

Early survival and development of coastal cutthroat trout (*Oncorhynchus clarki clarki*), steelhead (*Oncorhynchus mykiss*), and reciprocal hybrids

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Abstract: We compared the survival and development of embryos from fertilization to emergence of two populations of coastal cutthroat trout (*Oncorhynchus clarki clarki*), one population of steelhead (*Oncorhynchus mykiss*), and their reciprocal hybrids under controlled conditions ($10 \pm 1^\circ\text{C}$). The egg weight of the steelhead (0.110 ± 0.008 (SE) g) was nearly twice that of coastal cutthroat trout (0.056 ± 0.003 and 0.051 ± 0.001 g for the two populations). Fertilization success, survival, duration of hatching and emergence periods, yolk absorption relative to total alevin weight, and yolk conversion efficiency were similar among the cross types. There was no evidence of reduced hatchability or viability of hybrids despite maternal and paternal species effects on size and development. Fish with cutthroat dams had lower percent hatch, were smaller at hatch and emergence, grew more slowly, used less yolk per day, reached 50% yolk absorption earlier, and had a smaller percent yolk at hatch than those fish with steelhead dams. Fish sired by cutthroat males hatched and emerged earlier and took less time between the two stages than fish sired by steelhead. As a consequence, hybrids with cutthroat dams emerged late and had little yolk, while hybrids with steelhead dams emerged early and had an abundance of yolk.

Résumé : Nous avons comparé les taux de survie et de développement d'embryons, de la fécondation à l'émergence, de deux populations côtières de truite fardée (*Oncorhynchus clarki clarki*), d'une population de truite arc-en-ciel anadrome (*O. mykiss*) et de leurs hybrides réciproques dans des conditions particulières ($10 \pm 1^\circ\text{C}$). Le poids des oeufs de la truite arc-en-ciel ($0,110 \pm 0,008$ (é-t) g) était presque le double de celui de la truite fardée ($0,056 \pm 0,003$ g et $0,051 \pm 0,001$ g pour les deux populations). La réussite de la fécondation, la survie, la durée des périodes d'éclosion et d'émergence, l'absorption du vitellus par rapport au poids total de l'alevin et l'efficacité de conversion du vitellus étaient semblables chez les types de croisements. Rien n'indiquait une baisse de la capacité d'éclosion ou de la viabilité des hybrides en dépit des effets des espèces maternelles et paternelles sur la taille et le développement. Les poissons ayant pour mère une truite fardée présentaient un pourcentage d'éclosion inférieur, étaient plus petits au moment de l'éclosion et de l'émergence, avaient une croissance plus lente, utilisaient moins de vitellus par jour, atteignaient le taux d'absorption de 50% du vitellus plus rapidement et disposaient d'un plus petit pourcentage de vitellus à l'éclosion comparativement aux poissons dont la mère était une truite arc-en-ciel. Les poissons dont le père était une truite fardée présentaient une éclosion et une émergence plus hâtives et la période entre ces deux étapes était plus courte que chez les poissons dont le père était une truite arc-en-ciel. Les hybrides ayant pour mère une truite fardée émergeaient tardivement et disposaient de peu de vitellus tandis que les hybrides ayant une truite arc-en-ciel anadrome pour mère émergeaient plus tôt et disposaient de beaucoup de vitellus.

[Traduit par la Rédaction]

Introduction

Hybridization can be defined as the breakdown of isolating mechanisms between two reproductively isolated species or populations. In fishes, hybridization is common within many families, due in large part to external fertilization and weak pair bonds between the parents (Avisé 1994). There are many examples of hybridization among salmonids (re-

viewed by Verspoor and Hammar 1991). In a review of the fitness of hybrids relative to their parents, Arnold and Hodges (1995) provided examples from many taxa and genera and concluded that hybrids are not uniformly less fit than the parental species. Although the relative fitnesses of hybrid and pure-strain offspring have seldom been compared in salmonids, superior performance by hybrids is generally the exception (Leary et al. 1995). The relative fitness of F_1 hybrids determines whether occasional hybridization strengthens isolating mechanisms or leads to introgression of the gene pools (Avisé 1994).

Behnke (1988) divided cutthroat trout (*Oncorhynchus clarki*) into four major subspecies and 10 minor subspecies depending on historical biogeography and the magnitude of phylogenetic divergence. Introgression between many of the interior subspecies of cutthroat trout and introduced rainbow

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Fig. 1. Genetic design of cutthroat trout and steelhead crosses. The female parent is listed first in all cross types (C, cutthroat; S, steelhead). Subscripts indicate hatchery of origin for that parent (a, Aberdeen; s, Shelton). This design was repeated five times. Cutthroat stocks were subsequently pooled for analyses when no differences were found between them.

	C _a C _a Aberdeen cutthroat dam	C _s C _s Shelton cutthroat dam	SS steelhead dam
C _a C _a Aberdeen cutthroat sire	C _a C _a Aberdeen pure cutthroat	Cross not done	S _a C _a hybrid steelhead Aberdeen sire
C _s C _s Shelton cutthroat sire	Cross not done	C _s C _s Shelton pure cutthroat	S _s C _s hybrid steelhead Shelton sire
SS steelhead sire	C _a S _a hybrid cutthroat Aberdeen dam	C _s S _s hybrid cutthroat Shelton dam	SS pure steelhead

trout (*Oncorhynchus mykiss*) is widespread (Forbes and Allendorf 1991). This propensity to complete introgression suggests that because the interior subspecies of cutthroat trout were isolated from rainbow trout, they did not evolve isolating mechanisms to prevent hybridization (Behnke 1992).

Coastal cutthroat trout (*Oncorhynchus clarki clarki*) coexist with steelhead (the anadromous form of *O. mykiss*) throughout much of their native range. Although the timing of their phyletic divergence is not fully known (Utter and Allendorf 1994), Behnke (1992) concluded that cutthroat trout and steelhead shared a common ancestor as recently as 2 million years ago. As would be expected from this relatively recent divergence, cutthroat trout and steelhead share many characteristics. However, differences at protein-coding loci (e.g., Leary et al. 1987), in mtDNA sequence (Wilson et al. 1985), in karyotype (Gold 1977), and in morphology (e.g., Hawkins and Quinn 1996) can be used to distinguish between the species.

There are several differences between coastal cutthroat trout and steelhead that could lead to both pre- and post-mating reproductive isolating mechanisms that maintain the species' integrity. Spawning adults are generally separated both temporally and spatially (Trotter 1987; Burgner et al. 1992). In western Washington State, steelhead often spawn later in the season, using the deeper, faster water of larger rivers, whereas coastal cutthroat trout spawn earlier in smaller headwaters (Johnston and Mercer 1976). Steelhead are also often much larger at maturity, which could lead to assortative mating or possibly to directionality in pair formation in the event of heterospecific matings (Foote and Larkin 1988). Coastal cutthroat trout and steelhead also have different numbers of chromosomes (68 and 58–60, respectively; Behnke 1970), which could lead to mispairing of chromosomes and decreased viability in hybrid progeny. Furthermore, juvenile coastal cutthroat trout and steelhead use different stream habitats when sympatric and the hybrid progeny that do survive may be at a competitive disadvantage (Hawkins 1997).

Prior to 1981, hybridization between coastal cutthroat trout and steelhead was thought to be uncommon (Utter 1981), presumably due to the coevolution of coastal cutthroat trout and steelhead (Behnke 1992). However, Campton and Utter (1985) found a substantial number of hy-

brids in two streams in the Puget Sound area of Washington. Hybridization is cause for concern because it may contribute to the decline of western trout species (Busack and Gall 1981), and many cutthroat trout populations currently are declining (Johnson et al. 1994). Allendorf and Leary (1988) concluded that hybridization has been the main impact of introduced fish on native cutthroat trout populations. The incidence of natural hybrids between coastal cutthroat trout and steelhead, coupled with the apparent ability of the two species to maintain their genetic integrities in areas of natural sympatry, raises many questions regarding the existence and effectiveness of potential postzygotic isolating mechanisms.

Here, we examine (i) the relative viabilities of coastal cutthroat trout (subsequently referred to simply as cutthroat), steelhead, and their reciprocal hybrids when raised under controlled conditions from fertilization to 64 days postfertilization and (ii) the development of the embryos to distinct stages from fertilization to the voluntary emergence of the alevins. We report no evidence of decreased viability of hybrids, but did detect genetic differences in development rate.

Methods

Study populations

Adults from two populations of cutthroat trout (Lake Aberdeen Hatchery, Aberdeen, Wash., and Eels Springs Hatchery, Shelton, Wash.) and one population of steelhead (Lake Aberdeen Hatchery) were used to create the progeny for this experiment. Both populations of cutthroat originated from anadromous fish, but are currently maintained as captive broodstocks whereas the steelhead are released into the wild as smolts. The Lake Aberdeen cutthroat trout is the product of hybridization with steelhead in the past. Protein electrophoresis suggests that the majority of the adults used had cutthroat alleles at all diagnostic loci (Hawkins 1997).

Fertilization and rearing

Eggs and milt were obtained on 25 January 1995 from the two hatcheries and transported separately in plastic bags on ice to the University of Washington, School of Fisheries hatchery. Two hours later, the eggs from each cutthroat or steelhead female were divided and fertilized by milt from both cutthroat and steelhead males as detailed in Fig. 1. Five males and five females from each population were used, resulting in 35 families. The four major cross types were as follows: CC, pure cutthroat; CS, hybrids with cutthroat dams (referred to as hybrid cutthroat); SC, hybrids with steelhead dams (referred to as hybrid steelhead); SS; pure steelhead. Because two cutthroat populations were used, the crosses involving cutthroat can be subdivided into six minor cross types: C_aC_a, pure Aberdeen cutthroat; C_sC_s, pure Shelton cutthroat; C_aS_a, hybrid cutthroat with Aberdeen cutthroat dams; C_sS_s, hybrid cutthroat with Shelton cutthroat dams; S_aC_a, hybrid steelhead with Aberdeen cutthroat sires; S_sC_s, hybrid steelhead with Shelton cutthroat sires. This experimental design resulted in five independent replicates of a group of seven connected families. Within a group, the pure families (CC, SS) were not related; likewise, the hybrid cutthroat and hybrid steelhead (CS, SC) were not related. However, the hybrid types were related to two of the pure families and to like hybrids. Families within a row were paternal half-sibs and those within a column were maternal half-sibs (Fig. 1). Among the five replicates, none of the families were related.

Each family was incubated in a randomly assigned Heath tray in dechlorinated city water at 10 ± 1°C. At 21 days postfertilization, at the eyed-embryo stage of development, eggs and embryos from

all families were mechanically shocked by pouring them onto a damp tray from a height of 10 cm to facilitate identification and removal of all unfertilized eggs and dead embryos. All remaining embryos were counted for each family. All of the embryos from two cutthroat females from Aberdeen, accounting for two pure cutthroat and two hybrid cutthroat families, did not develop due to poor egg quality. One hundred embryos per family were then placed in two egg cups located in each Heath tray (50 per cup) to control for density and the amount of swimming space available to the alevins after hatching. Embryos were divided equally into the two cups for families with fewer than 100 embryos, and all embryos were placed in one cup for families with fewer than 50 embryos. These embryos were used to determine the rate of hatching, growth, and yolk absorption and the duration of the hatching period. The remaining embryos were left in the Heath tray for estimating survival. The cups were made from 10-cm-diameter PVC pipe with window screen on one side. The top screen of the Heath tray prevented embryos from entering or leaving the cups after hatching.

At the completion of hatching, 50 embryos (those in one egg cup) from each of three families per minor cross type and pure steelhead were placed into emergence chambers to determine the length of time from fertilization to 50% emergence, duration of emergence period (time from emergence of first to last fish), and length of time between 50% hatch and 50% emergence. The emergence chambers allowed the embryos to hide in the substrate and volitionally emerge (Hendry 1995). The remaining embryos were left in their egg cups (used for determination of growth and yolk absorption) or loose in the Heath trays. At this point, all embryos and alevins were switched from dechlorinated city water to Lake Washington water at $10 \pm 1^\circ\text{C}$. As the alevins in the Heath trays reached the swim-up stage of development (indicated by behavior), they were placed in 10-L buckets for continued rearing and survival measurements by family.

Data collection

Egg size was determined from 10 eggs per female collected prior to fertilization and preserved in 10% buffered formalin. After 2 months in formalin, each egg was blotted on paper towel to remove excess formalin and weighed to ± 0.001 g with a digital Mettler balance.

To minimize fungal growth, eggs or alevins that were not developing were removed and counted 1 day after fertilization, at the eyed-embryo stage, periodically until hatching, once every day during hatching, and every fourth day thereafter. All removed eggs were cleared with Stockard's solution to determine unfertilized eggs and stage of death of fertilized eggs. There was no particular stage at which development stopped within any of the cross types, and the results are not presented here. Survival was compared for all families at three intervals of development: fertilization to eyed embryo, eyed embryo to hatch, and hatch to 64 days of age. Percent mortality of each family was calculated as the number of dead eggs divided by the total starting number of eggs at each of the three intervals. Fertilization success was determined as the percentage of eggs that were fertilized after blank and atretic eggs (eggs that did not contain a yolk or a yolk that was partially resorbed) were removed from the total. Hatchability of each family was calculated as the percentage of fertilized eggs that hatched.

Throughout the hatching period, all hatched embryos were counted in each egg cup three times per day. Length of time from fertilization to 50% hatch and duration of hatching period (time from the first to the last embryo hatched) were determined for each family from the embryos in the egg cups. When the last embryo had hatched within a family, three hatched embryos were randomly selected to determine the body weight and amount of yolk at hatching. After preservation for at least 1 month, the yolk was dissected

from the body and both were dried for 24 h at 95°C to remove all moisture and then weighed to ± 0.001 g.

For the collection of the emergence data, the hatched embryos were added to the emergence chamber and within 15 min had all moved down into the marbles. The emergence chambers consisted of an inner chamber of water about 30% full of marbles with an exit notch at the top, within an outer chamber of water. As alevins emerged, they moved from the inner chamber to the outer chamber through the exit notch at the top of the inner chamber. Once in the outer chamber, they were unable to reenter the inner chamber because of the water level difference between the two (10 cm). Throughout the emergence period, alevins in the outer chamber were counted twice daily and removed. The fifth, 20th, and 45th alevins to emerge from each family were collected for determination of body weight and amount of yolk at emergence.

The absolute rate of yolk absorption, yolk absorption relative to total embryo weight, and rate of growth were determined from samples of the 50 embryos left in the remaining egg cups in the Heath trays. Beginning at 40 days postfertilization, three embryos from each family were sampled for body weight and amount of yolk every fourth day. Samples were taken until 64 days postfertilization, at which time, all fish in families had completely absorbed their yolk sacs. Length of time postfertilization to 50% yolk absorption and yolk conversion efficiency were also determined from these samples. Yolk conversion efficiency from day 40 to day 52 postfertilization (change in body weight/change in yolk weight) was calculated for all families with embryos in egg cups (except one hybrid steelhead family whose samples were lost during processing).

Statistical analyses

All measurements of percent fertilization, hatched, dead, and yolk were transformed using an arcsine square root transformation to normalize the data before either ANOVA or regression analysis. Because there were no differences between the two populations of cutthroat in either the pure cutthroat families or the reciprocal hybrid families (ANOVA), the Shelton and Aberdeen cutthroat populations were pooled for the analyses presented here.

Length of time to 50% hatch, 50% emergence, and 50% yolk were interpolated for each family using the regression equation from the linear portion of the graph of cumulative percent hatched, or emerged or percent yolk against number of days postfertilization.

A two-way ANOVA model was used to examine the effects of dam type, sire type, and interaction between dam and sire types among the four major cross types (CC, CS, SC, SS) (family means were used in all tests to avoid pseudoreplication):

$$Y_{ijk} = \mu_{..} + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}$$

where $\mu_{..}$ is a constant, α_i is the effect of the dam type, β_j is the effect of the sire type, $(\alpha\beta)_{ij}$ is the interaction effect between the dam and sire types, and ϵ_{ijk} is the independent error term.

When significant dam type effects were identified for a trait, simple linear regression of that trait on egg weight was carried out within each cross type to determine whether the results were due to species differences or could be explained by differences in egg size alone. ANCOVA was not appropriate because the egg sizes of cutthroat and steelhead did not overlap.

To examine the phenotypic correlations among the many traits measured in this study, Spearman correlation coefficients were calculated using the data from the 21 families for which all the traits were measured. The traits with significant dam type effects were generally correlated whereas the traits with significant sire type effects were correlated.

Table 1. Group means and results of statistical analyses for two-way ANOVA tests of dam type, sire type, and interaction effects.

Trait	CC mean (SE)	CS mean (SE)	SC mean (SE)	SS mean (SE)	Dam type effect	Sire type effect	Interaction effect dam type × sire type
Fertilization (%)	85.304 (7.074)	85.301 (6.038)	98.022 (0.896)	95.482 (3.155)	$F_{1,27} = 8.749$ $p = 0.006$	$F_{1,27} = 0.232$ $p = 0.634$	$F_{1,27} = 0.213$ $p = 0.648$
Fertilization to eyed-embryo mortality (%)	3.266 (1.421)	3.858 (2.683)	0.078 (0.053)	0.278 (0.278)	$F_{1,27} = 13.605$ $p = 0.001$	$F_{1,27} = 0.031$ $p = 0.862$	$F_{1,27} = 0.236$ $p = 0.631$
Eyed-embryo to hatch mortality (%)	0.885 (0.413)	0.866 (0.460)	0.181 (0.100)	0.100 (0.100)	$F_{1,27} = 4.871$ $p = 0.036$	$F_{1,27} = 0.028$ $p = 0.860$	$F_{1,27} = 0.060$ $p = 0.808$
Hatch to 64 days mortality (%)	1.918 (0.463)	1.755 (0.673)	1.295 (0.297)	0.716 (0.244)	$F_{1,27} = 2.334$ $p = 0.138$	$F_{1,27} = 0.799$ $p = 0.379$	$F_{1,27} = 0.238$ $p = 0.629$
Hatchability (%)	95.432 (1.506)	95.199 (2.908)	99.689 (0.131)	99.688 (0.138)	$F_{1,27} = 16.070$ $p < 0.001$	$F_{1,27} = 0.040$ $p = 0.843$	$F_{1,27} = 0.110$ $p = 0.743$
Duration of hatching (days)	1.381 (0.135)	1.469 (0.189)	1.295 (0.161)	1.970 (0.353)	$F_{1,27} = 1.078$ $p = 0.308$	$F_{1,27} = 3.638$ $p = 0.067$	$F_{1,27} = 2.160$ $p = 0.153$
Time to 50% hatch (days)	32.193 (0.175)	32.848 (0.219)	32.784 (0.113)	33.890 (0.280)	$F_{1,27} = 18.410$ $p < 0.001$	$F_{1,27} = 21.421$ $p < 0.001$	$F_{1,27} = 1.406$ $p = 0.246$
Body weight at hatch (g)	0.004 (0.0002)	0.0042 (0.0002)	0.0065 (0.0002)	0.0067 (0.0002)	$F_{1,27} = 153.021$ $p < 0.001$	$F_{1,27} = 0.588$ $p = 0.450$	$F_{1,27} = 0.003$ $p = 0.954$
Yolk at hatch (%)	78.39 (0.70)	78.04 (0.76)	84.03 (0.75)	83.34 (1.46)	$F_{1,27} = 37.529$ $p < 0.001$	$F_{1,27} = 0.324$ $p = 0.574$	$F_{1,27} = 0.039$ $p = 0.844$
Duration of emergence (days)	11.65 (1.76)	10.78 (1.40)	14.21 (1.52)	19.41 (1.25)	$F_{1,17} = 11.074$ $p = 0.004$	$F_{1,17} = 1.658$ $p = 0.215$	$F_{1,17} = 3.245$ $p = 0.089$
Time to 50% emergence (days)	56.40 (0.45)	58.57 (0.44)	55.55 (0.23)	58.81 (1.45)	$F_{1,17} = 0.285$ $p = 0.600$	$F_{1,17} = 23.045$ $p < 0.001$	$F_{1,17} = 0.912$ $p = 0.353$
Body weight at emergence (g)	0.013 (0.0007)	0.014 (0.0008)	0.024 (0.0014)	0.025 (0.0023)	$F_{1,17} = 78.117$ $p < 0.001$	$F_{1,17} = 0.627$ $p = 0.439$	$F_{1,17} = 0.061$ $p = 0.809$
Yolk at emergence (%)	16.46 (3.52)	9.81 (1.19)	39.33 (2.23)	35.31 (6.21)	$F_{1,17} = 60.490$ $p < 0.001$	$F_{1,17} = 3.247$ $p = 0.089$	$F_{1,17} = 0.425$ $p = 0.523$
Time between 50% hatch and 50% emergence (days)	24.16 (0.38)	25.85 (0.38)	22.63 (0.20)	24.90 (1.66)	$F_{1,17} = 4.729$ $p = 0.044$	$F_{1,17} = 12.048$ $p = 0.003$	$F_{1,17} = 0.263$ $p = 0.614$
Growth: 40–52 days postfertilization (g)	0.0068 (0.0003)	0.0067 (0.0004)	0.0130 (0.0009)	0.0135 (0.0010)	$F_{1,26} = 81.109$ $p < 0.001$	$F_{1,26} = 0.089$ $p = 0.768$	$F_{1,26} = 0.206$ $p = 0.654$
Yolk conversion efficiency: 40–52 days postfertilization	0.711 (0.033)	0.788 (0.052)	0.688 (0.019)	0.830 (0.039)	$F_{1,26} = 0.063$ $p = 0.804$	$F_{1,26} = 8.230$ $p = 0.008$	$F_{1,26} = 0.734$ $p = 0.399$
Time to 50% yolk (days)	42.84 (0.33)	43.68 (0.66)	47.53 (0.46)	48.49 (0.71)	$F_{1,27} = 73.763$ $p < 0.001$	$F_{1,27} = 2.680$ $p = 0.113$	$F_{1,27} = 0.010$ $p = 0.921$
Change in yolk weight: 40–52 days postfertilization (g)	0.0097 (0.0006)	0.0087 (0.0008)	0.0187 (0.0008)	0.0166 (0.0018)	$F_{1,26} = 77.741$ $p < 0.001$	$F_{1,26} = 2.663$ $p = 0.115$	$F_{1,26} = 0.379$ $p = 0.543$

Note: In all groups, the species of the dam is listed first (C, cutthroat; S, steelhead). The group means are untransformed means in the case of percentage data. All percentage data were transformed prior to ANOVA.

Results

The group means and ANOVA results are given in Table 1 for all of the traits except egg weights, which are given below.

Egg weight

The average egg weight of the two stocks of cutthroat was similar (Tukey $P > 0.8$; Aberdeen: 0.056 ± 0.003 SE g, range 0.053–0.063 g; Shelton: 0.051 ± 0.001 g, range 0.048–0.055 g) and less than that for steelhead (0.110 ± 0.008 g, range 0.091–0.134 g; $F_{2,10} = 38.270$, $P < 0.001$; Tukey $P < 0.001$ for steelhead compared with both Aberdeen and Shelton cutthroat).

Traits with significant dam type effects

Dam type effects were significant for the following traits: fertilization, mortality from fertilization to the eyed-embryo stage and from the eyed-embryo stage to hatching, hatchability, body weight and percent yolk at both hatching and emergence, duration of the emergence period, growth from 40 to 52 days postfertilization (Fig. 2A), length of time to 50% yolk, and yolk absorption from 40 to 52 days postfertilization (Fig. 2B) (Table 1).

Steelhead eggs were nearly twice the weight of cutthroat eggs. Consequently, maternal steelhead crosses were larger with a higher percentage of yolk at hatch, grew faster, and were larger with a higher percentage of yolk at emergence than maternal cutthroat (both pure and hybrid) (Figs. 2A and 2B). Because yolk absorption was proportional to body weight across species (larger fish use the same amount of yolk relative to their body weight, but use absolutely more yolk than smaller fish), the decrease in absolute yolk weight was higher in maternal steelhead than in maternal cutthroat. The combination of proportional absorption of yolk and a higher percent yolk at hatch in maternal steelhead resulted in a longer time from fertilization until 50% yolk absorption in maternal steelhead compared with maternal cutthroat (Fig. 2B).

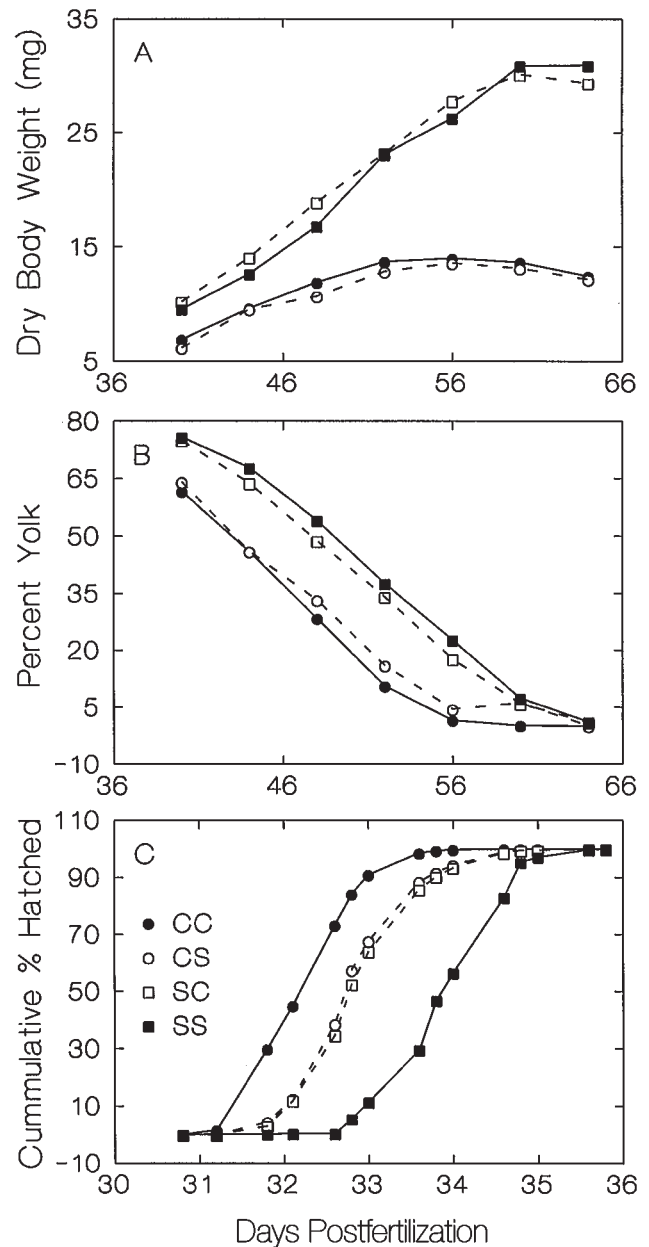
Traits with significant sire type effects

Sire type effects were significant for the time to 50% emergence and yolk conversion efficiency from 40 to 52 days postfertilization (Table 1).

Traits with significant dam and sire type effects

The following traits had both significant dam type and sire type effects (the interaction effects between the dam and sire types were not significant): length of time to 50% hatch and length of time between hatching and emergence (Table 1). These sex-specific effects acted together on hatching time. Steelhead sires and dams increased the time to hatching. Therefore, steelhead hatched later than all other cross types, cutthroat hatched earlier, and hybrids were generally intermediate (Fig. 2C). For the time between hatching and emergence the sex-specific effects acted in opposition within a species: steelhead sires increased the time and steelhead dams decreased the time. However, the sire type effect dominated so that pure steelhead took longer to emerge after hatching than pure cutthroat. Because of the opposing sex-specific effects on this trait, the means of the hybrids were

Fig. 2. (A) Plot of body weight by time for pure and reciprocal hybrids of steelhead, cutthroat trout, and reciprocal hybrids. (B) Rate of yolk absorption relative to total embryo weight for each cross type. For analyses presented in the text, slopes and change in body weight were calculated over the linear portion of the graph (40–52 days postfertilization). (C) Mean cumulative percent hatched for each cross type. The species of the dam is listed first in all cross types (C, cutthroat; S, steelhead).



extreme compared with the means of the pure species. Hybrid steelhead had the shortest time between hatching and emergence, due to the influence of steelhead dams and cutthroat sires, and hybrid cutthroat had the opposite influence from cutthroat dams and steelhead sires and took longer between hatching and emergence than all other cross types. There was also a strong sire type effect on time to emergence that enhanced the difference in time between hatching and emergence. Steelhead sires increased and cutthroat sires

Table 2. Simple linear regression analyses of each trait on egg weight for those traits with significant dam type effects.

Trait	CC	CS	SC	SS
Fertilization (%)	$r^2 = 0.009$ $p = 0.824$	$r^2 = 0.020$ $p = 0.735$	$r^2 = 0.235$ $p = 0.156$	$r^2 = 0.100$ $p = 0.604$
Fertilization to eyed-embryo mortality (%)	$r^2 = 0.010$ $p = 0.814$	$r^2 = 0.011$ $p = 0.804$	$r^2 = 0.097$ $p = 0.381$	$r^2 = 0.124$ $p = 0.561$
Eyed-embryo to hatch mortality (%)	$r^2 = 0.005$ $p = 0.866$	$r^2 = 0.095$ $p = 0.457$	$r^2 = 0.001$ $p = 0.932$	$r^2 = 0.561$ $p = 0.145$
Hatchability (%)	$r^2 = 0.002$ $p = 0.921$	$r^2 = 0.007$ $p = 0.842$	$r^2 = 0.009$ $p = 0.789$	$r^2 = 0.021$ $p = 0.815$
Time to 50% hatch (days)	$r^2 = 0.355$ $p = 0.119$	$r^2 = 0.000$ $p = 0.985$	$r^2 = 0.013$ $p = 0.749$	$r^2 = 0.129$ $p = 0.552$
Body weight at hatch (g)	$r^2 = 0.783$ $p = 0.004$	$r^2 = 0.403$ $p = 0.091$	$r^2 = 0.257$ $p = 0.135$	$r^2 = 0.105$ $p = 0.595$
Yolk at hatch (%)	$r^2 = 0.009$ $p = 0.823$	$r^2 = 0.588$ $p = 0.026$	$r^2 = 0.737$ $p = 0.001$	$r^2 = 0.921$ $p = 0.010$
Duration of emergence (days)	$r^2 = 0.005$ $p = 0.894$	$r^2 = 0.040$ $p = 0.703$	$r^2 = 0.169$ $p = 0.417$	$r^2 = 0.001$ $p = 0.980$
Body weight at emergence (g)	$r^2 = 0.378$ $p = 0.194$	$r^2 = 0.539$ $p = 0.097$	$r^2 = 0.738$ $p = 0.028$	$r^2 = 0.777$ $p = 0.313$
Yolk at emergence (%)	$r^2 = 0.006$ $p = 0.886$	$r^2 = 0.077$ $p = 0.594$	$r^2 = 0.469$ $p = 0.133$	$r^2 = 0.248$ $p = 0.668$
Time between 50% hatch and 50% emergence (days)	$r^2 = 0.057$ $p = 0.650$	$r^2 = 0.037$ $p = 0.717$	$r^2 = 0.137$ $p = 0.470$	$r^2 = 0.254$ $p = 0.664$
Growth: 40–52 days postfertilization (g)	$r^2 = 0.090$ $p = 0.471$	$r^2 = 0.332$ $p = 0.135$	$r^2 = 0.573$ $p = 0.018$	$r^2 = 0.743$ $p = 0.060$
Time to 50% yolk (days)	$r^2 = 0.233$ $p = 0.226$	$r^2 = 0.016$ $p = 0.762$	$r^2 = 0.577$ $p = 0.011$	$r^2 = 0.382$ $p = 0.267$
Change in yolk weight: 40–52 days postfertilization (g)	$r^2 = 0.152$ $p = 0.340$	$r^2 = 0.460$ $p = 0.064$	$r^2 = 0.643$ $p = 0.009$	$r^2 = 0.745$ $p = 0.059$

Note: All percentage data were transformed prior to analysis. In all groups, the species of the dam is listed first (C, cutthroat; S, steelhead).

decreased the time to emergence. Hybrid steelhead emerged first, cutthroat emerged second, hybrid cutthroat emerged third, and steelhead emerged last (Table 1).

Egg weight regression analyses

Egg weight was correlated with body weight at hatch (for CC) and emergence (for SC), growth (change in body weight from 40 to 52 days) (for SC), percent yolk at hatch (for CS, SC, SS), length of time to 50% yolk absorption (for SC), and the change in yolk weight from 40 to 52 days (for SC) (Table 2).

Discussion

Hybridization often has adverse effects on fitness characteristics such as survival, growth, and development (Leary et al. 1995). Comparative studies of salmonid hybrid survival and early development often come to conflicting conclusions as to the relative performance of hybrid and pure species. Depending in large part on which traits are used to evaluate the relative performance, whether reciprocal hybrids are examined, and possibly which species are observed, the data show no difference among hybrids and pure species (e.g., Ferguson et al. 1988; Wood and Foote 1990), intermediate performance of hybrids (e.g., Dumas et al. 1992), superior performance by hybrids (e.g., Halliburton et al. 1983), and in some cases mixed results including all of the previous

possibilities plus inferior performance by some hybrids (e.g., Halliburton et al. 1983; Ferguson et al. 1988; Wood and Foote 1990; Dumas et al. 1992; McGowan and Davidson 1992). In the study described here, the majority of the traits had a dam type effect with maternal cutthroat types similar to each other and different from maternal steelhead types. However, three measurements of development rate had a sire type effect, and thus, hybrids either grouped with the paternal species (two traits) or were intermediate (one trait) depending on the trait examined.

Relative survival as a component of fitness has been compared within species (e.g., Beacham 1988), between forms within species (e.g., Wood and Foote 1990, 1996), and between hybridizing species (e.g., Halliburton et al. 1983; Dumas et al. 1992; McGowan and Davidson 1992). Maternal effects accounted for most of the variation detected within species and between species; hybrids were not different from the maternally related pure species. Similarly, in this study, there was no evidence that fertilization success, hatchability, or subsequent survival of heterospecific crosses was less successful than for conspecifics in either steelhead or cutthroat trout. Therefore, decreased fertilization and viability of heterospecific embryos alone do not appear to function as postzygotic isolating mechanisms. For these three traits, there was a dam type effect with the hybrid crosses similar to the maternally related pure crosses. Maternal cutthroat demonstrated decreased fitness compared with mater-

nal steelhead under the conditions of gamete collection and rearing of this study.

Many studies have shown that larger eggs produce larger embryos with greater yolk reserves at hatch and that these larger embryos grow faster and are larger with greater yolk reserves at emergence (e.g., Hayashizaki et al. 1995). In the study described here the results were consistent with the literature results: maternal steelhead were larger with more yolk and hybrids were not clearly at any disadvantage.

Length of time postfertilization to hatch and emergence and time between hatch and emergence are all measures of development influenced by the sire (Wood and Foote 1990; McGowan and Davidson 1992). Furthermore, development rate is matched to the amount of yolk reserves available for development, which is an egg size (maternal) effect (Wood and Foote 1990; McGowan and Davidson 1992). Consistent with these studies within species in *Oncorhynchus* and between species in *Salmo*, body weight, yolk reserves, and growth all had a strong dam type effect in the reported data. Furthermore, in this study between species in *Oncorhynchus*, the three measures of development rate (time to 50% hatch and emergence and length of time between 50% hatch and emergence) had a strong sire type effect. It is the combination of the dam type influence on size and yolk reserves and the sire type influence on development rate that leads to the potential for mismatch in development rate and egg size in hybrids (Wood and Foote 1990). In this study, hatching time (in agreement with the results of Dumas et al. (1992) on brook trout (*Salvelinus fontinalis*) – Arctic char (*Salvelinus alpinus*) hybrids) and time between hatching and emergence had both dam and sire type effects, and emergence timing had sire type effects that affected the relationship of the hybrids to the pure species. Although the hybrids were intermediate compared with the pure species at hatching time, they were extreme compared with the pure species in the time between hatching and emergence and thus emerged similarly to the paternally related pure species.

Survival and development of salmonid fry from fertilization to emergence appear to be under strong natural selection both among and within populations (Brannon 1987). For example, spawning date in sockeye salmon (*Oncorhynchus nerka*) was correlated with the temperatures experienced by developing young. Further, within spawning populations, those fish that spawned earlier in the run developed more slowly than those that spawned at the end of the run.

Other studies have concluded that emergence timing, size, and amount of yolk at emergence have fitness consequences (e.g., Brannas 1995). Early-emerging fish may be better able to obtain and defend a feeding territory if food is available, but may not be able to survive if the food source is cyclical (Bagenal 1969). However, alevins with large orange yolk sacs that could exist on yolk reserves while waiting for a cyclic food source are more vulnerable to predation because they are both more visible and less maneuverable (Fresh and Schroder 1987). Smaller fish also tend to be more vulnerable to predation (Parker 1971), and alevins from same-sized eggs but with larger yolk sacs at emergence tend to be smaller because they have not fully converted the yolk into body tissue (Wood and Foote 1990).

Within either cutthroat or steelhead, the rate of development is presumably matched to the body size and yolk re-

serves of the alevin so that the alevins emerge at an appropriate time and state of yolk absorption (Brannon 1987). However, cutthroat–steelhead hybrids are mismatched with either a fast rate of development in a large egg or a slow rate of development in a small egg so that they emerge with either too much or too little yolk relative to the pure species. Hybrid cutthroat had the small egg of a cutthroat and the slow development rate of a steelhead. They therefore emerged later than cutthroat and at a smaller size than steelhead, but with less yolk than either pure species. Hybrid steelhead had the large egg of a steelhead with the fast development rate of a cutthroat and thus face the opposite problem at emergence. They emerge earlier than the other cross types, and although they are larger than cutthroat, they have a very large remaining yolk sac. Therefore, depending on the conditions of food and predation present at emergence, the possibility exists that even if hybrids are fully viable developmentally, they may experience increased mortality relative to the pure species because of a mismatch between egg size and development rate.

This study provides further evidence of the divergence in development rates of closely related species. This divergence has both a maternal (egg size) and a genetic component. While we found no evidence of reduced viability of hybrids, the limited introgression of coastal cutthroat and steelhead in the face of interbreeding (Campton and Utter 1985; Hawkins 1997) indicates that selection against the hybrids most likely occurs. Given the known adaptive significance of development rates in salmonids (Brannon 1987), the mismatch in various aspects of development in coastal cutthroat and steelhead may play an important role in limiting introgression between the species.

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References

- Allendorf, F.W., and Leary, R.F. 1988. Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Conserv. Biol.* **2**: 170–184.
- Arnold, M.L., and Hodges, S.A. 1995. Are natural hybrids fit or unfit relative to their parents? *Trends Ecol. Evol.* **10**: 67–71.
- Awise, J.C. 1994. *Molecular markers, natural history and evolution.* Chapman and Hall, Inc., New York.
- Bagenal, T.B. 1969. Relationship between egg size and fry survival in brown trout *Salmo trutta* L. *J. Fish Biol.* **1**: 349–353.
- Beacham, T.D. 1988. A genetic analysis of early development in pink (*Oncorhynchus gorbuscha*) and chum salmon (*Oncorhynchus keta*) at three different temperatures. *Genome*, **30**: 89–96.

- Behnke, R.J. 1970. The application of cytogenetic and biochemical systematics to phylogenetic problems in the family Salmonidae. *Trans. Am. Fish. Soc.* **99**: 237–248.
- Behnke, R.J. 1988. Phylogeny and classification of cutthroat trout. *Am. Fish. Soc. Symp.* **4**: 1–7.
- Behnke, R.J. 1992. Native trout of western North America. American Fisheries Society, Bethesda, Md.
- Brannas, E. 1995. First access to territorial space and exposure to strong predation pressure: a conflict in early emerging Atlantic salmon (*Salmo salar* L.) fry. *Evol. Ecol.* **9**: 411–420.
- Brannon, E.L. 1987. Mechanisms stabilizing salmonid fry emergence timing. In *Sockeye salmon (Oncorhynchus nerka) population biology and future management*. Edited by H.D. Smith, L. Margolis, and C.C. Wood. *Can. Spec. Publ. Fish. Aquat. Sci.* No. 96. pp. 120–124.
- Burgner, R.L., Light, J.T., Margolis, L., Okazaki, T., Tautz, A., and Ito, S. 1992. Distribution and origins of steelhead trout (*Oncorhynchus mykiss*) in offshore waters of the North Pacific Ocean. *Int. North Pac. Fish. Comm. Bull.* **51**: 1–92.
- Busack, C.A., and Gall, G.A.E. 1981. Introgressive hybridization in populations of Paiute cutthroat trout (*Salmo clarki seleniris*). *Can. J. Fish. Aquat. Sci.* **38**: 939–951.
- Campton, D.E., and Utter, F.M. 1985. Natural hybridization between steelhead trout (*Salmo gairdneri*) and coastal cutthroat trout (*Salmo clarki*) in two Puget Sound streams. *Can. J. Fish. Aquat. Sci.* **42**: 110–119.
- Dumas, L., Blanc, J.M., Audet, C., and de la Noue, J. 1992. The early development of hybrids between brook charr (*Salvelinus fontinalis*) and Arctic charr (*Salvelinus alpinus*). *Aquaculture*, **108**: 21–28.
- Ferguson, M.M., Danzmann, R.G., and Allendorf, F.W. 1988. Developmental success of hybrids between two taxa of salmonid fishes with moderate structural gene divergence. *Can. J. Zool.* **66**: 1389–1395.
- Foote, C.J., and Larkin, P.A. 1988. The role of male choice in the assortative mating of anadromous and non-anadromous sockeye salmon (*Oncorhynchus nerka*). *Behaviour*, **106**: 43–62.
- Forbes, S.H., and Allendorf, F.W. 1991. Associations between mitochondrial and nuclear genotypes in cutthroat trout hybrid swarms. *Evolution*, **45**: 1332–1349.
- Fresh, K.L., and Schroder, S.L. 1987. Influence of the abundance, size, and yolk reserves of juvenile chum salmon (*Oncorhynchus keta*) on predation by freshwater fishes in a small coastal stream. *Can. J. Fish. Aquat. Sci.* **44**: 236–243.
- Gold, J.R. 1977. Systematics of western North American trout (*Salmo*), with notes on the redband trout of Sheepheaven Creek, California. *Can. J. Zool.* **55**: 1858–1873.
- Halliburton, R., Pipkin, R.E., and Gall, G.A.E. 1983. Reproductive success of artificially hybridized golden trout (*Salmo aguabonita*) and rainbow trout (*Salmo gairdneri*). *Can. J. Fish. Aquat. Sci.* **40**: 1264–1269.
- Hawkins, D.K. 1997. Hybridization between coastal cutthroat trout (*Oncorhynchus clarki clarki*) and steelhead (*O. mykiss*). Ph.D. thesis, University of Washington, Seattle, Wash.
- Hawkins, D.K., and Quinn, T.P. 1996. Critical swimming velocity and associated morphology of juvenile coastal cutthroat trout (*Oncorhynchus clarki*), steelhead trout (*Oncorhynchus mykiss*), and their hybrids. *Can. J. Fish. Aquat. Sci.* **53**: 1487–1496.
- Hayashizaki, K., Hirohashi, M., and Ida, H. 1995. Effect of egg size on the characteristics of embryos and alevins of chum salmon. *Fish. Sci.* **61**: 177–180.
- Hendry, A.P. 1995. Sockeye salmon (*Oncorhynchus nerka*) in Lake Washington: an investigation of ancestral origins, populations differentiation and local adaptation. M.S. thesis, University of Washington, Seattle, Wash.
- Johnson, O.W., Waples, R.S., Wainwright, T.C., Neely, K.G., Waknitz, F.W., and Parker, L.T. 1994. Status review for Oregon's Umpqua River sea-run cutthroat trout. U.S. Dep. Commer. NOAA Tech. Memo. NMFS-NWFSC-15.
- Johnston, J.M., and Mercer, S.P. 1976. Sea-run cutthroat in saltwater pens: broodstock development and extended juvenile rearing (with a life history compendium). Wash. State Game Dep. Fish. Res. Rep. AFS-57.
- Leary, R.F., Allendorf, F.W., Phelps, S.R., and Knudsen, K.L. 1987. Genetic divergence and identification of seven cutthroat trout subspecies and rainbow trout. *Trans. Am. Fish. Soc.* **116**: 580–587.
- Leary, R.F., Allendorf, F.W., and Sage, G.K. 1995. Hybridization and introgression between introduced and native fish. *Am. Fish. Soc. Symp.* **15**: 91–101.
- McGowan, C., and Davidson, W.S. 1992. Artificial hybridization of Newfoundland brown trout and Atlantic salmon: hatchability, survival and growth to first feeding. *Aquaculture*, **106**: 117–125.
- Parker, R.R. 1971. Size selective predation among juvenile salmonid fishes in a British Columbia inlet. *J. Fish. Res. Board Can.* **28**: 1503–1510.
- Trotter, P.C. 1987. Cutthroat native trout of the west. Colorado Associated University Press, Boulder, Colo.
- Utter, F.M. 1981. Biological criteria for definition of species and distinct intraspecific populations of anadromous salmonids under the U.S. Endangered Species Act of 1973. *Can. J. Fish. Aquat. Sci.* **38**: 1626–1635.
- Utter, F.M., and Allendorf, F.W. 1994. Phylogenetic relationships among species of *Oncorhynchus*: a consensus view. *Conserv. Biol.* **8**: 864–867.
- Verspoor, E., and Hammar, J. 1991. Introgressive hybridization in fishes: the biochemical evidence. *J. Fish Biol.* **39**: 309–334.
- Wilson, G.M., Thomas, W.K., and Beckenbach, A.T. 1985. Intra- and inter-specific mitochondrial DNA sequence divergence in *Salmo*: rainbow, steelhead, and cutthroat trouts. *Can. J. Zool.* **63**: 2088–2094.
- Wood, C.C., and Foote, C.J. 1990. Genetic differences in the early development and growth of sympatric sockeye salmon and kokanee (*Oncorhynchus nerka*), and their hybrids. *Can. J. Fish. Aquat. Sci.* **47**: 2250–2260.
- Wood, C.C., and Foote, C.J. 1996. Evidence for sympatric genetic divergence of anadromous and nonanadromous morphs of sockeye salmon (*Oncorhynchus nerka*). *Evolution*, **50**: 1265–1279.