Exxon Valdez Oil Spill
State/Federal Natural Resource Damage Assessment Final Report

Impact of Oil Spilled from the *Exxon Valdez* on Survival and Growth of Dolly Varden and Cutthroat Trout in Prince William Sound, Alaska

Fish/Shellfish Study Number 5
(Restoration Study Number 90)

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Study History: Fish/Shellfish Study Number 5 was included in detailed study plans for 1989-1991 (Injury to Dolly Varden Char and Cutthroat Trout in Prince William Sound) and was closed out in 1992 by Restoration Study Number 90 (same title).

Abstract: Five emigrating populations of Dolly Varden and cutthroat trout were intercepted in 1989-1991 during seaward migration to Prince William Sound following the *Exxon Valdez* oil spill; two into the spill area, three into non-spill areas. Study populations were comprised of tagged adults and subadults. Survival rates were estimated with log-linear models of capture histories of tagged fish. We used a two-stage simulation based on bootstrapping and Monte Carlo techniques to compare average survival rates in study populations that were and were not associated with spilled oil. Growth and survival rates were significantly lower in study populations associated with spilled oil. Growth from 1989-1990 was on average less in study populations that emigrated into the spill area: 24% and 22% less for recaptured subadult and adult Dolly Varden and 36% and 43% less for subadult and adult cutthroat trout. This difference persisted through 1991 for cutthroat trout. Averages of estimated survival rates from 1989-1990 were less in populations associated with spilled oil: 36% and 40% less for subadult and adult Dolly Varden and 28% less for adult cutthroat trout. Results are consistent with the occurrence of a deleterious impact on growth and survival of emigrating species, although unable to be confirmed as results emanated from observation, not experiment.

Key Words: Cutthroat trout, Dolly Varden char, *Exxon Valdez* oil spill, growth, migration, *Oncorhynchus clarki*, recapture, *Salvelinus malma*, survival, tagging.

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Executive Summary

When crude oil spilled from the grounded tanker *Exxon Valdez* into Prince William Sound, Alaska in late March, 1989, anadromous Dolly Varden (*Salvelinus malma*) and cutthroat trout (*Oncorhynchus clarki*) were residing in lakes. Five populations of each species were intercepted with five weirs in 1989, 1990, and 1991 during their annual seaward emigration in the spring. Two populations emigrated into the wake of the spill, while three emigrated into waters free of spilled crude oil. Because anadromous Dolly Varden and cutthroat trout feed during the summer near the shores of Prince William Sound, fish emigrating into the path of the spill spent their summers near stranded oil and possibly ingested contaminated food. Of the 11,995 Dolly Varden and cutthroat trout marked at weirs in 1989 and the 41,550 marked in 1990, 24% and 18% were recaptured in 1990 and 1991. Almost all (97%) recaptured emigrants were recaptured at the same weirs in 1990 and 1991 at which they were released a year earlier. However, on average an estimated 39% of survivors of both species evaded recapture in 1990 at fish-tight weirs that year. In populations associated with spilled oil, growth from 1989-90 was 24% and 22% slower for recaptured subadult and adult Dolly Varden and 36% to 43% slower for subadult and adult cutthroat trout. This difference persisted through 1991 for cutthroat trout, but not for Dolly Varden; growth during 1990-91 of recaptured Dolly Varden in populations not associated with oil slowed. Estimates of mean length by age group for emigrating cutthroat trout in 1989 indicated that their growth had been uniform across Prince William Sound before the spill. Although averages of estimated survival rates from 1989 to 1990 are less in study populations associated with spilled oil (32% and 22% less for subadult and adult Dolly Varden and 28% less for adult cutthroat trout), none of the differences are statistically significant. Chronic starvation and direct exposure to petrogenic hydrocarbons were hypothesized as the pathways that spilled crude oil could have slowed growth of Dolly Varden and cutthroat trout.

Introduction and Objectives

In 1989, many Dolly Varden (*Salvelinus malma*) and cutthroat trout (*Oncorhynchus clarki*) in Prince William Sound, Alaska, were exposed to crude oil spilled from the tanker *Exxon Valdez*. Forty thousand metric tons of unrefined oil composed of aliphatic hydrocarbons such as the paraffins and water-soluble aromatic hydrocarbons such as benzene, toluene, xylene, and the napthalenes, spread across Prince William Sound and out into the Gulf of Alaska. As the oil spread to the southwest, anadromous Dolly Varden and cutthroat trout were still in the lakes and streams that ring Prince William Sound. By late April, Dolly Varden and cutthroat trout began leaving these sanctuaries and moving into estuaries and bays and along shorelines. During summer, members of both species utilize near-shore and estuarine waters for feeding (Scott and Crossman 1979, Armstrong and Morrow 1980, Morrow 1980, see Trotter 1989), areas in which the spillage of petrogenic hydrocarbons can be especially perturbing to biological communities (Teal and Howarth 1984; Highsmith and Stekoll, this volume).

Under controlled conditions, exposure to crude oil has proven toxic or lethal to Dolly Varden, cutthroat trout, and to members of closely related species. In static bioassays using the water-soluble fractions of crude oils from Prudhoe Bay and Cook Inlet in seawater, the 96-h TLm was 1.30-1.80 mg/L (ppm) for smolts, juveniles, and adult Dolly Varden from Southeastern Alaska (Moles et al. 1979, Rice et al. 1979). In 90-d tests in flow-through bioassays with freshwater, 48% of the cutthroat trout fry held at concentrations of 0.52 mg/L of the water soluble fraction of crude oil died (Woodward et al. 1981). Although there were few mortalities of fry held at lower concentrations, growth was inhibited even at the lowest concentration in this study (0.10 mg/L). Growth of rainbow trout (*O. mykiss*) held in freshwater was significantly slowed when fed *ad libitum* a 1%-diet of some of the aliphatic compounds found in crude oil (Luquet et al. 1983, 1984).

Toxicity under controlled conditions in the laboratory, however, is not a sufficient reason to expect impairment in the productivity of unconfined Dolly Varden and cutthroat trout in Prince William Sound. By June, 1989, levels of aromatic hydrocarbons suspended in the water column in Prince William Sound (Neff 1990, Short and Rounds 1993) were a few μg/L (ppb), well below levels found toxic in bioassays for Dolly Varden. Many species of fish, including Dolly Varden and rainbow trout, can readily metabolize and excrete the aromatic and aliphatic compounds in crude oil if concentrations are sublethal (Pedersen et al. 1976, Varanasi and Malins 1977, Neff 1979, Thomas and Rice 1981). Since salmonids can detect concentrations of aromatic compounds down to a few mg/L (Rice 1973, Maynard and Weber 1981, Weber et al. 1981), members of this family might have avoided any significant contact with crude oil in Prince William Sound. Yet these detectable levels of petrogenic hydrocarbons did not affect migrations of salmonids in experiments (Brannon et al. 1986, Nakatani and Nevissi 1991). Dolly Varden and cutthroat trout, especially immature individuals, do not feed so much in the water column as along the shoreline (Narver and Dahlberg 1965, see Trotter 1989) where crude oil is entrapped in sediments, macrophytes, and transported through the food chain (see Varanasi and Malins 1977, Lee 1978, Teal and Howarth 1984). Relevance of these and similar studies is ambiguous because the studies are based on individual fish under circumstances dissimilar to those in Prince William Sound in

1989. Before the productivity of populations of Dolly Varden and cutthroat trout in Prince William Sound can be judged damaged, there must be a demonstrable diminution in survival or growth of fish in populations in the path of the spill, and not elsewhere.

The objectives of our study are to test the hypotheses that growth and survival of Dolly Varden and cutthroat trout were significantly diminished in the path of the spill. A coordinated suite of studies to provide specific information on the impact of crude oil from the *Exxon Valdez* on the productivity of natural resources, including Dolly Varden and cutthroat trout in Prince William Sound, began immediately after the spill (see State/Federal Natural Resource Damage Assessment Plan, 1989). Studies were planned in accordance with the Clean Water Act and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Because baseline data on populations of Dolly Varden and cutthroat trout in Prince William Sound were limited, and because there was no control of which populations were exposed to crude oil, our investigation is observational, not experimental (Cochran 1983, Eberhardt and Thomas 1991). Our sampling was predicated on the unusual migratory behavior of both these species.

Methods

Our sampling design was based upon the model of migratory behavior of anadromous Dolly Varden developed by Armstrong (1970, 1974, 1984) and Armstrong and Morrow (1980). Juvenile Dolly Varden remain in their natal stream for up to four years, then smolt to sea during spring. In late summer or early fall, all surviving Dolly Varden return to freshwater to spend the winter. Dolly Varden spawned in watersheds with lakes (lacustrine watersheds) return to their natal watershed to overwinter in one of its lakes; fish spawned in watersheds without lakes (fluvial watersheds) enter lacustrine watersheds in search of lakes suitable as their winter residence. Each spring, adults and immature Dolly Varden again migrate to the sea with adults preceding younger fish. Once a Dolly Varden has spent the winter in a lake, it returns to that lake each winter if it survives. Mature Dolly Varden return to their natal streams to spawn in the fall. The migratory habits of anadromous cutthroat trout are similar to those of Dolly Varden in that they both emigrate from lakes in the spring and return in the summer and fall, however, cutthroat trout spawn in the spring (Scott and Crossman 1979, Morrow 1980, see Trotter 1989). Timing of spring emigration of cutthroat trout corresponds to that of smolting Dolly Varden. Because Dolly Varden and cutthroat trout annually leave the ocean and return to the same lacustrine watershed to overwinter, all survivors of the emigration from such a watershed in one year would be expected to emigrate from that watershed in the next.

Our study was designed to detect differences in survival rates and growth among several groups of marked fish (study populations). Dolly Varden and cutthroat trout emigrating from five lacustrine watersheds were marked in 1989 and 1990 and the survivors recaptured during their spring emigrations in 1990 and 1991. Study populations were named according to where they were captured as emigrants: in Makaka Creek (Makaka), in unnamed streams flowing into Boswell and Rocky bays (Boswell and Rocky), in an unnamed stream on Green

Island (Green), and in Eshamy Creek (Eshamy) (see map in xx, this volume). Of the 143 streams surveyed in Prince William Sound during 1989 in the search for spawning salmon (Sharr 1990), only 14 lacustrine watersheds contained anadromous Dolly Varden and 10 anadromous cutthroat trout (Sam Sharr, unpublished data). Only two of these lacustrine watersheds with cutthroat trout emptied into the path of the spill (the watershed on Green Island and Eshamy Creek).

Emigrating Dolly Varden and cutthroat trout were captured during the spring with weirs located just above tidewater. Weirs were constructed of inclined aluminum panels comprised of parallel vertical rods 1.25 cm in diameter set with 1.25-cm gaps. Each panel measured 2.0 m long and 1.0 m high and rested vertically against wooden tripods spaced approximately 2 m apart. Each weir completely blocked the stream and directed emigrating fish into a holding pen. Weirs were installed from 22 April through 15 May in 1989, and as soon as streams were ice-free in 1990 and 1991; weirs were dismantled at the end of June each year. Large numbers of Dolly Varden were captured as soon as weirs became fish-tight in 1989; few or no cutthroat trout were caught until several days had passed beyond installation of the weirs in all years. Weirs remained fish-tight through the emigrations in 1990 except for 2 d in mid June on Makaka Creek and after 14 June for the Boswell population when high water compromised these weirs. These breaches occurred after the emigration of Dolly Varden, but during the end of the emigration of cutthroat trout. In 1991, weirs lost integrity due to high water at Eshamy Creek (5 d in late May to early June) and at Green Island (4 d in early May); all other weirs remained fish-tight through the emigration that year.

Every captured fish was measured to the nearest mm fork length (FL) with those $\geq$200 mm marked with an individually numbered Floy, t-bar tag (Model 68-B) and by excision of their adipose fin. Tags were inserted between fin rays at the base of the dorsal fin approximately 15 mm forward of the posterior end of the fin on the left side. Fish were not anesthetized. Marked fish were retained for five minutes to cull any immediate mortalities. Study populations consisted of all marked fish released alive in 1989 and 1990 to continue their emigration. Eight percent of Dolly Varden recaptured from the Boswell population in 1990 had lost their tags while $< 2\%$ recaptured at other weirs in 1990 and 1991 had shed tags. All recaptured cutthroat trout retained their tags. According to observations from other studies on both species (Armstrong 1970, 1974, see Trotter 1989), almost all fish between 200 mm and 270 mm FL emigrating in the spring are immature (subadults); smaller fish are smolts and most larger are adults.

Ages of fish emigrating in 1989 and 1990 were determined from scales collected from samples of cutthroat trout and from sagittal otoliths from samples of Dolly Varden. Cutthroat trout were systematically sampled. The lowest sampling rate across populations and years was one out of every three captured cutthroat trout; the highest rate was almost every emigrating fish. Scales were collected from the left side of cutthroat trout approximately two rows above their lateral line and on the diagonal row downward from the posterior insertion of the dorsal fin. Scales were impressed onto acetate under heat or mounted whole onto glass slides. All impressions and whole mounts were scanned under

40x magnification in a microfiche reader by the same person within the last three months of 1992. Recognition of annuli followed criteria in Fuss (1982) and Jearld (1983). Ages were determined twice for each sampled cutthroat trout; all samples were scanned once before any sample was scanned twice. Only samples with matching estimates were used in subsequent analyses; all samples with regenerated scales or discordant estimates were excluded. The weekly frequency of samples retained for the analysis remained roughly proportional to the weekly frequency of cutthroat trout emigrating past weirs in 1989 and 1990. Although otoliths were collected from Dolly Varden in 1989, these samples proved to be unrepresentative of emigrating populations. Analysis of samples indicated that larger Dolly Varden emigrated first regardless of their age, and we probably did not sample the larger Dolly Varden in each age group with our late, staggered start in 1989. Although weirs were installed in 1990 before the emigration of Dolly Varden began, sampling at Green Island and Eshamy Creek was delayed, and the largest Dolly Varden in these populations were not sampled. No otoliths and few scales were collected in 1991.

Analyses of variance (ANOVA) and covariance (ANCOVA) were used to test hypotheses that growth of fish had been the same regardless of their apparent association with spilled crude oil. Estimates of mean length by age group of emigrant cutthroat trout sampled in 1989 and 1990 were compared across study populations in different analyses using the same design, an unbalanced 2-way parametric ANOVA with study population (γ) as the independent variable (a random effect) and age (ψ) as an exogenous variable (ANOVA Model: $l_{ijk} = \mu + \gamma_i + \psi_j + \sigma_{ij} + \epsilon_{ijk}$ with μ as the overall mean length and σ and ϵ as true error and sampling error, each distributed normally with mean 0). Because of their migratory behavior, information on mean length by age group of cutthroat trout collected in 1989 is *a priori* information on populations never exposed to crude oil. Significance of differences in directly measured growth of recaptured Dolly Varden during each period of freedom (1989 to 1990 and 1990 to 1991) was tested with a two-way unbalanced parametric ANOVA with nested effects [ANOVA Model: $\Delta l_{ijtk} = \mu + \tau_i + \phi_j + \gamma(\tau_i)_t + \sigma_{ijt} + \epsilon_{ijtk}$]. The difference between the length of an individual fish as measured in consecutive years (Δl) was the dependent variable, and association with spilled crude oil was the independent variable (a random effect). Study populations were nested within association with spilled oil (τ) as random effects. Starting length of Dolly Varden in 1989 or 1990 was the exogenous variable. Since starting length proved poorly correlated with growth for Dolly Varden, the Δl were segregated according to their starting lengths into two blocks (ϕ: adults and subadults) to maximize power in tests of significance (Cox 1957). A nested ANOVA could not be used to test the significance of differences in growth for cutthroat trout because few cutthroat trout in the Green population were recaptured in either 1990 or 1991. An unbalanced parametric ANCOVA with study population as the independent variable (a random effect) and Δl the dependent variable was used instead [ANCOVA Model: $\Delta l_{ijk} = \mu + \gamma_i + \beta(l_{ijk}) + \sigma_{ij} + \epsilon_{ijk}$]. Because starting length (l) of recaptured cutthroat trout was highly correlated with their growth, starting length was treated as a covariate to maximize power of tests (Cox 1957). Before any analysis of growth for either species began, all Δl that fell outside 1.5 interquartiles away from the edge of box plots for each group (combination of population, species, and period of freedom) were considered as outliers due to measurement error [as per guidelines in Tukey (1977), p. 30-44] and were not used in analyses. Approximately 6% of the data were excluded on this basis with no more than 19% coming from a single study population (4 Dolly Varden removed from a sample of 25 from Green from 1989 to 1990).

Estimated survival rates were averaged across study populations with like association to the spill to determine if fish had similar expectations of mortality regardless of their association. Because immature and mature Dolly Varden and cutthroat trout typically have different survival rates (Armstrong 1974, Sumner 1953), study populations were divided into adults and subadults, and the analysis was repeated for each group. Because high water had compromised some of the weirs for a few days in 1990, survival rates were estimated with log-linear models following Cormack (1989). The *M* tagged and released fish that comprised each group in a study population in 1989 were tallied according to their capture history: 1) never recaptured (m_{100}); 2) recaptured in 1991, but not in 1990 (m_{101}); 3) recaptured in 1990, but not in 1991 (m_{110}); and 4) recaptured in 1990 and 1991 (m_{111}). The maximum likelihood estimates of the probability of capturing a marked fish at the weir in 1990 ($\hat{q}$) and the survival rate from 1989 to 1990 ($\hat{S}$) for each group are:

$$\mathrm{MLE}[q] = \hat{q} = \frac{m_{111}}{m_{101} + m_{111}} \tag{1}$$

$$\mathrm{MLE}[S] = \hat{S} = \frac{m_{111} + m_{110}}{M\,\hat{q}} \tag{2}$$

Precision of average survival rates across populations was estimated through a two-stage Monte Carlo simulation based on empirical distributions for $\hat{S}$ derived through bootstrapping capture histories (Efron 1982, Sauermann 1989, Buckland and Garthwaite 1991). Each empirical distribution of estimated survival rates consisted of statistics calculated from 1,000 bootstrap samples for each study population. Each bootstrap sample consisted of *M* records randomly drawn with replacement from the original data to create a new set of capture histories $\{m^*\}$. Estimates of $\hat{q}$ and $\hat{S}$ were calculated for each bootstrap sample in which $m^*_{111} > 0$ ($m^*_{111} = 0$ in only 0.23% of the bootstrap samples drawn). The difference between the average of the survival rates from the bootstrap samples and the original estimate for the same population is a measure of the mathematical bias in the latter statistic (Efron 1982). These empirical distributions of survival rates based on bootstrapping were used in eight Monte Carlo simulations, one for each combination of species, maturity, and association with spilled oil. Each simulation had 1,000 iterations, and each iteration had two stages. Choosing *n* study populations at random with replacement from the *n* available populations in the combination was the first stage in each iteration; the second consisted of drawing a single statistic at random from each of the empirical distributions of survival rates for the chosen populations, then averaging these statistics across populations to produce a simulated survival rate. Each simulation produced 1,000 such simulated rates for each combination of species, maturity, and association with oil.

The significance of differences in estimated survival rates across associations with spilled oil was judged relative to their precision. Each of the four combinations of species and maturity corresponded to two distributions of simulated rates: one for study populations that were associated with spilled oil and one for study populations that were not. An angular transformation was applied to all simulated rates to create normally distributed data, and a mean θ and variance $V[\theta]$ were calculated for each transformed distribution. The difference between means for populations associated with spilled oil (θ_t) and those not (θ_c) is a measure of the potential impact of spilled oil. The probability P of making a Type I error in judging a difference in survival rates to be real was based on a one-tailed alternative hypothesis $H_a:\theta_c > \theta_t$ and the test statistic:

$$Z = \frac{\theta_c - \theta_t}{\sqrt{V[\theta_c] + V[\theta_t]}} \tag{3}$$

Results

Size and Migrations of Study Populations

Ten thousand nine hundred ninety seven Dolly Varden and 1,086 cutthroat trout were tagged and released in 1989 and 39,160 and 2,390 in 1990; 40,914 Dolly Varden and 2,441 cutthroat trout were inspected for tags in 1990; and 28,657 Dolly Varden and 5,062 cutthroat trout were inspected in 1991 (Table 1). Because of the late start of the project in 1989 and the proclivity of larger Dolly Varden to emigrate earlier, disproportionately more immature fish were captured in 1989 than in other years (Figure 1). The trimodal distributions of emigrating fish of both species corresponded to smolts (80 to 160 mm FL), immature fish (160 to 270 mm FL), and adults ($\geq$ 270 mm FL). Except for the Green population, adults of both species comprised a higher fraction of study populations in 1990 (Figure 1) and 1991.

There was little evidence that Dolly Varden and cutthroat trout had strayed into or out of areas in Prince William Sound covered by the spill. Of those fish recaptured during emigration in 1990 and 1991 (10,242), 97% were recaptured at the same weir at which they had been released a year earlier (Figure 2). Of those fish that had strayed (298 of 10,242), few Dolly Varden and no cutthroat trout had been recaptured in watersheds across the boundary of the spill. Of the 336 fish that were recaptured and reported by persons working or recreating around Prince William Sound during the summers of 1989 and 1990, few Dolly Varden (18) and no cutthroat trout had been caught across the boundary of the spill. The median distances between release and recapture for these 336 fish were 26 km for Dolly Varden and 2 km for cutthroat trout; the maximum distances were 248 km and 55 km, respectively.

Table 1. Numbers of Dolly Varden and cutthroat trout in study populations tagged and released during their spring emigrations in 1989 and 1990 and inspected for tags in 1990 and 1991. Adults of both species were $\geq$ 270 mm FL upon release while subadults were 200 mm $\leq$ FL < 270 mm.

		Dolly Varden				Cutthroat Trout			
		Tagged & Released		Inspected		Tagged & Released		Inspected	
Populations	Group	1989	1990	1990	1991	1989	1990	1990	1991
Not Associated with Spilled Oil:									
Boswell	Subadults	68	2,678	2,911	927	59	468	498	465
	Adults	7	4,051	4,373	2,260	53	853	868	1,220
Makaka	Subadults	4,114	3,188	3,290	6,717	491	263	264	1,487
	Adults	1,178	9,311	9,660	7,862	254	570	571	1,667
Rocky	Subadults	3,721	6,745	6,977	3,080	7	16	16	0
	Adults	692	8,960	9,220	6,016	3	8	9	0
Associated with Spilled Oil:									
Green	Subadults	180	1,006	1,040	301	4	6	6	10
	Adults	83	106	111	427	5	2	3	2
Eshamy	Subadults	684	2,148	2,290	817	75	48	48	36
	Adults	182	967	1,042	250	135	156	158	175

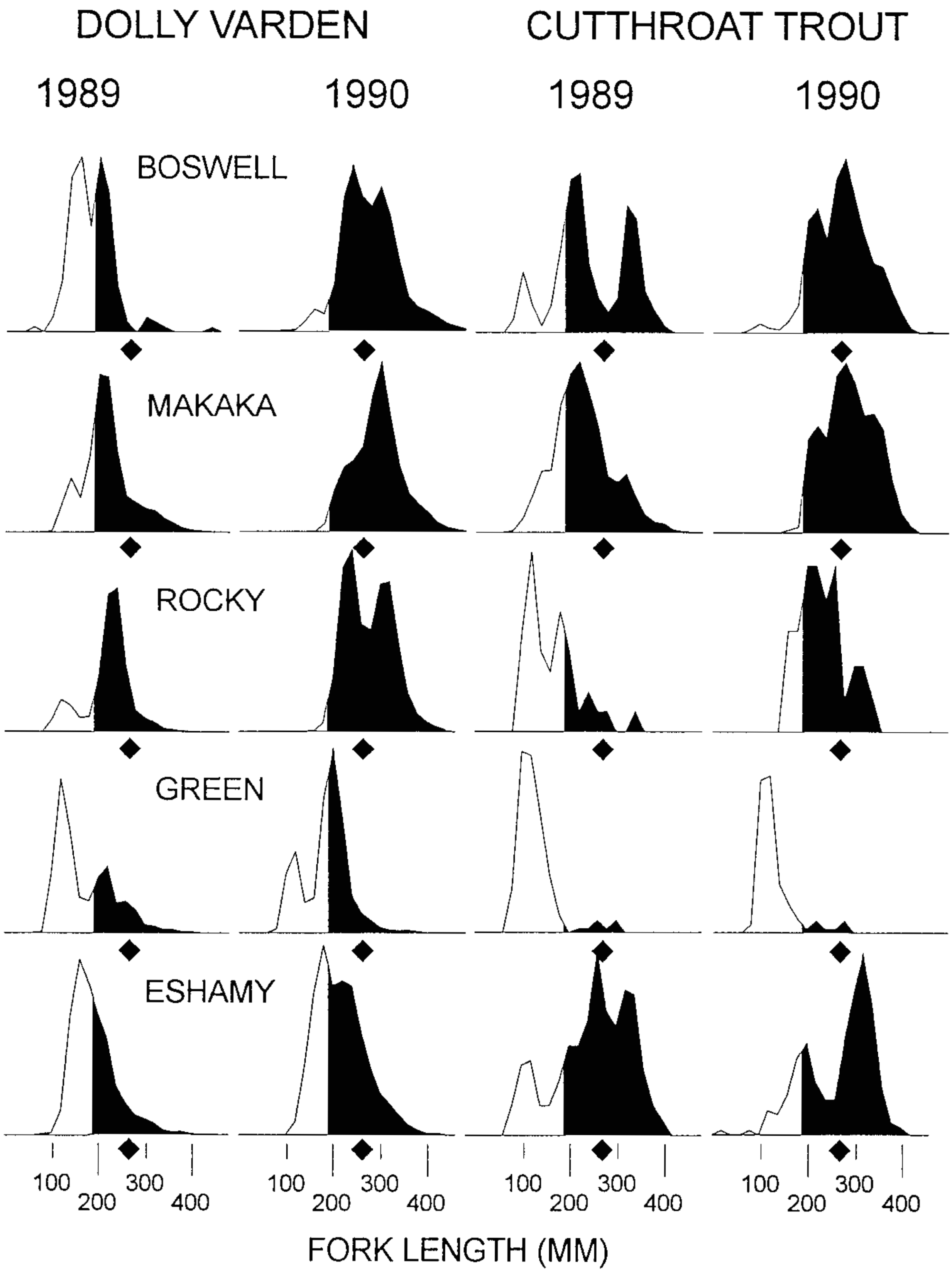

Figure 1. Relative length frequency by study population of Dolly Varden and cutthroat trout caught at weirs while emigrating to the sea in the spring, 1989 and 1990. Individual frequencies are based on 20-mm cells. Black areas correspond to fish released with tags. Black diamonds correspond to the length that separates adults from subadults (270 mm FL).

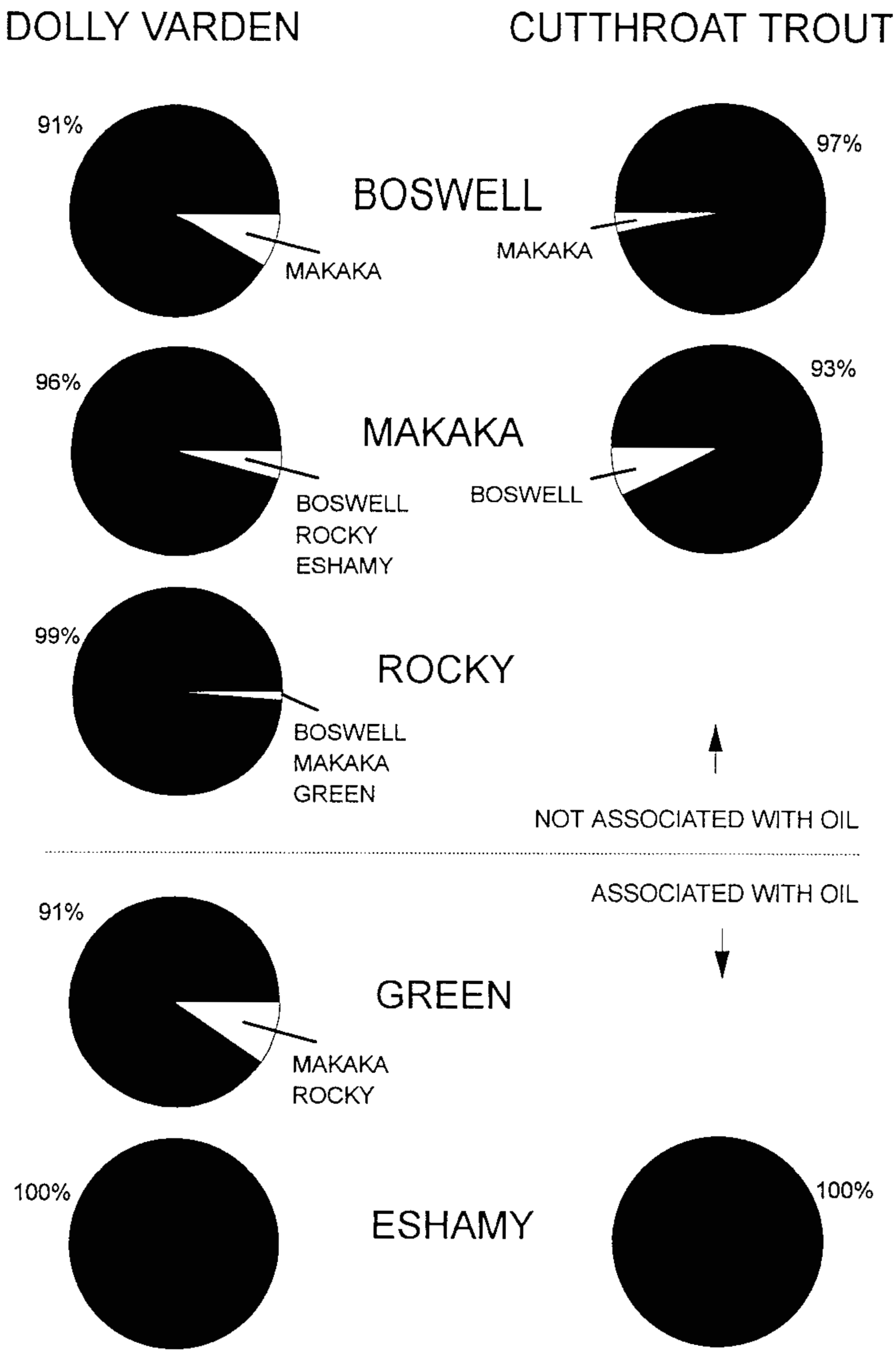

Figure 2. Distribution by study population of Dolly Varden and cutthroat trout recaptured during emigration in 1990 and 1991 (combined) relative to where they were released a year earlier. Black areas correspond to fish recaptured at the same location a year later; clear areas correspond to fish recaptured elsewhere at the specified locations.

Differences in Growth

Estimates of mean length by age group for 1989 indicate cutthroat trout had similar rates of growth across Prince William Sound prior to that year (Figure 3). Estimates of mean length at age 4 (years) are similar for emigrants from Eshamy and Makaka creeks and emigrants to Boswell Bay; for older ages, estimates from cutthroat trout sampled at Eshamy Creek are midway between statistics for emigrants at the other two sampling locations. Although the means are significantly different among these three locations (two-way ANOVA, $P<0.001$), the only paired comparison of significance (least-significant difference, $\alpha = 0.05$) is between statistics for emigrants to Boswell Bay and emigrants from Makaka Creek. Averages of statistics for emigrants at these two eastern locations followed the same pattern as did statistics for emigrants from Eshamy Creek on the western rim of Prince William Sound. Comparison of statistics was limited to emigrants past these three sampling locations because there were only a few emigrants leaving Green Island or entering Rocky Bay in 1989.

Growth of fish recaptured in 1990 and 1991 and estimates of mean length by age group in 1990 showed: 1) that growth of Dolly Varden and cutthroat trout during 1989-1990 was appreciably slower in study populations associated with spilled oil, and 2) that this depression in growth persisted into the next year for cutthroat trout, but not for Dolly Varden (Figure 3, Table 2). Although estimates of mean length by age group in 1990 are significantly different among cutthroat trout emigrating from Eshamy and Makaka creeks and to Boswell Bay [$P < 0.001$ in a two-way ANOVA with all possible paired comparisons significantly different (Scheffe's test, $\alpha = 0.05$)], only statistics for emigrants from Eshamy Creek are meaningfully different (Figure 3). Statistics for 1990 are 21 and 28 mm lower and 5 mm higher for emigrants ages 4 through 6 from Eshamy Creek than are the corresponding statistics for 1989. Average statistics from 1989 and 1990 are similar for study populations not associated with spilled oil: 223 vs. 219 mm FL for age 4; 272 vs. 275 mm FL for age 5; and 316 vs. 316 mm FL for age 6. Directly measured growth of recaptured fish during 1989-1990 (Figure 4) was significantly less for Dolly Varden in those populations associated with oil ($P = 0.01$ in a nested ANOVA) and for cutthroat trout ($P < 0.001$ in an ANCOVA). Paired comparisons based on least-square means in the ANCOVA showed similar growth by cutthroat trout in Makaka and Boswell populations and lower in the Eshamy population ($\alpha = 0.05$). Growth was 24% less for subadult and 22% less for adult Dolly Varden associated with oil; growth was 36% less for subadult and 43% slower for adult cutthroat trout. A year later (1990-1991), growth of recaptured cutthroat trout was still significantly less in the population associated with spilled oil ($P < 0.001$ in an ANCOVA), but not for recaptured Dolly Varden ($P = 0.88$ in a nested ANOVA) (Figure 4). Parity in growth of Dolly Varden across Prince William Sound in 1990-1991 came not through increased growth of Dolly Varden in the Eshamy and Green populations, but through decreased growth by Dolly Varden emigrating into areas not in the wake of the spill (Figure 4). Adult Dolly Varden marked in 1989 were similar in size to those marked in 1990 (324 versus 329 mm FL on average across all five study populations).

Table 2. Mean annual growth (mm) of recaptured Dolly Varden and cutthroat trout in study populations associated with spilled oil (Green and Eshamy) relative to study populations not so associated (Boswell, Makaka, and Rocky). Growth of individual fish were averaged within each study population, and these statistics were averaged across study populations. Adults of both species were $\geq$ 270 mm FL upon release while subadults were 200 mm $\leq$ FL < 270 mm.

	1989-1990		*1990-1991*	
	Dolly Varden	Cutthroat Trout	Dolly Varden	Cutthroat Trout
SUBADULTS:				
Not Associated with Oil	77	69	55	65
Associated with Oil	59	44	51	45
Relative Difference	-24%	-36%	-7%	-30%
ADULTS:				
Not Associated with Oil	57	44	44	42
Associated with Oil	44	25	41	22
Relative Difference	-22%	-43%	-9%	-47%

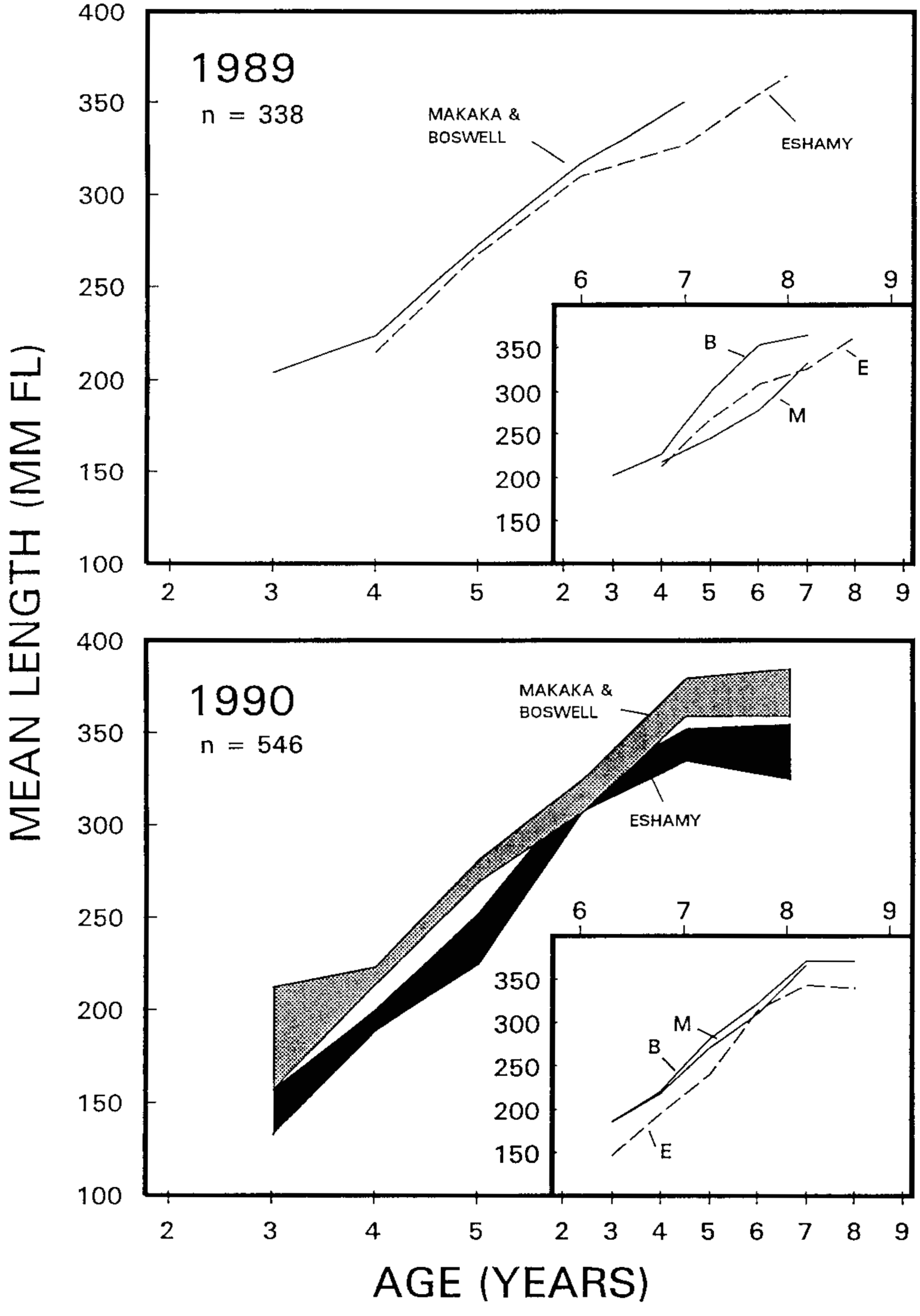

Figure 3. Estimated mean length by age group in 1989 and 1990 for cutthroat trout sampled at weirs on Eshamy and Makaka creeks and on a stream near Boswell Bay. Lines in insets correspond to statistics for the three individual sampling locations. The lines and the bands in the larger panels correspond to statistics for emigrants from Eshamy Creek and averages of statistics for emigrants at the other two locations. Bands in the lower panel correspond to approximate confidence intervals (±2xSE).

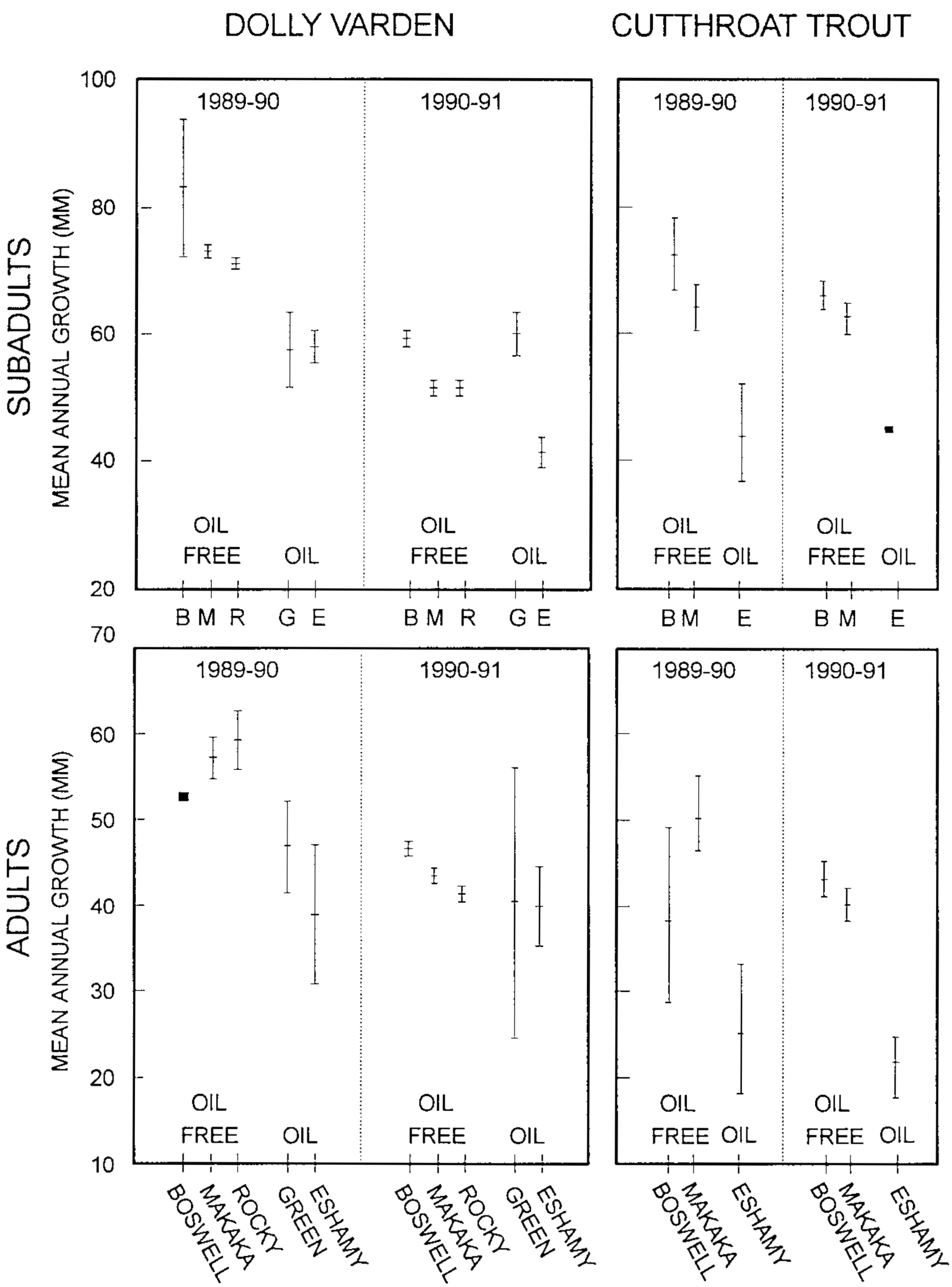

Figure 4. Average, annual growth (mm) from 1989 to 1990 and from 1990 to 1991 for recaptured Dolly Varden and cutthroat trout by study population. Study populations were divided into subadults (200 mm $\leq$ FL < 270 mm) and adults ($\geq$ 270 mm FL). Points correspond to measurement of a single fish while intervals are 95% confidence intervals. Terms "oil" and "oil free" correspond to populations associated with spill and to those not.

Differences in Survival Rates

Averages of estimated survival rates from 1989 to 1990 were 24% to 33% lower in those populations of Dolly Varden and adult cutthroat trout associated with spilled oil (Table 3), however, these differences were not significant (all $P \geq 0.16$, Figure 5) when compared against standard thresholds of acceptable risk ($\alpha = 0.10$). Under the precision attained in this study, the chances of wrongly concluding lower survival rates for study populations associated with spilled oil are two-in-seven for adult Dolly Varden, one-in-six for subadult Dolly Varden, and one-in-three for adult cutthroat trout (Figure 5). Because of small sample sizes, survival rates could not be estimated for some study populations, and these populations were excluded from the Monte Carlo simulation (Table 4). Differences between estimated survival rates and average rates from bootstrapping ranged from 0.01 and 0.02, indicating bias in estimated rates to be negligible for all populations save one. This exception concerns subadult cutthroat trout for which the difference between the average rate from bootstrapping for the Eshamy population (0.60) is considerably lower than the corresponding estimated rate from the original data (0.72 in Table 3). Precision and bias in the latter statistic are such that the frequency distribution of simulated averages for the Eshamy completely swamps the corresponding distribution for the other populations (Figure 5). Even though weirs remained fish-tight through the spring emigration at most sites in 1990, relatively large numbers of fish recaptured in 1991 were not recaptured in 1990 (m_{101}) in many populations (Table 4). Average estimates of the probability of capture ($\hat{q}$) in 1990 at all weirs are 49% and 58% for subadult and adult cutthroat trout and 65% and 68% for subadult and adult Dolly Varden.

Discussion

The reduced growth of Dolly Varden and cutthroat trout that we observed in the wake of the spill meet the necessary conditions to show that exposure to crude oil had damaged the resources that these fish represent. If growth of Dolly Varden had been similar across Prince William Sound after 1989, the hypothesis of damage would have been rejected. The same consequence holds for cutthroat trout. This was not the case. Growth was consistently better for those fish not associated with spilled oil in 1989. Estimates of mean length by age group are consistent with populations of cutthroat trout having similar growth rates across Prince William Sound before the spill, yet after the spill, growth was slower for those populations in its wake. Differences in growth according to association with crude oil persisted for cutthroat trout into 1990, but not for Dolly Varden. Surprisingly, the difference for Dolly Varden was negated, not through acceleration of growth for fish from Green and Eshamy populations, but through apparent deceleration of growth in populations not associated with spilled oil. Either Dolly Varden experienced annual variation in growth rates that cutthroat trout did not, or spilled crude oil extended its influence in 1990.

There is evidence supporting both reduction of food and direct exposure as mechanisms by which spilled oil could have affected growth of Dolly Varden and cutthroat trout. Crustaceans and other small invertebrates dominate the diet of subadult Dolly Varden and cutthroat trout while adults eat mostly fish. During residence in Alaskan estuaries, smaller

Table 3. Estimated survival rates for study populations of adult ($\geq$ 270 mm FL) and subadult (200 mm $\leq$ FL $<$ 270 mm) Dolly Varden and cutthroat trout from 1989 to 1990 averaged over populations associated with spilled crude oil (Green and Eshamy) and those not (Boswell, Makaka, and Rocky).

	Dolly Varden		Cutthroat Trout	
	Adults	Subadults	Adults	Subadults
Not Associated with Oil:	0.21	0.39	0.31	0.39
Associated with Oil:	0.16	0.26	0.22	0.72
Relative Difference:	-24%	-33%	-29%	+85%

Table 4. Number released in 1989 (M), number of these fish recaptured in 1990 and 1991 (m_{111}), number recaptured in 1991 but not in 1990 (m_{101}), number recaptured in 1990 but not in 1991 (m_{110}), estimated probabilities of being recaptured in 1990 ($\hat{q}$), and estimated survival rates from 1989 to 1990 ($\hat{S}$) for adult ($\geq$ 270 mm FL) and subadult (200 mm $\leq$ FL $<$ 270 mm) Dolly Varden and cutthroat trout released in 1989. Those groups with no estimated survival rates $\hat{S}$ were excluded from the Monte Carlo simulation to measure precision of average statistics.

		M	m_{111}	m_{101}	m_{110}	$\hat{q}$	$\hat{S}$
DOLLY VARDEN:							
Not Associated with Spilled Oil:							
Boswell	Subadults	68	2	1	20	0.67	0.49
	Adults	7	0	0	1		
Makaka	Subadults	4,114	130	52	830	0.71	0.32
	Adults	1,178	18	4	149	0.81	0.17
Rocky	Subadults	3,721	153	40	921	0.79	0.36
	Adults	692	23	5	111	0.82	0.24
Associated with Spilled Oil:							
Green	Subadults	180	2	3	15	0.40	0.24
	Adults	83	3	4	1	0.43	0.11
Eshamy	Subadults	684	4	2	128	0.67	0.29
	Adults	182	2	1	24	0.67	0.21
CUTTHROAT TROUT:							
Not Associated with Spilled Oil:							
Boswell	Subadults	59	10	3	10	0.77	0.44
	Adults	53	3	3	6	0.50	0.34
Makaka	Subadults	491	33	30	54	0.52	0.34
	Adults	254	12	4	40	0.75	0.27
Rocky	Subadults	7	0	0	1		
	Adults	3	0	0	0		
Associated with Spilled Oil:							
Green	Subadults	4	0	0	0		
	Adults	5	0	1	0		
Eshamy	Subadults	75	1	5	8	0.17	0.72
	Adults	135	3	3	12	0.50	0.22

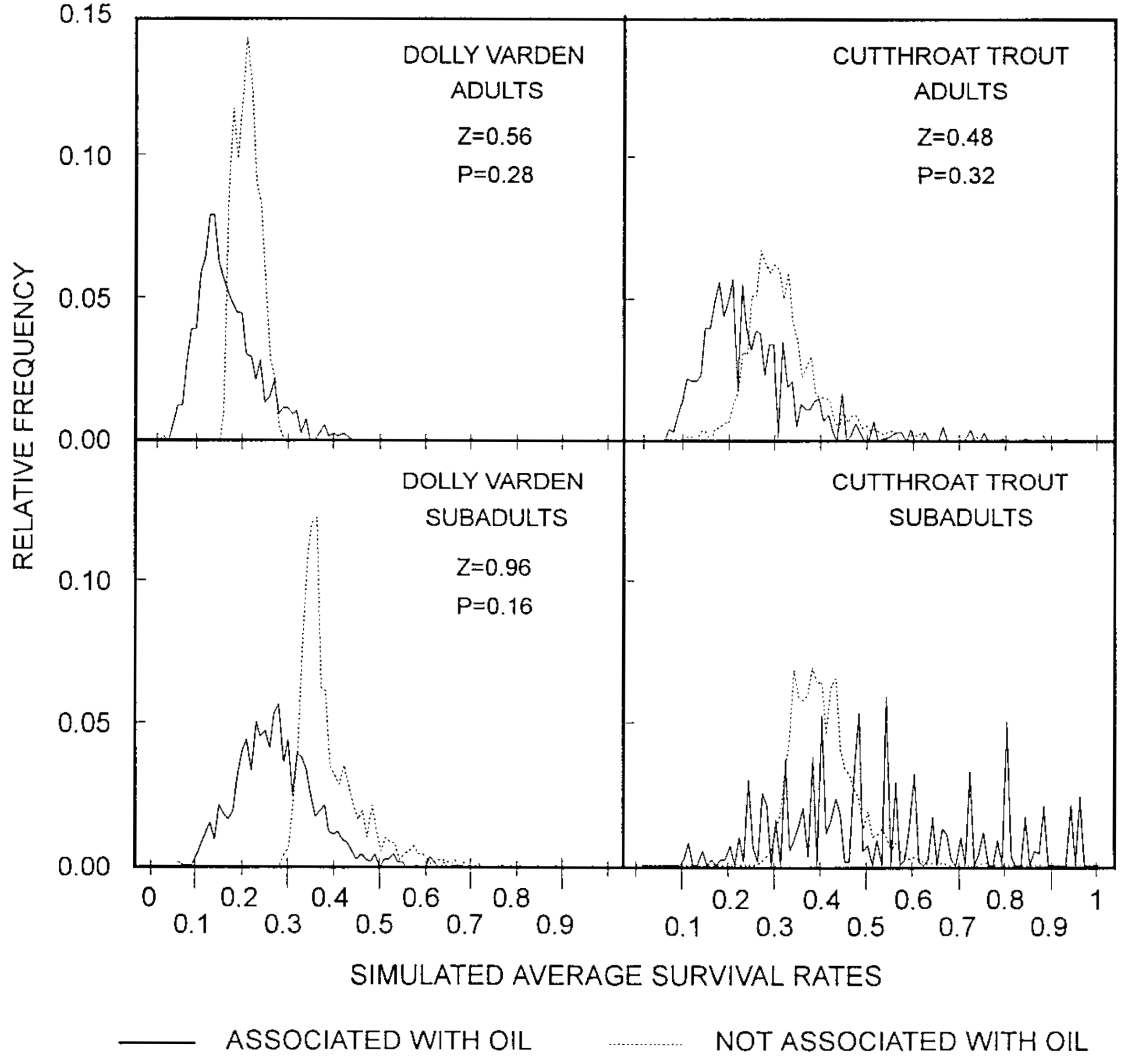

Figure 5. Distributions of simulated average survival rates from 1989 to 1990 for those study populations associated with spilled crude oil and those not. Distributions were built on 1,000 simulated averages each based on random sampling of empirical distributions of estimated survival rates built by bootstrapping the capture histories of individual study populations. Adults of both species were $\geq$ 270 mm FL upon release while subadults were 200 mm $\leq$ FL $<$ 270 mm.

Dolly Varden mostly eat gammarid amphipods, euphausiids, megalopae, isopods, mysids, and polychaetes while larger Dolly Varden prey on capelin (*Mallotus villosus*), sand lance (*Ammodytes hexapterus*), herring (*Clupea harengus pallasi*), greenling (*Hexagrammos stelleri*), young salmon (*Oncorhynchus* spp.), rockfish larvae (*Sebastes* spp.), and sculpin (*Blepsias cirrhosus*) (Lagler and Wright 1962, Narver and Dahlberg 1965). In Alaska, small cutthroat trout eat some of the same estuarine foods as small Dolly Varden: gammarid amphipods, euphausiids, capelin, sand lance, and young salmon (Armstrong 1971). Estuarine diets of cutthroat trout in Canada, Washington, and Oregon are similar to those of cutthroat trout and Dolly Varden in Alaska (see Trotter 1989). When exposed to petrogenic hydrocarbons, the subtidal invertebrate community shifts to smaller epifauna, usually with markedly fewer amphipods (Teal and Howarth 1984). Such a shift occurred in Prince William Sound in 1989 and 1990 in the path of the spill (Jewett and Dean 1993, Jewett et al. 1993).

Dolly Varden collected along shorelines in the wake of the spill in another study had been exposed to crude oil (Collier et al. 1993). Large Dolly Varden were captured in shallow subtidal areas with gill nets and beach seines. Those fish captured in the wake of the spill in 1989 had exceptionally high levels of metabolites of petrogenic hydrocarbons, such as biliary fluorescent aromatic hydrocarbons (FACs). Enzymes for metabolizing aromatic hydrocarbons, such as hepatic cytochrome P4501A, were also elevated. In contrast, contents of their stomachs were free of petrogenic hydrocarbons, indicating that absorption is more likely than digestion as the pathway of their exposure. Levels of FACs and hepatic P4501 in Dolly Varden captured along shorelines in 1990 were considerably reduced from levels observed a year earlier. No cutthroat trout or small Dolly Varden were tested for FACs or hepatic P4501A in this or any other study.

While estimated differences in growth met the necessary conditions to show damage from oil spilled by the *Exxon Valdez*, estimated differences in survival rates did not. The late installation of weirs in 1989 and the breach of the weir on Eshamy Creek in 1991 kept sample sizes low and subsequently reduced the power of hypothesis tests for Dolly Varden. These factors were not as critical in studies of cutthroat trout; sample sizes for this species were small because their populations are small. Prince William Sound is the extreme northern edge of the observed range of coastal cutthroat trout (Scott and Crossman 1979). Unexpected migratory behavior of Dolly Varden may have also affected estimation of survival rates with log-linear models. Equations 1 and 2 are based on all tagged fish experiencing the same survival rates during the winter of 1989-1990 regardless of their location. Dolly Varden with capture history (*101*) may have resided in habitats different from other tagged fish. If survival rates had been less for wayward Dolly Varden than for their peers, m_{101} would be depressed, the estimated probability of capture $\hat{q}$ would be biased high, and the estimated survival rate $\hat{S}$ would be biased low as calculated with Equations 1 and 2. If survival rates for wayward Dolly Varden had been better, m_{101} would be elevated, $\hat{q}$ biased high, and $\hat{S}$ biased low. In either instance, there would be an inverse relationship between $\hat{q}$ and $\hat{S}$. Casual inspection of Table 4 shows no such relationship, indicating that if there had been different survival rates for Dolly Varden with different capture histories during the winter of 1989-1990, all study populations were similarly affected. Since different survival rates only invalidates the log-linear model for capture history (*101*) and since only the value of m_{101} is affected, $\hat{q}$ and $\hat{S}$ are relatively insensitive to bias from all but the largest differences in rates.

Since our study was observational and not experimental, interpretation of its results is subject to two kinds of criticism, the first concerns experimental design and the second natural variation. Whenever treatments (in our case association with crude oil) are not randomly assigned to experimental units (populations), spatial or temporal trends in parameters (survival rates and growth) can be confused with effects of treatments (Green 1979, Hurlbert 1984). One way to avoid this problem is to estimate parameters for several years before the treatment is applied. Although we could not do this for survival rates, we could and did for growth. Our *a priori* information on mean length by age group in 1989 showed a history of near uniform growth across Prince William Sound for cutthroat trout. While we did not directly measure growth prior to 1989, mean length by age group is an integration of growth over the extant year classes extending backward several years. This uniformity of growth is the consequence of the size of Prince William Sound; its uniformity of climate, limnology, and oceanography; and the mixture of reproductively distinct stocks in most of our study populations. There were no *a priori* estimates of survival rates. The second common criticism of observational studies is that natural variation in parameters can be confused with the effects of treatments when sample size is small (Eberhardt and Thomas 1991). Although populations in our study are few, they represented much of the suitable winter habitat, and by inference, many of the anadromous Dolly Varden and cutthroat trout in Prince William Sound. Of the 14 lacustrine watersheds with anadromous Dolly Varden and 10 with anadromous cutthroat trout, five are in this study, including the only two lacustrine watersheds that empty into the path of the spill (Green and Eshamy) that contain anadromous cutthroat trout.

One final note is warranted on those cutthroat trout and Dolly Varden with capture history (*101*). Fish with this history could only have escaped capture at fish-tight weirs in 1990 by remaining in the lake past spring, by spending the winter in a different watershed, or by spending the winter at sea. Observations of cutthroat trout at sea during the winter have been reported in the scientific literature (see Trotter 1989), but not observations of Dolly Varden. All three of these possible explanations for not recapturing significant numbers of Dolly Varden in 1990 are at odds with the "migration hypothesis" for this species (see Armstrong and Morrow 1980), which makes the low probabilities of capture that year anomalies for this hypothesis.

Conclusions

Our findings are consistent with oil spilled from the *Exxon Valdez* having retarded the body growth of anadromous Dolly Varden and cutthroat trout in 1989. Our evidence is also consistent with lingering effects of this spilled oil having reduced body growth of cutthroat trout through at least 1990 as well. Although estimated survival rates of Dolly Varden and cutthroat trout are lower for those fish emigrating into the path of the spill in 1989, differences are not significant given a reasonable statistical risk.

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