

Thermally induced chronic developmental stress in coho salmon: integrating measures of mortality, early growth, and developmental instability

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Developmental stability, or homeostasis, facilitates the production of consistent phenotypes by buffering against stress. Fluctuating asymmetry is produced by developmental instability and is manifested as small random departures from bilateral symmetry. Increased fluctuating asymmetry is thought to parallel compromised fitness, in part, because stress promotes energy dissipation. Compensatory energy expenditures within the organism are required to complete development, thus promoting instability through reductions in homeostasis. Increased heterozygosity may enhance developmental stability by reducing energy dissipation from stress through increased metabolic efficiency, possibly by providing greater flexibility in metabolic pathways.

Traditionally, fluctuating asymmetry has been used as a bioindicator of chronic stress, provided that selective mortality of less fit individuals did not reduce stress-mediated increases in fluctuating asymmetry to background levels produced by natural developmental error, or create data inconsistencies such as higher asymmetry in groups exposed to lower stress. Unfortunately, absence of selective mortality and its effects, while often assumed, can be difficult to substantiate. We integrated measures of early growth, mortality, fluctuating asymmetry (mandibular pores, pectoral finrays, pelvic finrays, and gillrakers on the upper and lower arms of the first branchial arch) and directional asymmetry (branchiostegal rays) to assess chronic thermal stress (fluctuating temperatures as opposed to ambient temperatures) in developing eggs from two different coho salmon (*Oncorhynchus kisutch*) stocks and their reciprocal hybrids. Hybridization provided insight on the capacity of heterozygosity to reduce stress during development.

Although egg losses were consistently higher in crosses exposed to fluctuating temperatures, egg mortality was predominantly a function of maternal stock of origin. Post-hatch losses were higher in crosses exposed to ambient temperatures than in crosses exposed to fluctuating temperatures during embryogenesis. Observed patterns of early growth revealed no heterosis, but instead reflected maternal effects, with some crosses slowing growth and yolk utilization when exposed to fluctuating temperatures. Analyses of fluctuating asymmetry also showed no effects from heterosis. While analyses of composite asymmetry scores and branchiostegal rays were inconclusive, analyses of individual characters showed significantly higher fluctuating asymmetry in pelvic finray counts and a marginal change in the numbers of fish asymmetric for this character in crosses exposed to chronic thermal stress. In contrast, the fluctuating asymmetry in lower gillraker counts was significantly higher in crosses exposed to ambient temperatures and there were significantly more fish asymmetric for this character. Data on mortalities and fluctuating asymmetry indicate pelvic finray development was thermally stressed, while the heightened fluctuating asymmetry in lower gillraker counts under ambient temperatures was due to a greater frequency of less fit fish that had not been culled by thermal stress. Changes

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in early growth patterns in response to developmental stress yielded no parallel responses in meristic characters.

We conclude that chronic thermal stress produced both selectively lethal and sublethal effects that directly shaped fluctuating asymmetry and fitness profiles in these crosses. Implicit in this conclusion is that developmental instability analyses can detect more than just chronic sublethal stress, thus providing substantial credence for using instability studies as proactive bioassessment methodologies.

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All definitions of biotic stress share a common theme: a stimulus acting on and evoking a reaction from a biological system. This action-reaction phenomenon is basic to all biological processes regardless of whether the organizational level being examined is the individual, population, community, or ecosystem. These responses are defined as deviations from some point of reference, where the magnitude of change is presumed to indicate the level of stress. For example, stress proteins (catecholamines and corticosteroid hormones) are considered normal and adaptive responses to commonly encountered situations, but are indicators of stress when they occur at elevated levels (Wedemeyer and McLeay 1981). Alternatively, some compounds can be functionally inhibited under stress. The activity of erythrocyte δ -amino levulinic acid dehydratase, for example, is reduced in the presence of lead (Hodson et al. 1984) and brain acetylcholinesterase activity is impeded by organophosphate pesticides (Neff 1985).

Stress is categorized as acute or chronic, with both forms having the potential for lethality. Studies of acute stress involve short time-period influences and reflect responses at the specific life-history stage used at the time of study. Post-exposure responses are usually immediate, or can be delayed for a few hours or days. Chronic stress (especially chronic sublethal stress) is more common, but difficult to assess because it involves continuous or periodic exposure to low levels of a stressor(s) over long time-periods and encompasses more than one life-history stage.

One useful method of measuring chronic sublethal stress has evolved from the concept of developmental stability, the ability of an organism to produce a consistent phenotype under given environmental conditions. Development is imperfect and small deviations from any developmental plan occur routinely. These errors are produced by developmental instability and are manifested as small random departures from phenotypic symmetry, such as the right-left asymmetries of bilaterally symmetric characters (i.e., fluctuating asymmetry) (Van Valen 1962, Palmer and Strobeck 1986).

Developmental disruption occurs because stress in organisms is considered to be energy dissipative (Aleksieva et al. 1992, Ozernyuk et al. 1992). Such energy diffusion challenges homeostatic mechanisms, thus reducing fitness and increasing instability (e.g. Allendorf and Leary 1986, Palmer and Strobeck 1986, Zakharov and Graham 1992, Freeman et al. 1993, Graham et al. 1993a, b). Heterozygosity has been reported to be positively associated with developmental stability because it may reduce energy dissipation from stress by increasing metabolic efficiency (e.g. Soulé 1979, Leary et al. 1983, 1984, 1985, Mitton and Grant 1984, Mitton 1994).

To assess developmental instability, the portion of phenotypic variance due to developmental disturbances must be estimated. Detection and interpretation of stress-induced changes in developmental instability have traditionally required the satisfaction of four conditions (Campbell and Emlen 1996). (1) Stress is present throughout all or most of character development, such that the influence of stress on the development of selection characters is maximized as is the number of characters affected. (2) Traits can be accurately measured, thus reducing the added data variability caused by measurement (observer) error. (3) Mortality is less sensitive as an indicator of stress than developmental instability. Selective removal of less fit individuals may reduce, or even reverse differences in average developmental instability between samples. (4) Samples are collected randomly.

We measured mortality, early growth, energy allocation (as measured by changes in dry yolk and body weight) and developmental instability (as measured by fluctuating and directional asymmetry) to detect evidence of chronic thermal stress occurring throughout embryogenesis in progeny from two coho salmon (*Oncorhynchus kisutch*) stocks and their reciprocal hybrids. The different expressions and sensitivities of each measure provided insight on integrating multi-character responses over different life-history stages and ascer-

Table 1. Statistical summary of egg diameters and total lengths for all five early growth samples. If interaction is significant, Tukey test results are reported for all thermally treated crosses, otherwise only those levels within the significant factor are presented (S = Skykomish, U = Univ. of Washington, A = ambient thermal treatment, F = fluctuating thermal treatment, $\alpha = 0.05$, maternal stock source first in each designated cross; $n = 60$).

Sample number	Source	df	F	Pr > F	Tukey grouping	Mean length (mm)	Cross/Treatment
1 Egg diameter	Treatment	1	0.48	0.4909		6.8	SSF
	Cross	3	240.58	0.0001		6.7	SUF
	Interaction	3	4.28	0.0054		6.7	SUA
						6.7	SSA
						6.1	USA
						6.1	UUA
						6.0	UUF
					6.0	USF	
2 Egg diameter	Treatment	1	2.71	0.1007		6.8	SS
	Cross	3	268.51	0.0001		6.8	SU
	Interaction	3	0.22	0.8792		6.1	US
						6.0	UU
3 Total length	Treatment	1	13.92	0.0002		20.5	Ambient
	Cross	3	48.2	0.0001		20.1	Fluctuating
	Interaction	3	1.44	0.23			
						20.8	SS
						20.8	SU
						19.9	UU
						19.5	US
4 Total length	Treatment	1	1.7	0.1923		26.1	SU
	Cross	3	46.3	0.0001		25.9	SS
	Interaction	3	1.38	0.2493		24.6	UU
						24.5	US
5 Total length	Treatment	1	11.43	0.008		31.4	SUA
	Cross	3	59.25	0.001		31.4	SSA
	Interaction	3	4.8	0.0026		30.8	SSF
						30.8	SUF
						29.8	USA
						29.7	UUF
						29.3	UUA
					29.1	USF	

taining whether presumptive increases in heterozygosity act to reduce developmental stress.

Materials and methods

Gametes were collected from 15 adult male and 15 adult female coho salmon obtained from the Skykomish and from the Univ. of Washington fish hatcheries located in northwestern Washington state. Adult coho returning to spawn at the Univ. of Washington hatchery are smaller than those returning to the Skykomish hatchery (A. Appleby, Washington State Dept of Fisheries, pers. comm.; G. Yokoyama, Univ. of Washington School of Fisheries, pers. comm.). This difference is due to more intensive feeding and higher rearing temperatures at the university which lead to accelerated growth, producing juveniles that smolt within their first year (Brannon et al. 1982). Although the two stocks originate from contiguous watersheds, suggesting some genetic divergence between the two

stocks is probable, they overlap sufficiently in spawning migration times to permit crossbreeding.

Eggs and sperm were crossed in a 2×2 factorial design to obtain all possible cross combinations, and yielded approximately 7500 eggs per cross. Throughout embryogenesis, half of the eggs in each cross were exposed to temperatures regularly fluctuating between 7°C and 12°C , while the other half experienced ambient temperatures (Fig. 1). Eggs exposed to fluctuating temperatures experienced ambient temperatures for 8 h during each shift between minimum and maximum temperatures to reduce adverse effects from thermal shock.

Early growth

To compare patterns of early growth, five samples were collected from each thermally treated cross before the initiation of exogenous feeding. Sixty individuals were collected per sample at similar levels of cumulative temperature units (TU) calculated daily as $^{\circ}\text{F} - 32$

(Leitritz and Lewis 1980), and preserved in 10% neutral buffered formalin (Heming and Preston 1981, Fleming and Ng 1987). Before dissection, egg diameters were estimated by averaging the measurements along the short and long axes of the egg, while sac-fry were measured for total length to the nearest 0.5 mm. Yolk and body tissue were then separated, dried for 48 h at 60°C (Heming 1982), cooled in a desiccator and weighed to the nearest 0.0001 g. Using SAS (SAS Institute Inc., Carey, NC), egg diameters, total lengths, dry yolk weights and dry body weights were analyzed using a fixed effects two-factor analysis of variance and multiple comparisons (Tukey test). Percentage yolk absorption.

$$\%YA = [(Y_0 - Y_t)/Y_0]100$$

and yolk absorption efficiency (i.e., efficiency of the conversion of absorbed yolk into body tissue)

$$YA \text{ Efficiency} = [(F_t - F_0)/(Y_0 - Y_t)]100$$

were calculated over all samples (where Y_0 and F_0 are the average dry weights of the first yolk and embryo samples, respectively, and Y_t and F_t are the average dry weights of the final yolk and fry samples, respectively). These values were then arcsine-transformed and statistically examined using two-factor non-replicated analyses of variance (Zar 1984).

Meristic characters

At six months of age, 75 parr were collected from each thermally treated cross and preserved in 10% neutral buffered formalin for meristic analysis. The specimens were cleared and stained according to Potthoff (1984). This procedure differentially stains cartilage and bone (blue and red, respectively), while enzymatically digesting the soft tissues. The result is a highly transparent specimen containing a strongly color contrasted skeletal architecture. Meristic elements can be counted with great accuracy and precision, and counts are more complete because rudimentary structures are revealed, an advantage not provided by less intensive methods of enhancing character visibility.

Six bilateral meristic characters were counted: mandibular pores, pectoral finrays, pelvic finrays, anterior gillrakers on the lower first branchial arm (which included those rakers straddling the arch between the upper and lower limbs), anterior gillrakers on the upper first branchial arm, and branchiostegal rays. While the first five characters exhibit fluctuating asymmetry, the last trait is directionally asymmetric (Hubbs and Hubbs 1945, Landrum 1966).

To verify the presence or absence of fluctuating asymmetry, distributions of differences in counts between the sides were tested for deviations from a mean of zero and non-normality (Palmer and Strobeck 1986, 1992). Using SAS (SAS Institute, Carey, NC), fixed effects two-factor analyses of variance and multiple comparisons (Tukey test) were applied to the magnitude of asymmetry ($|R_i - L_i|$) for each character to test for differences from the effects of thermal treatment or cross. Identical enumeration and statistical procedures were used to analyze branchiostegal ray directional asymmetry. Further, the number of characters displaying fluctuating asymmetry in each fish was examined among treated crosses using a fixed effects two-factor analysis of variance, as was the total asymmetry across characters (a composite asymmetry score obtained by adding the absolute values of the individual character asymmetry scores within each fish). For descriptive purposes, individual scores for the number of asymmetric characters and individual asymmetry scores were then summed over all fish in a sample and each divided by the sample size to obtain population mean scores (Wagner 1996). Numbers of fish asymmetric for each character and the total number of asymmetric fish were compared among samples using non-replicated two-factor analyses of variance (Leary et al. 1992).

When calculated over all individuals in a sample, high correlations (r) between the sides for a character imply fluctuating asymmetry is contributing less to meristic variance than lower correlations. Hence, the reciprocal value $1 - r$ estimates the contribution to meristic variance from fluctuating asymmetry (Bader 1965, Leary et al. 1992). To test the importance of this contribution, product-moment correlation coefficients (r) were calculated between the sides for each character, normalized using Fisher's Z -transformation and analyzed using modified chi-square and multiple comparisons tests (Zar 1984).

Mortality

Mortalities were removed and recorded up to the time at which samples for meristic analysis were collected. Total deaths, expressed as proportions of the total egg allotments to each thermally treated cross, were statistically examined using chi-square analysis while arcsine-transformed proportions were used in subsequent multiple comparisons (Zar 1984). The total mortality in each treated cross was then divided into those individuals that died as eggs (pre-hatch) and those that died as fish (post-hatch). These proportions were also subjected to identical chi-square analysis and multiple comparisons procedures. A significance level of 5% was established for all statistical analyses.

Table 2. Statistical summary of dry yolk weights for all five early growth samples. If interaction is significant, Tukey test results are reported for all thermally treated crosses, otherwise only those levels within the significant factor are presented (S = Skykomish, U = Univ. of Washington, A = ambient thermal treatment, F = fluctuating thermal treatment, $\alpha = 0.05$, maternal stock source first in each designated cross; $n = 60$).

Sample number	Source	df	F	Pr > F	Tukey grouping	Mean length (mm)	Cross/Treatment
1	Treatment	1	7.94	0.0051		0.0956	SUF
	Cross	3	288.43	0.0001		0.0949	SSF
	Interaction	3	10.08	0.0001		0.0899	SSA
						0.088	SUA
				0.0702	USA		
					0.0684	UUF	
					0.0674	UUA	
					0.0658	USF	
2	Treatment	1	0	0.9739		0.0908	SS
	Cross	3	387.38	0.0001		0.0908	SU
	Interaction	3	0.4	0.7547		0.0651	US
						0.0644	UU
3	Treatment	1	9.27	0.0025		0.0711	Fluctuating Ambient
	Cross	3	286.52	0.0001		0.0687	
	Interaction	3	2.09	0.1003		0.0819	SS
						0.0808	SU
				0.0584	US		
					0.0584	UU	
4	Treatment	1	0.89	0.3459		0.0679	SS
	Cross	3	274.61	0.0001		0.0664	SU
	Interaction	3	1.07	0.3604		0.0471	UU
						0.046	US
5	Treatment	1	12.97	0.0004		0.0444	SUF
	Cross	3	280.96	0.0001		0.0433	SSF
	Interaction	3	3.83	0.0099		0.0404	SSA
						0.039	SUA
				0.0243	USF		
					0.0242	USA	
					0.0239	UUF	
					0.0233	UUA	

Results

Analysis of total mortalities among treated crosses (Fig. 2) revealed significant differences ($\chi^2 = 1233$, $df = 7$, $P < 0.001$). Multiple comparisons showed little apparent effect from thermal treatment, but did indicate strong maternal effects (S = Skykomish, U = Univ. of Washington, A = ambient thermal treatment, F = fluctuating thermal treatment; maternal stock source first in each designated cross),

SSA SSF SUF SUA UUA UUF USA USF

Analysis of pre-hatch (egg) mortalities also showed significant differences ($\chi^2 = 1492$, $df = 7$, $P < 0.001$), revealing a similar pattern of multiple comparison groupings to that from total mortality,

SSA SSF SUA SUF UUA USA UUF USF

suggesting losses at this stage of development were primarily responsible for the total mortality profile. In addition, crosses exposed to fluctuating temperatures throughout embryogenesis had consistently higher egg mortality (Fig. 2).

Analysis of post-hatch mortalities revealed significant differences among thermally treated crosses ($\chi^2 = 408.6$, $df = 7$, $P < 0.001$).

SSF UUF USF SUF USA UUA SSA SUA

Crosses exposed to fluctuating temperatures during embryogenesis showed consistently lower mortality during post-hatch development compared to those crosses exposed to ambient temperatures over the same developmental period (Fig. 2).

Table 3. Statistical summary of dry body weights for all five early growth samples. If interaction is significant, Tukey test results are reported for all thermally treated crosses, otherwise only those levels within the significant factor are presented (S = Skykomish, U = Univ. of Washington, A = ambient thermal treatment, F = fluctuating thermal treatment, $\alpha = 0.05$, maternal stock source first in each designated cross; $n = 60$).

Sample number	Source	df	F	Pr > F	Tukey grouping	Mean weight (g)	Cross/Treatment
1	Treatment	1	20.12	0.0001		0.0042	SSA
	Cross	3	55.04	0.0001		0.004	SUA
	Interaction	3	16.75	0.0001		0.0033	SSF
						0.0032	SUF
						0.003	USF
						0.0029	USA
						0.0028	UUF
						0.0025	UUA
2	Treatment	1	19.34	0.0001		0.0067	SSA
	Cross	3	52.14	0.0001		0.0054	SSF
	Interaction	3	4.63	0.0033		0.0052	SUA
						0.005	SUF
						0.0044	USA
						0.0043	UUA
						0.004	UUF
						0.0039	USF
3	Treatment	1	0.34	0.5588		0.0105	SS
	Cross	3	114.17	0.0001		0.0103	SU
	Interaction	3	0.81	0.487		0.0086	UU
						0.0082	US
4	Treatment	1	0.27	0.6058		0.023	SS
	Cross	3	82.88	0.0001		0.0229	SU
	Interaction	3	1.57	0.1954		0.0189	UU
						0.0188	US
5	Treatment	1	14.93	0.0001		0.0435	SUA
	Cross	3	89.85	0.0001		0.0426	SSA
	Interaction	3	5.37	0.0012		0.0398	SSF
						0.0394	SUF
						0.0342	UUF
						0.0341	UUA
						0.034	USA
						0.0339	USF

Early growth

Mean temperatures during the experiment were similar (fluctuating = 9.5°C, ambient = 9.1°C), suggesting the differing profiles of thermal variance contributed different levels of stress during egg development (Fig. 1).

Due to their smaller adult size, crosses with Univ. of Washington dams had significantly lower mean egg diameters, mean dry yolk weights, mean dry body weights and shorter lengths than crosses with Skykomish dams over most of the examination period (Figs 3–5, Tables 1–3). Crosses with Skykomish dams that were exposed to ambient temperatures used more yolk than those exposed to fluctuating temperatures, resulting in sac-fry that were consistently longer and significantly heavier by the end of thermal treatment. In contrast, patterns of dry yolk weight, dry body weight and total length were relatively invariant among crosses with Univ. of Washington dams.

Although yolk absorption efficiencies (Table 4) were not significantly different ($F_{\text{thermal}} = 0.608$, $df = 1$, $P =$

0.493; $F_{\text{cross}} = 0.404$, $df = 3$, $P = 0.762$), efficiencies were divided among crosses with Skykomish dams. Those crosses exposed to ambient temperatures had higher efficiencies than those exposed to fluctuating temperatures. In comparison, divergent patterns among crosses with Univ. of Washington dams were not evident. While no significant effects from thermal treatment were observed for yolk absorption ($F_{\text{thermal}} = 7.368$, $df = 1$, $P = 0.073$), significant cross effects were recorded ($F_{\text{cross}} = 126.158$, $df = 3$, $P = 0.001$). Multiple comparisons revealed crosses with Univ. of Washington dams absorbed significantly more yolk over the course of thermal treatment.

Fluctuating asymmetry

No significant effects from thermal treatment or cross were detected in the fluctuating asymmetry of mandibular pores, pectoral finrays, or gillrakers on the upper arm of the first branchial arch (Tables 5 and 6). In

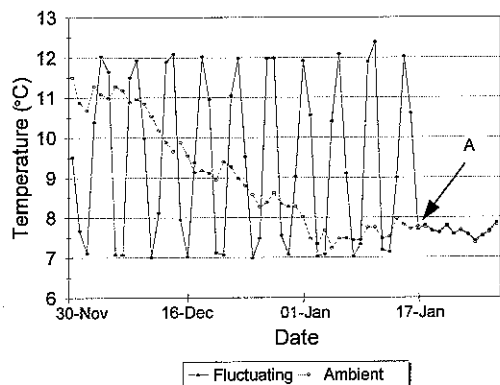


Fig. 1. Plot of thermal data collected throughout treatment of the coho crosses with ambient and fluctuating temperatures. Values plotted are the means of three daily temperature readings (A = termination of fluctuating thermal treatment).

contrast, significant differences in fluctuating asymmetry due to thermal treatment were detected in pelvic finrays and gillrakers on the lower arm of the first branchial arch. More fluctuating asymmetry was revealed in pelvic finrays in fish exposed to fluctuating temperatures, while fluctuating asymmetry in lower gillrakers was higher in fish exposed to ambient temperatures. Although some variation in the mean number of asymmetric characters (\pm SD) was evident among scores for each treated cross (UUA = 1.72 ± 1.05 , USA = 1.77 ± 0.89 , SUA = 1.48 ± 0.96 , SSA = 1.64 ± 1.19 , UUF = 1.83 ± 1.1 , USF = 1.69 ± 0.99 , SUF = 1.79 ± 1.0 , SSF = 1.6 ± 0.97), no significant effects from thermal treatment or cross were detected ($F_{\text{thermal}} = 0.77$, $df = 1$, $P = 0.380$; $F_{\text{cross}} = 0.81$, $df = 3$, $P = 0.489$; $F_{\text{interaction}} = 1.1$, $df = 3$, $P = 0.349$). The average asymmetry across characters (\pm SD) also showed some variability among scores for each treated cross (UUA = 1.96 ± 1.3 ; USA = 1.91 ± 1.02 ; SUA = $1.76 \pm$

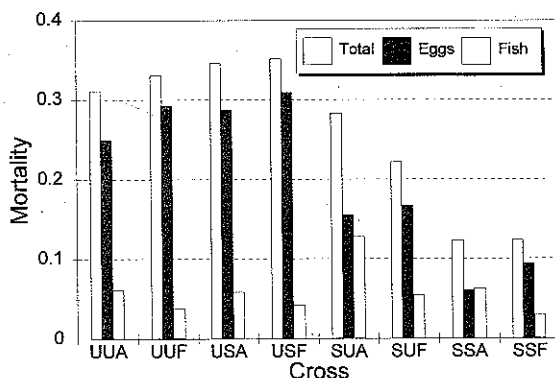


Fig. 2. Mortality levels as a proportion of the total number of individuals within each thermally treated cross (=Total) and those mortality proportions occurring as eggs, or fish (S = Skykomish, U = Univ. of Washington, A = ambient thermal treatment, F = fluctuating thermal treatment, maternal stock source first in each designated cross).

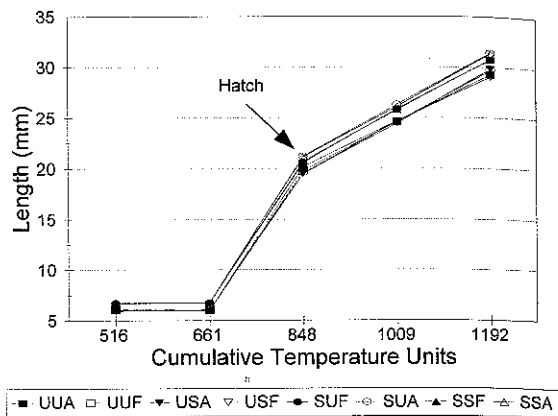


Fig. 3. Average egg diameters (first two samples) and total lengths (last three samples) for all five early growth samples collected at increasing levels of cumulative temperature units (S = Skykomish, U = Univ. of Washington, A = ambient thermal treatment, F = fluctuating thermal treatment, maternal stock source first in each designated cross).

1.39; SSA = 1.85 ± 1.34 ; UUF = 1.96 ± 1.22 ; USF = 1.99 ± 1.34 ; SUF = 1.97 ± 1.14 ; SSF = 1.85 ± 1.16), but no significant differences were observed ($F_{\text{thermal}} = 0.52$, $df = 1$, $P = 0.471$; $F_{\text{cross}} = 0.29$, $df = 3$, $P = 0.835$; $F_{\text{interaction}} = 0.25$, $df = 3$, $P = 0.865$). Tests for changes in the magnitude of directional asymmetry in branchiostegal rays among treated crosses also were inconclusive ($F_{\text{thermal}} = 0.21$, $df = 1$, $P = 0.648$; $F_{\text{cross}} = 0.39$, $df = 3$, $P = 0.760$; $F_{\text{interaction}} = 0.28$, $df = 3$, $P = 0.842$).

Approximately 87% of the fish examined were asymmetric for one or more characters (Table 7), and fish asymmetric for mandibular pores were more frequent than those asymmetric for gillrakers or finrays. Analyses showed no significant effects from thermal treatment or cross on the total number of asymmetric fish or on the number of fish asymmetric for pectoral finrays

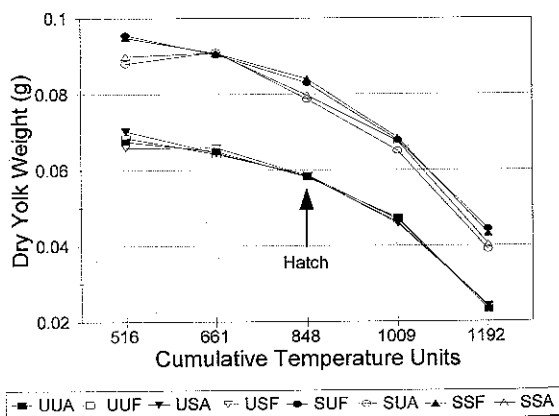


Fig. 4. Average dry yolk weights for all five early growth samples collected at increasing levels of cumulative temperature units (S = Skykomish, U = Univ. of Washington, A = ambient thermal treatment, F = fluctuating thermal treatment, maternal stock source first in each designated cross).

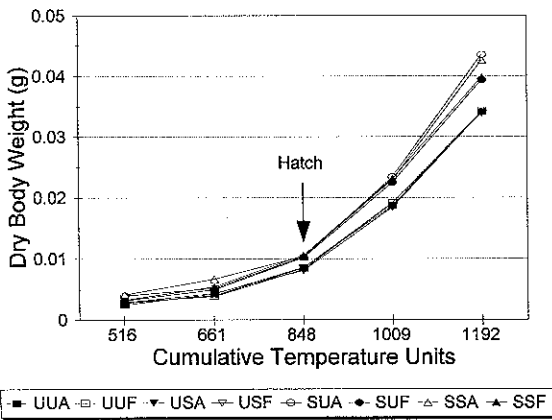


Fig. 5. Average dry body weights for all five early growth samples collected at increasing levels of cumulative temperature units (S = Skykomish, U = Univ. of Washington, A = ambient thermal treatment, F = fluctuating thermal treatment, maternal stock source first in each designated cross).

or upper gillrakers (Table 8). Marginally significant treatment differences were observed for the number of fish asymmetric for pelvic finrays and mandibular pores, favoring increased numbers of asymmetric fish from exposure to fluctuating temperatures. Tests on lower gillrakers showed significantly higher numbers of fish asymmetric for this character were produced under ambient temperatures and multiple comparisons tests revealed marginally significant similarities based on paternal contribution.

Chi-square analyses of the normalized correlation coefficients (as a surrogate for $1 - r$) (Table 9) revealed no significant differences among treated crosses for mandibular pores ($\chi^2 = 3.259$, $df = 7$, $0.9 > P > 0.75$), lower gillrakers ($\chi^2 = 7.867$, $df = 7$, $0.5 > P > 0.25$), upper gillrakers ($\chi^2 = 9.819$, $df = 7$, $0.25 > P > 0.1$), or pectoral finrays ($\chi^2 = 12.26$, $df = 7$, $0.1 > P > 0.05$), while similar tests on pelvic finrays showed significant differences ($\chi^2 = 68.32$, $df = 7$, $P < 0.001$)

(SUF SSF UUF SSA UUA USF USA SUA)

Table 4. Percentage yolk absorption and yolk absorption efficiency for each thermally treated cross (S = Skykomish, U = Univ. of Washington, A = ambient thermal treatment, F = fluctuating thermal treatment, maternal stock source first in each designated cross).

Cross	% yolk absorption	Yolk absorption efficiency
UUA	65.43	71.66
USA	65.53	67.61
UUF	65.06	70.56
USF	63.07	74.46
SSA	55.06	77.58
SUA	55.68	80.61
SSF	54.37	70.74
SUF	53.56	70.7

Over all characters, mandibular pores showed the lowest correlations (i.e., the highest contribution to meristic variation from asymmetry), while pectoral and pelvic finrays showed the highest (i.e., fluctuating asymmetry contributed the least to meristic variance).

Discussion

Although a sizable literature base exists on the positive associations between increased heterozygosity, growth and developmental stability (e.g. Soulé 1979, Leary et al. 1983, 1984, 1985, Mitton and Grant 1984, King 1985, Allendorf and Leary 1986, Danzmann et al. 1986, 1988, 1989, Mitton 1994), responses reflecting positive relationships were not observed in this study. Three reasons for this lack of concordance can be suggested. First, there may have been little or no genomic difference between the source stocks, yielding marginal increases in heterozygosity in the reciprocal hybrids. However, Campbell (1995) showed vertebral fusions among fish from the same study had different distribution patterns along the spine depending on cross. In fish sampled from the Skykomish pureline cross, fusions were randomly distributed, while in fish from the Univ. of Washington pureline cross, the fusions were significantly clustered anteriorly and posteriorly. Reciprocal hybrids displayed similar, but significantly biased intermediate profiles. Coupled with the differences in early growth and life-histories, the assumption that the parental stocks did not differ genetically is not supported, but instead suggests differing character sensitivities to hybridization. Second, qualitative phenotypic analyses, such as those performed in this study, may not be sensitive enough to detect weak associations between developmental stability and heterosis, or those effects connected to a few specific gene loci (e.g. see Clarke 1994, Britten 1996, Palmer 1996, Vøllestad and Hindar 1996). Third, maternal effects appear to have been present during early growth. These effects have been noted during early development in salmonids (e.g. Hitzeroth et al. 1968, Withler and Morley 1970), suggesting the ability to dominate genetic expression early in life. Such a process could mask the effects from heterosis on the expression of fluctuating asymmetry later in development.

Although reciprocal hybridization appeared ineffective at increasing developmental stability, thermal treatment did induce measurable responses. Whereas crosses with Univ. of Washington dams absorbed more yolk and showed very similar responses to thermal treatment during early growth, crosses with Skykomish dams absorbed less yolk and displayed divided responses; fish exposed to ambient temperatures used yolk more efficiently and grew larger than those exposed to fluctuating temperatures. This difference in responses between

Table 5. Levels of fluctuating asymmetry (SD) by character and thermally treated cross (S = Skykomish, U = Univ. of Washington, A = ambient thermal treatment, F = fluctuating thermal treatment; maternal stock source first in each designated cross; $n = 75$).

Cross	Character				
	Mandibular pores	Lower gillrakers	Upper gillrakers	Pectoral finrays	Pelvic finrays
UUA	0.747 (0.77)	0.653 (0.56)	0.253 (0.44)	0.227 (0.45)	0.067 (0.25)
USA	0.787 (0.68)	0.533 (0.58)	0.293 (0.46)	0.253 (0.44)	0.067 (0.25)
SUA	0.64 (0.73)	0.72 (0.78)	0.173 (0.38)	0.227 (0.58)	0.027 (0.16)
SSA	0.693 (0.7)	0.587 (0.64)	0.307 (0.46)	0.227 (0.42)	0.04 (0.2)
UUF	0.813 (0.63)	0.533 (0.55)	0.32 (0.47)	0.2 (0.4)	0.093 (0.29)
USF	0.853 (0.73)	0.467 (0.58)	0.32 (0.81)	0.24 (0.43)	0.107 (0.31)
SUF	0.773 (0.69)	0.507 (0.55)	0.24 (0.43)	0.333 (0.47)	0.107 (0.31)
SSF	0.787 (0.78)	0.387 (0.59)	0.24 (0.43)	0.293 (0.54)	0.147 (0.36)

Table 6. Analysis of variance results on comparisons of fluctuating asymmetry among thermally treated crosses for each bilateral character ($n = 75$; $\alpha = 0.05$). If significant differences among levels of a factor are indicated, then mean values for those levels are listed.

Character	Source	df	F	Pr > F	
Pectoral finrays	Treatment	1	0.37	0.5424	
	Cross	3	0.51	0.6734	
	Interaction	3	0.7	0.5552	
Pelvic finrays	Treatment	1	8.06	0.0047	Fluctuating = 0.113 Ambient = 0.05
	Cross	3	0.26	0.8538	
	Interaction	3	0.68	0.5662	
Lower gillrakers	Treatment	1	9.13	0.0026	Fluctuating = 0.473 Ambient = 0.623
	Cross	3	1.68	0.171	
	Interaction	3	0.49	0.6925	
Upper gillrakers	Treatment	1	0.33	0.5684	
	Cross	3	1.12	0.3389	
	Interaction	3	0.59	0.6207	
Mandibular pores	Treatment	1	2.19	0.1391	
	Cross	3	0.74	0.5285	
	Interaction	3	0.05	0.9862	

Table 7. Numbers of fish that were asymmetric for one or more characters (overall), or for specific characters (MP = mandibular pores, LGR = lower gillrakers on the first branchial arch, UGR = upper gillrakers on the first branchial arch, Pc = pectoral finrays, Pv = pelvic finrays; S = Skykomish, U = Univ. of Washington, A = ambient thermal treatment, F = fluctuating thermal treatment; maternal stock source first in each designated cross; $n = 75$).

Character	Cross							
	UUA	USA	SUA	SSA	UUF	USF	SUF	SSF
Overall	63	70	61	62	68	65	69	66
MP	43	49	39	42	53	51	47	48
LGR	46	38	43	38	38	32	36	26
UGR	19	22	13	23	24	18	18	18
Pc	16	19	14	17	15	19	25	17
Pv	5	5	2	3	7	8	8	11

stocks during early growth may represent a genetically based adaptive mechanism to reduce stress.

Despite suggestions that composite indices of fluctuating asymmetry are more convenient and informative measures of developmental instability (e.g. Wagner 1996, Leung and Forbes 1997), significant differences among treated crosses were not observed in this study. Such results may have been caused by several factors.

First, different bilateral characters may have alternate developmental timing and, hence, different developmental (genetic) controls. Second, differences in developmental control may produce differing stress sensitivities among characters, leading to differing magnitudes of response. Third, the first two points highlight the necessary assumption of character independence and that character responses may not be additive.

Table 8. Analysis of variance results on the total number of fish asymmetric for one or more characters and those asymmetric for specific characters. If a factor is found to be significant, the total number of fish observed to be asymmetric within each level of that factor are presented (S = Skykomish, U = Univ. of Washington; $n = 75$).

Character	Source	df	F	Pr > F	
Total	Treatment	1	1.149	0.3623	
	Cross	3	0.277	0.8404	
Pectoral finrays	Treatment	1	0.773	0.444	
	Cross	3	0.423	0.7511	
Pelvic finrays	Treatment	1	11.901	0.0409	Fluctuating = 34
	Cross	3	0.385	0.7733	Ambient = 15
Lower gillrakers	Treatment	1	39.361	0.0082	Fluctuating = 132
	Cross	3	11.602	0.037	Ambient = 165
					UU = 84 SU = 79 US = 70 SS = 64
Upper gillrakers	Treatment	1	0.008	0.9333	
	Cross	3	0.934	0.5218	
Mandibular pores	Treatment	1	14.486	0.0319	Fluctuating = 199
	Cross	3	3.314	0.1757	Ambient = 173

Table 9. Pearson product-moment correlation coefficients (r) of counts between right and left sides among specimens for each character within each thermally treated cross (MP = mandibular pores, LGR = lower gillrakers on the first branchial arch, UGR = upper gillrakers on the first branchial arch, Pc = pectoral finrays, Pv = pelvic finrays; S = Skykomish, U = Univ. of Washington, A = ambient thermal treatment, F = fluctuating thermal treatment; maternal stock source first in each designated cross; $n = 75$).

Cross	Character				
	MP	LGR	UGR	Pv	Pc
UUA	0.15	0.35	0.57	0.58	0.55
USA	0.29	0.32	0.43	0.7	0.61
SUA	0.23	0.16	0.64	0.89	0.64
SSA	0.38	0.16	0.3	0.49	0.65
UUF	0.34	0.46	0.47	0.48	0.71
USF	0.26	0.34	0.38	0.6	0.68
SUF	0.22	0.44	0.61	0.18	0.49
SSF	0.21	0.37	0.49	0.39	0.4

Table 10. Coefficients of variation for each character within each thermally treated cross (MP = mandibular pores, LGR = lower gillrakers on the first branchial arch, UGR = upper gillrakers on the first branchial arch, Pc = pectoral finrays, Pv = pelvic finrays; S = Skykomish, U = Univ. of Washington, A = ambient thermal treatment, F = fluctuating thermal treatment; maternal stock source first in each designated cross).

Character	Cross							
	UUA	USA	SUA	SSA	UUF	USF	SUF	SSF
MP	7.39	8.34	7.29	9.13	8.52	8.67	7.36	8.03
UGR	5.63	5.04	5.17	4.33	5.6	7.48	5.57	4.73
LGR	4.65	4.09	4.62	3.76	4.83	4.04	4.49	3.89
Pc	3.2	3.49	4.32	3.52	3.71	3.82	3.26	3.04
Pv	2.54	3.03	3.46	1.51	2.63	3.36	1.95	2.95

Fourth, composite scores blend character responses, resulting in a loss of character-specific information. Last, increases in fluctuating asymmetry may be a product of increases in one or a few characters, of low-level increases across many characters, or of greater numbers of affected fish. Consequently, we do not

recommend using composite scores of fluctuating asymmetry unless the scores also can be decomposed into their component parts; hence, testing their sensitivity.

Thermal stress was evident in the fluctuating asymmetry data, but was represented in two strikingly different manners. Fluctuating asymmetry in pelvic finrays

was significantly higher in fish exposed to fluctuating temperatures. In contrast, fish exposed to ambient temperatures showed significantly higher fluctuating asymmetry in lower gillrakers. Data gathered on the numbers of fish asymmetric for these two characters remedy this apparent contradiction. Analyses of the numbers of fish possessing asymmetric pelvic finrays showed only marginal significance for the higher numbers observed under fluctuating temperatures. In comparison, analyses of the numbers of fish asymmetric for lower gillrakers were strongly significant, with higher numbers detected under exposure to ambient temperatures. Thus, the observed increase in pelvic finray fluctuating asymmetry appears to be a function of increases in magnitude within individuals, while the increase in fluctuating asymmetry of lower gillrakers appears to be a function of greater numbers of asymmetric fish. This observation implies that stress is operating either very differently depending on character, or that fitness differs among fish between the two thermal treatment groups. A revisit of the mortality data reveals fluctuating temperatures inflicted consistently higher egg mortality compared to ambient temperatures during embryogenesis. Examination of the post-hatch mortality data reveals that elimination of higher numbers of presumably less fit eggs from chronic thermal stress resulted in consistently and significantly fewer post-hatch losses. Thus, lower egg mortality under ambient temperatures appears to have left behind greater numbers of less fit fish which may have been responsible for the higher losses during post-hatch and the greater remaining numbers of fish asymmetric for lower gillrakers (see Møller 1997). In other words, fish asymmetric for lower gillrakers were not stressed during character development to cause a detectable increase in the magnitude of fluctuating asymmetry, but instead reflect the differential action of selective mortality between treatments.

Campbell and Emlen (1996) have shown that spring chinook salmon having high levels of bacterial kidney disease (BKD) were larger, had significantly lower directional asymmetry in branchiostegal rays, lower proportions of unusable scales and reduced numbers of circlus errors. These results challenged traditional developmental instability theory by suggesting individuals under greater stress can have lower developmental instability than individuals experiencing less stress. The problem was resolved by positing that the greater apparent fitness of high-BKD fish was caused by the stress-induced selective death of less fit fish. The absence of significant differences in fluctuating asymmetry suggested insufficient stress from BKD infection was present to destabilize bilateral character development. In the present study, changes in branchiostegal ray directional asymmetry in coho were inconclusive despite significant combined effects from chronic thermal stress and selective mortality. The lack of significant

changes in directional asymmetry coupled with the cessation of thermal stress at hatch suggests that branchiostegal rays in coho salmon may have been incompletely developed at hatch, thus preventing observation of selective changes in population fitness based on directional asymmetry measurements. In both studies, however, characters associated with oral function were most affected by selective mortality. Increased directional or fluctuating asymmetry in orally related traits may reflect mechanical interference with feeding and gill ventilation, leading to reduced fitness.

While the foregoing analyses describe how stress and selective mortality affect fluctuating and directional asymmetry, they do not explain why only pelvic finrays responded to developmental stress, especially when coefficients of variation (Table 10) and the meristic variation contributed by asymmetry (Table 9) were the lowest of all five bilateral traits. These low values would normally suggest pelvic finrays to be the most canalized and resistant to change. To address this question, we must refer to the concept of character vestigialization (Fong et al. 1995). The process involves the relaxation of selection on a trait, leading to an evolutionary reduction or eventual loss of that character. In salmonids, the pelvic fins are located in a primitive postero-ventral position. Compared to pectoral fins, pelvic fins are reduced in size and restricted in function (acting more as stabilizers) (Lindsey 1978, Bond 1979). If this difference is a product of evolutionary reduction in the functional value of pelvic fins, then the apparent canalization of pelvic finray counts could reflect a stable, but simplified intermediate stage in the serial attrition process (i.e., partial fin atrophy leading to the loss of some finrays). The stability (characterized by relatively low levels of meristic variation) is conferred because any further reduction in finray counts is constrained as it would require non-adaptive alterations in more canalized portions of the pelvic fins. As a result, meristic variance is minimal, simulating a canalized condition. Such low meristic variance may provide a greater opportunity to witness changes in fluctuating asymmetry caused by developmental stress, compared to other meristic characters where higher levels of background variance could mask additional asymmetry contributed by increased stress.

Conclusion

Clearly, thermal treatments directly affected mortality, early growth and fluctuating asymmetry, but there was insufficient statistical evidence linking metabolic efficiency (i.e., stress-induced energy dissipation manifested as changes in yolk absorption efficiency) to changes in, or forms of fluctuating asymmetry. This weak association is most likely a product of interference from mater-

nal effects, differing degrees and forms of character sensitivity and response to stress, number and choice of characters, and diffuse associations between character responses and heterozygosity. In addition to stress-mediated increases in fluctuating asymmetry, the data analyses support a significantly influential connection between fluctuating asymmetry and selective mortality. Therefore, all three measures provide a more complete picture of thermal stress effects than any single measure alone. Furthermore, despite the cessation of thermal treatment at hatch, effects from thermal stress extended much farther, eventually shaping the fitness profiles among the populations of parr.

The conclusions presented in this report support previous research results that show developmental instability analyses can detect stress. More importantly, these results substantiate the hypothesis that lethal and sublethal chronic stress can alter phenotypic profiles of populations in predictable and detectable ways. By using multiple traits with differing developmental histories to provide a more complete picture of stress influences, analyses of developmental instability also will facilitate the detection of influences from selective mortality, an event that is not measurable in most field research. In doing so, these results may help clarify data sets that have been considered inconclusive, or ambiguous. Given this information, we can more effectively address concerns regarding how much stress is too much by using developmental instability measures as proactive bioindicators. We highly recommend using juveniles (parr or presmolts, provided the characters being measured have been completely determined and differentiated) rather than adults, especially when life-history records are unavailable. Despite the greater apparency and easier measurement of characters produced by large size in older cohorts, age-related selective mortality may have weeded out a substantial proportion of the available asymmetry (e.g. Beacham and Withler 1987, Møller 1995). Last, we strongly promote the clearing and staining of meristic characters in juveniles as a means of nullifying the effects of measurement (observer) error, thereby allaying concerns regarding difficulties with data interpretation.

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