissolved oxygen (DO) was monitored at periodic intervals and flow manually regulated to ensure DO remained above 6 ppm. Fish were fed Biodiet starter mix, Hatchery diet, Promoter I diet (Whitecrest Mills), and Promoter II diet (Whitecrest Mills) at specific times during the 2-year period. Feed was dispensed by automatic feeders every half hour during daylight at a daily rate of 3.8–1.0% body weight per fish, decreasing progressively over time. Fish reared in indoor tanks were fed the same diets but under a variety of feeding regimes for a growth rate experiment. Mortality was very low during the experiments and was recorded throughout the 2-year period. Terminal sampling took place from 14 September to 6 October 1993. Sex was determined by visual inspection of the gonads.

Fig. 1. Daily incubation temperature during development for sockeye and kokanee (*Oncorhynchus nerka*) crosses from several populations throughout British Columbia. Takla Lake 1991 crosses underwent a temperature manipulation in which trays were moved to heated water on day 41 (8.4–10.5°C) and back to ambient water on day 78 (11.7–8.4°C). All other populations were reared in ambient well water. Arrows along the *x* axes indicate time of transfer ("ponding") of alevins from trays to rearing tanks maintained on the same ambient well water. Fertilization and ponding dates for Kennedy 1991 sockeye are shown relative to those of Kootenay kokanee and Kootenay × Takla "hybrid" kokanee from 1991.



Control populations

Several additional populations in which no temperature manipulations occurred have been reared under near identical conditions in the same hatchery since 1986. In 1989 pure and reciprocal hybrid crosses between sockeye and kokanee (five families each of PS and HS, eight families of PK) were generated with fish from Narrows Creek, Takla Lake (see Wood and Foote 1995 for details of rearing methodology). These crosses serve as a partial control for possible population and cross type effects, assuming that there was no year effect. In 1991 crosses were also made with fish from several additional populations from throughout British Columbia, and these served as a partial control for possible year effects. These fish were reared under the same conditions as the Takla 1991 and 1989 crosses but differed from the Takla 1991 crosses in that they experienced no shift in incubation temperature. The additional populations consisted of (*i*) 1991 pure kokanee crosses (10 families) from Kootenay Lake, British Columbia, on the Columbia River system, (*ii*) "hybrids" (4 families) between Kootenay Lake and Takla Lake (Fraser River system) kokanee (Kootenay Lake female from the wild × Takla Lake male from the 1989 Takla crosses), and (*iii*) pure sockeye crosses (20 families) from Kennedy Lake on Vancouver Island, British Columbia.

Sex ratio data were also available for pure kokanee crosses (12 families) from Kootenay Lake in 1989 (see Foote et al. 1994 for rearing methodology). These fish were reared in a similar manner to the 1991 Kootenay crosses, serving as a between-year comparison (1989 and 1991) of the same population undergoing no temperature manipulation. In addition, these were the same years as the between-year comparison within Takla Lake, in which 1991 fish experienced a temperature manipulation and 1989 fish did not. Also, sex ratio data were available for pure and reciprocal hybrid crosses of sockeye and kokanee (12 families of each of the four cross types) from Shuswap Lake in 1986 (see Foote et al. 1992 for rearing methodology), and pure sockeye crosses (7 families) from Babine Lake in 1992.

Complete sex ratio data were available for all of these populations with the exception of the Babine Lake population, which represented a random sample of fish taken on 3 October 1994 at 2 years of age. Though detailed descriptions of rearing methodology are not presented, these populations were maintained under standard hatchery conditions and sampled under a variety of protocols. Hatchery conditions (such as density, diet, and photoperiod) were remarkably constant across years and rearing temperature was consistently between 8 and 10°C (Fig. 1).

Table 1. Percentage of female progeny from crosses of sockeye salmon and kokanee (*Oncorhynchus nerka*) from Takla Lake, 1991.

Population	Brood year	Rearing protocol	*n*	PK	*n*	HK	*n*	HS	*n*	PS
Takla	1991	Outdoor channels	127	78.0*** (69.7–84.8)	132	82.6*** (75.0–88.6)	394	74.9*** (70.3–79.1)	288	61.8** (55.9–67.4)
		Indoor tanks	148	76.2*** (65.8–80.5)	127	81.1*** (73.2–87.5)	111	82.9*** (74.6–89.4)	199	70.4*** (63.5–76.6)
		Outdoor tank	56	83.9*** (71.7–92.4)	0	—	58	74.1* (61.0–84.7)	57	70.2* (56.6–81.6)
		Total	330	78.2*** (73.3–82.5)	259	81.9*** (76.6–86.4)	563	76.4*** (72.6–79.8)	544	65.8*** (61.7–70.0)
Takla	1989		98	54.1 ns (43.7–64.2)	35	54.3 ns (36.6–71.2)	135	45.2 ns (36.6–54.0)	78	60.3 ns (48.5–71.2)
Kennedy	1991		0	—	0	—	0	—	552	56.6 ns (51.5–60.0)
Kootenay	1991		152	39.8 ns (31.0–47.0)	0	—	0	—	0	—
Kootenay	1989		209	53.1 ns (46.1–60.0)	0	—	0	—	0	—
Kootenay × Takla[a]	1991		158	54.4 ns (46.3–62.4)	0	—	0	—	0	—
Babine	1992		0	—	0	—	0	—	419	48.2 ns (43.3–53.1)
Shuswap	1986		93	44.1 ns (33.8–54.8)	121	44.6 ns (35.6–53.9)	128	40.6 ns (32.0–49.7)	95	50.5 ns (40.1–60.9)

Note: PK, pure kokanee; HK, hybrid kokanee (kokanee female × sockeye male); HS, hybrid sockeye (sockeye female × kokanee male); PS, pure sockeye. Results of χ^2 analysis for deviation from a sex ratio of 1:1 are also shown (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns, nonsignificant). Binomial confidence limits are given in parentheses.

[a]Hybrids between Kootenay Lake female kokanee from the wild (1991 brood) and Takla Lake male kokanee maturing in the hatchery (1989 brood).

Table 2. Mortality from 16 August 1991 to 6 October 1993 and adjusted sex ratio (% female assuming all male mortality) by cross type for Takla 1991 crosses.

Cross type	Number of embryos	Number of mortalities[a]	Percent mortality	Percent female
PK	1158	57	4.9	66.7*** (61.7–71.3)
HK	849	101	11.9	58.9* (53.6–64.0)
HS	5164	93	1.8	65.5*** (61.8–69.2)
PS	3887	49	1.3	60.4*** (56.3–64.3)

Note: PK, pure kokanee; HK, hybrid kokanee (kokanee female × sockeye male); HS, hybrid sockeye (sockeye female × kokanee male); PS, pure sockeye. Results of χ^2 analysis for deviation from a sex ratio of 1:1 are also shown (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns, nonsignificant). Binomial confidence limits are given in parentheses.

[a]Includes any unfertilized eggs.

Data analysis

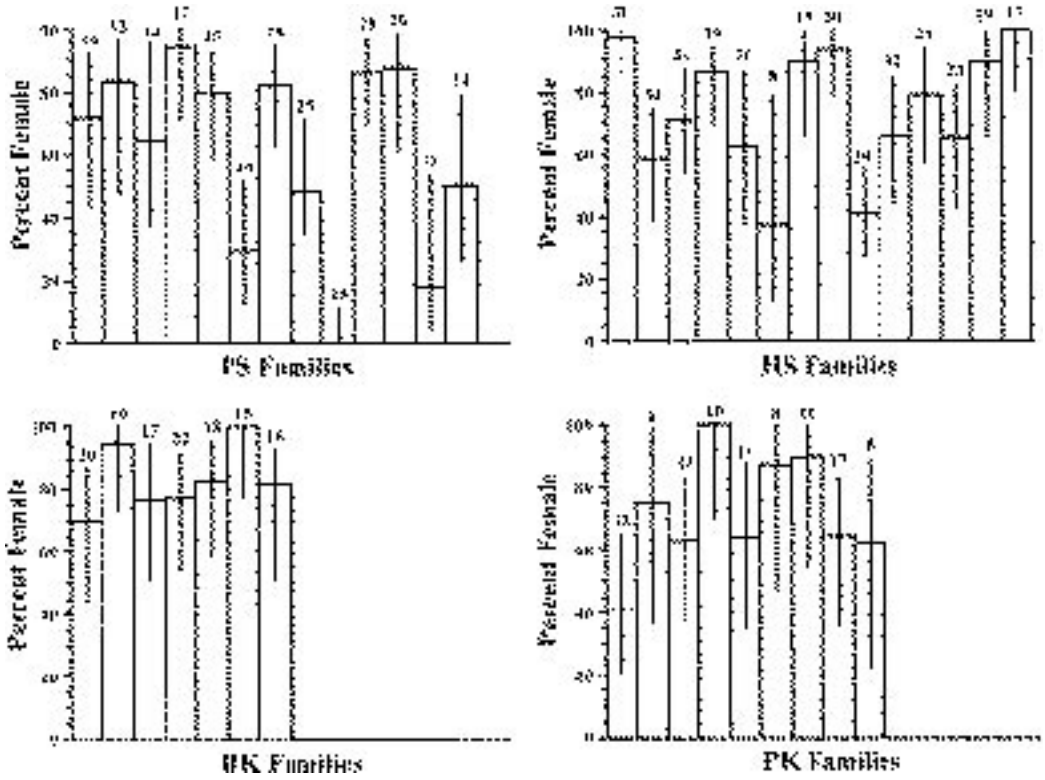
All fish were initially pooled by cross type and sex ratios were individually tested within each cross type for deviations from 1:1 by χ^2 analysis with Yates continuity correction (Zar 1984). Heterogeneity χ^2 analysis was used to examine differences in sex ratio among the three rearing protocols (indoor tanks, outdoor channels, outdoor tank sampled periodically during 1993) and the four cross types (PK, HK, HS, PS) from the Takla 1991 brood. This is a conservative analysis as deviations from a 1:1 sex ratio were tested repeatedly within each rearing protocol and cross type before pooling (see Results). Family sex ratio data were available for the four cross types from the Takla 1991 brood reared in the outdoor channels. χ^2 analysis was used to examine deviations from a 1:1 sex ratio within each family as well as variation in sex ratio among families. Fish of unknown sex accounted for a minor proportion of the data set (9 of 1696 individuals for Takla 1991 crosses; 0.5%) and were conservatively assumed to be males.

Results

Sex ratios shifted drastically in 1991 sockeye and kokanee crosses from Takla Lake (Table 1). Across the four cross types and rearing locations, sex ratios ranged from 61.8 to 83.9% female (all $P < 0.03$). Within a particular cross type, there was no difference in sex ratio among fish reared in outdoor channels, indoor tanks, or those sampled periodically during 1993 (Table 1, all $P > 0.35$). Therefore, data were pooled over the three sampling protocols. Even when all mortalities were combined with the males, a female-biased sex ratio remained (Table 2, all $P < 0.03$).

While sex ratios were similar among the three rearing protocols, the four cross types differed in sex ratio ($P < 0.02$). HK

Fig. 2. Sex ratio by family for pure sockeye (PS), hybrid sockeye (HS; sockeye female × kokanee male), hybrid kokanee (HK; kokanee female × sockeye male), and pure kokanee (PK) crosses from Takla Lake in 1991. Numbers above binomial confidence limits represent family sample sizes.



(81.9% female), PK (77.0% female), and HS (76.4% female) crosses had similar sex ratios,while PS had a less skewed sex ratio (65.8% female). This pattern was mirrored at the family level with female-biased sex ratios in 100% of HK, 89% of PK, 86% of HS, and 77% of PS families (Fig. 2). Thirty-five of the 43 families (79.1%) exhibited female-biased sex ratios (58.0–100.0% female, $P < 0.01$; Fig. 2), though only 15 were individually significantly different from 1:1 ($P < 0.05$). Further, families within cross type were significantly heterogenous in sex ratio for PS ($P < 0.001$) but not the other three cross types (all $P > 0.4$).

Populations reared under nearly identical conditions but undergoing no temperature manipulation exhibited balanced sex ratios (all $P > 0.05$; Table 1). Indeed, pure and reciprocal hybrid crosses from Narrows Creek, Takla Lake, in 1989 exhibited sex ratios that did not differ from 1:1 (all $P > 0.2$). PK from Kootenay Lake in 1989 and 1991, the same 2 years in which Takla Lake fish were reared, maintained sex ratios of 1:1 ($P = 0.58$ and 0.06, respectively; Table 1). In addition, hybrids between Takla Lake kokanee and Kootenay Lake kokanee, as well as pure sockeye from Kennedy Lake, generated in 1991 maintained sex ratios of 1:1 ($P = 0.56$ and 0.06, respectively; Table 1). Further, sockeye and kokanee crosses maintained under standard hatchery conditions from Babine and Shuswap lakes, British Columbia, two separate freshwater systems (Skeena and Fraser rivers, respectively), exhibited normal sex ratios ($P = 0.63$ and all $P > 0.15$, respectively; Table 1).

Discussion

We observed strongly female-biased sex ratios in crosses of sockeye and kokanee from Takla Lake in 1991. The female-biased sex ratio was observed in each of the four cross types and in the majority of families, despite the fact that sockeye and kokanee from Takla Lake are genetically distinct populations (Foote et al. 1989). Therefore, the result was replicated not only across families but across genetically independent populations and their hybrids. The consistency of sex ratios across environments and sampling protocols suggests that sex was irreversibly determined at some common point during early development.

Incubation temperature is probably the factor responsible for the shift in sex ratio. Populations reared under nearly identical conditions but undergoing no temperature manipulation exhibited balanced sex ratios. In addition, similar crosses from Takla Lake in 1989 exhibited balanced sex ratios. The parents of these crosses were collected at the same creek location and nearly the identical date (August 14) as in 1991, indicating that the female-biased sex ratio of 1991 Takla fish was not the result of a population or cross type effect. Further, PK from Kootenay Lake reared in the same 2 years as the Takla crosses but undergoing no temperature manipulation exhibited a normal sex ratio, strongly suggesting that the biased ratio of 1991 Takla fish was the result of the temperature manipulation. That sockeye and kokanee crosses from five disparate locations and three distinct freshwater systems (Fraser, Columbia, and

Skeena rivers) maintained sex ratios of 1:1 with the exception of pure sockeye and kokanee crosses (and their hybrids) undergoing a temperature manipulation, strongly suggests that temperature was the factor responsible for the shift in sex ratio of Takla 1991 crosses.

Other factors that might result in the skewed sex ratio of 1991 Takla fish can largely be ruled out. Culling was random with respect to sex and mortality was very low at all stages. Since a female-biased sex ratio remained after all mortalities were assumed to be males, the biased ratio could not be the result of differential mortality by sex. Although a control independent of temperature is not available for the actual movement of trays to and from the heated water, it is unlikely that slight mechanical agitation significantly affected the sex ratio as all control populations were subject to mechanical agitation at a similar stage to identify dead eggs. In addition, all embryos had hatched and alevins were mobile by the time trays were moved back to ambient water. Other environmental factors that might be responsible for the shift (photoperiod, dissolved oxygen, pH, contaminants) were either invariant or similiar among populations and years. As the hatchery is supplied by groundwater, contamination from surface pollutants (e.g., environmental estrogens) is unlikely and would have affected the other 1991 populations as well. Given the consistency of hatchery conditions across years, the magnitude of the shift in sex ratio, the replication of the effect across genetically distinct populations, the low mortality observed, and evidence for temperature effects on sex determination in other fishes (Harrington 1968; Conover and Kynard 1981), the temperature manipulation remains the most plausible cause of sex ratio distortion observed in the Takla 1991 crosses.

It is not clear what aspect(s) of the temperature manipulation was (were) responsible for the shift in sex ratio. The effect could have resulted from the the shift to an elevated incubation temperature (8.4–10.5°C), the actual incubation temperature itself (range 10.5–12.0°C) following the shift, and (or) the shift back to water of ambient temperature (11.7–8.4°C) (Fig. 1). It would appear unlikely that incubation temperature per se was responsible. Incubation temperature undoubtedly varies among hatcheries (see Table 7, Burgner 1991) and though few studies report sex ratios, it is improbable that such a strongly biased ratio would go unreported or unnoticed. It is more probable that the shift itself occurred during a critical point in development, inhibiting the phenotypic expression of maleness. During the sexual development of many fishes the gonads develop first as female-like ovaries and subsequently a proportion (usually 50%) further develop as testes (Yamazaki 1983). Perhaps the underlying physiological process(es) responsible for male development was (were) inhibited by the temperature shift. Embryos underwent an immediate temperature increase from 8.4 to 10.5°C at 360 degree-days followed by a decrease from 11.7 to 8.4°C at 776 degree-days. Sex determination may be sensitive to temperature within a developmental window during this period.

The four cross types differed in sex ratio, with PS exhibiting a less biased ratio than the other cross types. As sockeye and kokanee differ in development rate owing to both male and female genetic effects (Wood and Foote 1990), the cross types may have been at slightly different developmental stages when the temperature shift occurred. Alternatively, sockeye and kokanee may be polymorphic for labile sex determination such that they differ in sensitivity to temperature and (or) have different developmental windows during which temperature exerts an effect.

The majority of families (79.1%) of the four cross types had female-biased sex ratios though many were not significantly different from 1:1, probably because of small family sample sizes (8–40 fish/family). In addition, a small proportion of families were male biased, some extremely so (Fig. 2). For example, one PS family contained all males (n = 32) whereas two other families were strongly male biased (70.8 and 81.8% male; n = 24 and 11, respectively). That family sex ratios were either strongly male or female biased with few families exhibiting sex ratios approaching 1:1 suggests that a temperature-dependent, major sex determining gene may be segregating in the population (see Lagomarsino and Conover 1993). The existence of such a gene could account for the heterogeneous sex ratios observed among PS families.

Some populations of sockeye and kokanee exhibit morphologically distinct sex chromosomes (sockeye, Thorgaard 1978; kokanee, Udea and Ojima 1984). Sockeye and kokanee from Takla Lake have not been cytologically examined. However, assuming dimorphic sex chromosomes are present in this population as well, presumably some of the females from the Takla 1991 brood had XY sex chromosomes. Therefore, XY females when mated to XY males could give rise to XX, XY, or YY offspring, even under normal rearing conditions.

It is not clear whether ESD may be adaptive in *O. nerka* or, alternatively, some genetically determined, physiological process was simply inhibited in our experiments by temperature shock. Conover and Kynard (1981) demonstrated that higher rearing temperatures result in a greater proportion of males in silversides and the magnitude of this response varies latitudinally among allopatric populations (Lagomarsino and Conover 1993). In addition, ESD in this species appears to be adaptive (Conover 1984) and under the control of major and minor sex determining genes as well as temperature (Conover and Heins 1987; Conover et al. 1992; Lagomarsino and Conover 1993). Adaptive explanations also have been postulated for ESD in reptiles (Deeming and Ferguson 1988; Janzen and Paukstis 1991).

That a species with morphologically distinct sex chromosomes (Thorgaard 1978; Ueda and Ojima 1984) appears labile for sex determination is intriguing. Given the growing recognition of the effects of environmental factors on sex determination, the possible effects of global climate change and human-induced temperature shifts in localized environments, and the economic importance of salmonid aquaculture, the possibility of temperature-dependent sex determination in salmonids deserves further investigation.

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