Costs of living for juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in an increasingly warming and invaded world

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Abstract: Rapid environmental change in freshwater ecosystems has created a need to understand the interactive effects of multiple stressors, with temperature and invasive predators identified as key threats to imperiled fish species. We tested the separate and interactive effects of water temperature and predation by non-native smallmouth bass (*Micropterus dolomieu*) on the lethal (mortality) and sublethal (behavior, physiology, and growth) effects for juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in seminatural stream channel experiments. Over 48 h trials, there was no difference in direct predation with warmer temperatures, but significant interactive effects on sublethal responses of juvenile salmon. Warmer temperatures resulted in significantly stronger and more variable antipredator responses (surface shoaling and swimming activity), while physiological indicators (plasma glucose, plasma cortisol) suggested suppression of physiological mechanisms in response to the combined stressors. These patterns corresponded with additive negative growth in predation, temperature, and combined treatments. Our results suggest that chronic increases in temperature may not increase direct predation over short periods, but can result in significant sublethal costs with negative implications for long-term development, disease resistance, and subsequent size-selective mortality of Pacific salmon.

Résumé : Des changements environnementaux rapides affectant les écosystèmes d'eau douce découle la nécessité de comprendre les effets interactifs de stresseurs multiples, la température et les prédateurs envahissants constituant les principales menaces identifiées pour les espèces de poissons en péril. Nous avons testé les effets isolés et interactifs de la température de l'eau et de la prédation par l'achigan à petite bouche (*Micropterus dolomieu*), une espèce non indigène, sur les effets létaux (mortalité) et sublétaux (comportement, physiologie et croissance) pour les saumons quinnat (*Oncorhynchus tshawytscha*) juvéniles dans des expériences en chenal semi-naturel. Au cours d'essais de 48 h, aucune différence associée à l'augmentation de la température n'a été observée sur le plan de la prédation directe, bien que des effets interactifs significatifs sur les réponses sublétales des saumons juvéniles aient été notés. L'augmentation des températures s'est traduite par des réponses anti-prédation (formation de bancs en surface et activité natatoire) significativement plus fortes et plus variables, alors que des indicateurs physiologiques (glucose plasmatique, cortisol plasmatique) semblaient indiquer la suppression de mécanismes physiologiques en réponse aux stresseurs combinés. Ces patrons correspondent à une croissance négative additive dans les traitements de prédation, de température et de ces facteurs combinés. Nos résultats suggèrent que, si des augmentations chroniques de la température n'entraînent pas nécessairement une augmentation de la prédation directe à court terme, elles peuvent entraîner des coûts sublétaux significatifs ayant des répercussions négatives sur le développement à long terme, la résistance aux maladies et la mortalité subséquente dans certaines classes de tailles de saumons du Pacifique.

[Traduit par la Rédaction]

Introduction

Coping with stress is a natural part of life; however, human activities have caused unprecedented rates and magnitudes of environmental change that may push animals to their limits (Ellis 2011). Concerns regarding these changes have elevated the need for investigation into the ecosystem effects from multiple interacting drivers of ecological change, such as those associated with habitat, climate, pollution, and invasive species (Sih et al. 2004; Brook et al. 2008; Crain et al. 2008). Although there is little consensus whether these effects (additive, synergistic, or antagonistic) can be reasonably predicted (Christensen et al. 2006; Darling and Côté 2008), there is an emerging need for research that bridges the (generally dire) theoretical and landscape-scale predictions of species persistence with understanding of behavioral and physiological capacities to respond or adapt to environmental change (Thrush et al. 2009). Not surprisingly, the potential for synergistic effects of climate change with other large-scale stressors is a primary impetus for research in responses to stress at the level

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of the whole organism (Fuller et al. 2010). Recent inquiry has also illuminated a particular dearth of research on impacts of multiple stressors in freshwater systems (Strayer 2010); this is troubling as freshwater organisms may be especially vulnerable to the projected effects of climate change and invasive species (Rahel and Olden 2008).

As ectothermic animals whose body temperature fluctuates according to the surrounding environment, freshwater fish are particularly sensitive to the effects of changing water temperatures (McCullough et al. 2009). Loss of riparian vegetation and greater impervious land cover elevates water temperatures (Allan 2004), dam operations modify downstream thermal regimes (Olden and Naiman 2010), and both past trends and climate change models indicate warmer water temperatures for many regions (Nelson et al. 2009; Kaushal et al. 2010). Prior work on the effects of temperature on freshwater fish has emphasized lethal limits (Lutterschmidt and Hutchison 1997) and acute changes associated with hydropower systems (Schreck 2000), leaving the impacts of chronic temperature stress on growth, development, and disease resistance less well studied (but see Morgan et al. 2001; Marine and Cech 2004). These sublethal temperature effects are particularly important given evidence that some fish populations are already living at or near limits of positive growth potential (McCarthy et al. 2009).

Coincidental with temperature, predation also affects species persistence; however, the extent to which temperature may mediate predator–prey interactions has rarely been considered and tested experimentally. This represents an important knowledge gap for at least two reasons. Species ranges and distributions have already responded to past climate change by showing shifts to higher latitudes and elevations (Hickling et al. 2006); this is expected to change the spatiotemporal overlap of many predators and their prey. Spread of non-native fish species into new systems and habitats are also occurring, creating novel predator–prey encounters (Rahel and Olden 2008; Kuehne and Olden 2012) that may result in greater predation pressure than by native predators (Salo et al. 2007). Further, studies of aquatic predators based on bioenergetic modeling support and predict substantially greater predation pressure on juvenile fish populations during warmer climate regimes (Rogers and Burley 1991; Petersen and Kitchell 2001).

In this study, we tested the effects of increased water temperature on the vulnerability of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) to both direct mortality and sublethal (behavior, physiology, and growth) effects of predation by nonnative smallmouth bass (*Micropterus dolomieu*) using large, seminatural stream channels. Our choice of predator and prey species reflects substantial management interest in the impacts of warmwater invaders in freshwater ecosystems (Vander Zanden and Olden 2008) and, more specifically, the synergistic impacts of climate and non-native predators on threatened Pacific salmon (*Oncorhynchus* spp.) representing cold-water stenotherms highly sensitive to climate-induced warming (Schindler et al. 2008; Sanderson et al. 2009). Smallmouth bass were initially transplanted from eastern North America to the Pacific Northwest in the 1920s; subsequent decades saw continued stocking by state agencies and private citizens, with establishment of self-sustaining populations in lake and river systems. Smallmouth bass have become an increasingly conspicuous predator of juvenile Pacific salmon over the last two decades (Carey et al. 2011). Although estimated predation

rates vary across the region, analysis over several outmigration periods (March–June) suggests that smallmouth bass predation could account for between 4% and 35% of wild fall-run Chinook smolt mortality in the lower Yakima River, depending on environmental conditions (Fritts and Pearsons 2004). The negative effects of bass on both abundance and diversity of native fish communities in other regions is well documented (Jackson 2002); this problem is compounded by the forecasted expansion of thermally suitable habitat for smallmouth bass in the future (e.g., Sharma et al. 2007). For these reasons the interactive effects of temperature and predation for this and other invasive warmwater species is of considerable concern for management and conservation of salmon populations.

Our study objectives were to test (*i*) the hypothesis that warmer temperatures would result in increased rates of predation by smallmouth bass and (*ii*) the hypothesis that temperature and predation would result in variable interactive effects — additive, synergistic, or antagonistic in manifestation — on different types of organism response. We intended to address a knowledge gap by measuring responses to multiple stressors across behavior, physiology, growth and mortality in an ecologically realistic setting (Helmuth et al. 2005). In the past, the focus on response to multiple stressors has largely been on mortality (Darling and Côté 2008); although understandable, this does not take into account that animals accept substantial sublethal costs to avoid mortality, such as growth, optimal habitat, or mating opportunities (Werner and Peacor 2003). (Note that we have used the terms "lethal effect" to describe mortality due to predation and "sublethal effects" for behavior and physiological responses, including growth. Consumptive and nonconsumptive effects have also been used to describe these types of responses to predators.) The "risk-sensitive hypothesis" has been well studied in predator–prey interactions, demonstrating that animals behave in ways that prioritize and balance costs; this prioritization process can drive patterns of animal distribution, growth, and survival at landscape scales (Preisser et al. 2005; Biro et al. 2006). By taking a comprehensive approach that examines multiple responses, we can identify whether different kinds of stressors (e.g., biotic or abiotic) exert pressure on different or similar aspects of biological response, and we can also examine how individuals prioritize and respond to multiple threats.

Materials and methods

Test fish

Spring-run (Yakima River population) Chinook salmon eggs were acquired by the Cle Elum Supplementation Research Facility from 15 natural origin families (wild broodstock) spawned during September 2009. The eggs were transferred to the Northwest Fisheries Science Center (National Oceanic and Atmospheric Administration, Seattle, Washington) and incubated at 5 °C until they began feeding in mid-February 2010. In early April, the fish (approximate fork length 40 mm) were transported to the Western Fisheries Research Center (WFRC, US Geological Survey, Seattle, Washington) and reared in sand-filtered and ultraviolet (UV)-treated fresh water from Lake Washington at ambient intake temperatures (mean ± standard deviation, SD = 10.7 ± 1.0 °C) in a 700 L circular tank. Juvenile Chinook salmon were held on a natural photoperiod regime and fed twice daily ad libitum (Bio Oregon bioVita) until trials began in early August, when fish measured 85.0 ± 7.0 mm (fork length: mean ± SD).

Fig. 1. (*a*) Above-water view of stream channel inside of covered pavilion. (*b*) Underwater longitudinal view of channel showing smallmouth bass and juvenile Chinook salmon in foreground. (*c*) Schematic of stream channel during the acclimation period; predators were acclimated separately and downstream from salmon prey. Predation periods (48 h) were initiated by removing the net and allowing predators and salmon to freely access the entire channel.
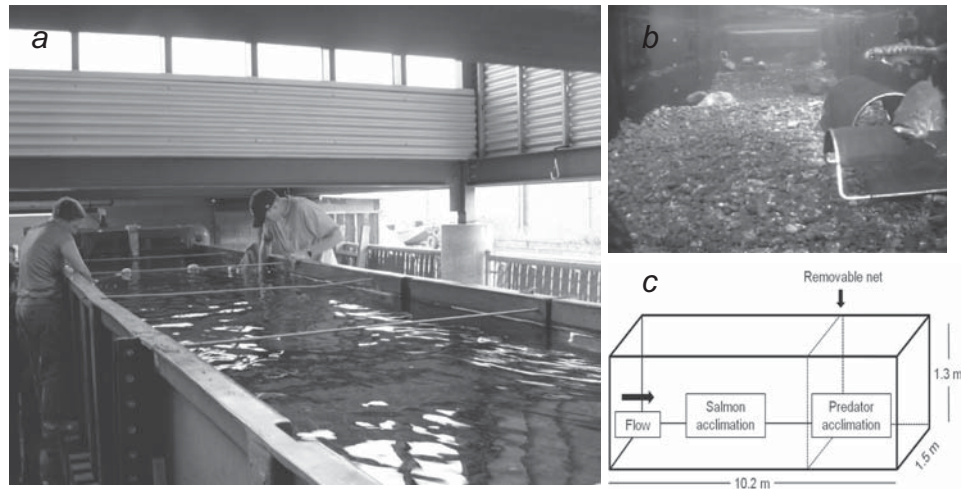
Fifteen smallmouth bass predators (362 ± 23 mm) were captured by angling from Lake Washington and housed in an unused stream channel at ambient temperatures (mean ± SD = 14.8 ± 1.4 °C) until the trials began. This allowed them to habituate in a very similar environment to the experimental channels for 6–8 weeks prior to trials. Predators were maintained on a mixed diet (earthworms, crayfish, Chinook salmon) and were only used in a trial if they regularly consumed live prey; as the majority (12 out of 15) habituated to the lab and only 10 predators were required, no predator was used more than once. The length ratio of prey to predator did not exceed 26% of any individual predator, well within the 50% maximum reported for smallmouth bass preying on salmonids in the wild (Fritts and Pearsons 2006). Preliminary trials also confirmed that smallmouth bass were effective and efficient predators on juvenile Chinook salmon in this size range (L. Kuehne, unpublished data).

Experimental arenas

Trials took place in four fiberglass experimental channels (1.5 m width × 10.2 m length × 1.3 m depth) under a covered outdoor pavilion on the WFRC facility, which conveyed partial light through skylights (Fig. 1*a*). The water was supplied from nearby Lake Washington through sand and UV filters and circulated continuously by pumps at 0.1 m·s⁻¹ within the channels. Channel temperatures were set at 15 °C (hereafter "cool") and 20 °C (hereafter "warm"). Beckman et al. (2000) estimated this salmon population commonly encountered water temperatures of 17–18 °C in the mainstem Yakima River during peak summer months, although many fish may rear in cooler tributaries. We therefore determined a sustained 48 h warm treatment of 20 °C would likely pose a chronic temperature stress, as well as represent midsummer temperatures expected to occur with greater frequency and duration under regional climate change scenarios (Mote et al. 2003).

Within channels, a mixture of pebble and cobble was landscaped into upper (0.8 m depth), middle (1 m depth), and lower (0.8 m depth) sections (ordered downstream); each section contained two groups of large cobble affording some refuge from predators without concealing fish from the observer. Channel interiors were viewable through six regularly spaced 25 cm × 22 cm acrylic windows. An underwater camera (Speco Technologies, model CVC321WP) was mounted at the head of each channel to record predator activity for subsequent analysis (Fig. 1*b*). A removable seine net was positioned two-thirds of the way down each channel to create separate acclimation areas for prey (upstream) and predators (downstream) (Fig. 1*c*).

A trial consisted of four separate treatments: (*i*) cool, (*ii*) cool + predator, (*iii*) warm, and (*iv*) warm + predator. Temperature and light levels in each channel were recorded every 2 h during trials (Onset Corp, UA-002-08). Although incoming lake temperatures were subject to some variation, temperatures between trials were highly consistent (cool (mean ± SD): 14.9 ± 0.7 °C, warm: 20.2 ± 0.8 °C). Because channel temperatures could not be changed easily, channels alternated (cool and warm) in order of arrangement but remained constant between trials; predator treatments were randomized within cool and warm channels. To minimize potential temporal effects, trials were conducted as closely together in time as was feasible; a single trial took 4 days to complete, including 1 day to reset channels. The five consecutive trials were conducted 7–27 August 2010.

Behavior and physiology data collection

Predator-naïve juvenile Chinook salmon were acclimated to cool and warm treatment temperatures over 7 days at rates of 0.5 and 1.3 °C·day⁻¹, respectively, to avoid stress responses due to heat shock (G.E. Sanders, WFRC, US Geological Survey, 6505 NE 65th Street, Seattle, Washington, personal communication, 2010). Preliminary trials indicated that juvenile salmon took approximately 16–24 h to establish feeding territories, consistent with other stream channel studies (Taylor 1988). At the start of each trial (0700), 20 salmon were transferred to each experimental channel and allowed to acclimate for 24 h (acclimation period) in their upstream area

Table 1. Behavioral metrics for salmon prey and smallmouth bass predators.

Variable	Description
Chinook salmon	
Time swimming	Time in seconds of continuous and directional movement resulting in net displacement
Vertical position	Score of vertical position in water column using index of (0) bottom 25 cm, (1) middle 25 cm, (2) top 25 cm, and (3) surface 5 cm
Feed strikes	Biting either in the water column or on the substrate
Aggression	Nips, charges, or chases
Shoaling index	Score using index of (1) further than two body lengths from any other individual fish to (5) within one body length of three or more individuals
Smallmouth bass	
Transits	Crossings among upper, middle, and lower sections in channel
Attacks	Attacks on salmon prey

Note: Behaviors were assessed every 3 hours during trials in 1 min focal follow surveys of juvenile salmon (real time) and 10 min surveys of smallmouth bass (video footage). Ordinal metrics for juvenile salmon prey were characterized every 10 s during focal follows.

(Fig. 1c). At dusk (1900), single smallmouth bass were transferred to the predation treatments behind block nets; in nonpredator treatments this disturbance was mimicked using an empty net. The block nets were removed at 0700 the following morning, and predators and prey then freely accessed the entire channel for 48 h (predation period).

During predation periods, behavioral surveys of Chinook salmon were conducted every 3 h between 0730 and 1930. Three individual focal fish were selected randomly starting in the lower, middle, and upper sections, ensuring representation throughout the stream channel. For each separate focal follow, we quantified for a 1 min period the (i) number of seconds spent swimming, (ii) degree of shoaling based on an index, (iii) vertical position in the water column, (iv) number of feed strikes, and (v) number of aggressive encounters (Table 1). Vertical position in the water column was scored every 10 s by the observer (based on channel wall markings) using an index of (0) bottom 25 cm, (1) middle 25 cm, (2) upper 25 cm, and (3) 5 cm surface layer. Shoaling was also scored every 10 s using an index from 1 (further than two body lengths from any other fish) to 5 (within one body length of three or more fish). Observations were made from a downstream position whenever possible. In general, juvenile salmon appeared relatively undisturbed by an observer; only 3 of 600 focal surveys were abandoned because a fish exhibited abnormal behavior attributed to the observer. Ability of the observer to locate all fish in the channel during surveys was high (mean ± SD: 91% ± 5% of fish per survey), and all observations during all trials were made by the same observer (LMK).

The natural supply of invertebrate prey (mean ± SD: 0.13 ± 0.07 L^{-1}) in the channels, consisting primarily of cladocerans, copepods, and chironomids, was supplemented with chironomid larvae delivered every 6 h and introduced after surveys to avoid recording acute behavioral changes in response to increased food. Frozen cubes (3 g) were added to a perforated bottle upstream of the screen between the pump and experimental area. Water flowing through the bottle gradually carried chironomids into the channel; although exit times were variable, typical dispersion occurred within 60 min. This amounted to an approximate daily food ration of 5% body mass delivered to each channel; although this food was unlikely to be evenly obtained by individuals, it provided a standardized ration across trials and treatments in addition to invertebrate prey occurring in situ.

At the end of trials, all fish were removed from channels and blood samples collected from salmon for analysis of plasma cortisol and plasma glucose. These indicators, well-studied in salmonids, were chosen to offer insight into primary and secondary physiological stress response (Barton 2002). Smallmouth bass were quickly netted; surviving salmon prey were removed by electrofishing (Smith-Root, model L-24) and placed directly in buffered 220 mg·L^{-1} tricaine methanesulfonate (MS222). Length measurements were taken for all fish and blood collected with a heparinized Natelson capillary tube at the severed caudal peduncle. Removal of fish and sampling were done rapidly to avoid acute increases in plasma cortisol; time to obtain samples after initiating electrofishing was 4–6 min. Blood samples were centrifuged for 10 min at 5 °C, and plasma was extracted and stored at –20 °C until analysis.

Plasma glucose and plasma cortisol were measured on different individuals, as the volume collected from individuals (mean ± SD = 40 ± 20 μL) was sufficient for only one assay; sample sizes for each assay were maximized based on the number of survivors across all trials and cost of running samples. Plasma glucose levels ($n = 7$ for each trial and treatment) were measured in duplicate using a hexokinase kit (Stanbio Laboratory, Boerne, Texas). Samples were read at 340 nm on a spectrophotometer (Thermo Electron Corporation, Madison, Wisconsin), and intra- and inter-assay coefficients of variation (CV) were less than 5%. Plasma cortisol concentration ($n = 5$ for each trial and treatment) was measured using a radioimmunoassay (Siemens Diagnostics, Los Angeles, California); samples were run by the Washington State University Center for Reproductive Biology Assay Core Lab (Pullman, Washington). Samples were twice ether-extracted (extraction efficiency 75%), and extracts were dried and reconstituted in the same matrix as the standards. Two additional standards of 2.5 and 5 ng·mL^{-1} were added to the standard curve; these standards bound at 94% and 88%, respectively, and were within the reported assay sensitivity of 2 ng·mL^{-1}. All cortisol samples were assayed together, with a CV of 3.82%.

Growth and mortality data collection

For each trial, initial length and mass estimates were obtained from 10 additional salmon (five cool and five warm) acclimated for the purpose of establishing initial size without introducing handling stress by anesthetizing and measuring all fish prior to stocking to stream channels. At the end of each trial, final mass was measured for a subsample of five fish from each treatment during blood sampling. A specific growth rate (SGR, percent body mass·day^{-1}) was then calculated using the mean mass of the initial and final subsamples as

$$\text{SGR} = (\log_n \text{mass}_2 - \log_n \text{mass}_1)/\text{time}$$

where mass$_2$ = final mass (mean of five fish, subsample) and mass$_1$ = initial mass (mean of five fish, subsample). The time

Fig. 2. Principal component analysis (PCA) of mean behavioral responses for each trial and treatment combination ($n = 5$). Treatment groups are delineated with ordination hulls (95% confidence interval) and labeled as cool (●), cool + predator (○), warm (▼), and warm + predator (Δ). The inset indicates the importance of each behavioral metric to the first and second axes, which jointly explain 66.9% of the variation in the behavioral data set. Behavioral metrics from focal follows of individual fish (Table 1) are coded as follows: time spent swimming (swim), number of feeding attempts (feed), degree of shoaling (shoal), vertical position in the water column (vert), and number of aggressive encounters (agg).
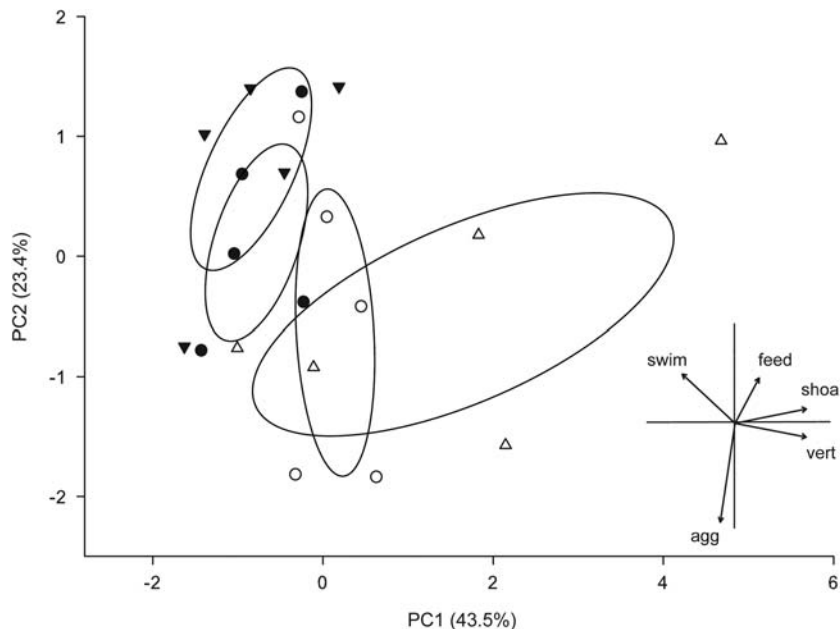
interval was 3 days, which included stream channel acclimation and predation periods.

Direct predation of juvenile Chinook salmon by smallmouth bass was inferred from the number of survivors remaining in channels, and smallmouth bass activity was later quantified using video recordings from the underwater cameras. For the 10 min time period corresponding to behavioral surveys of salmon, an observer recorded the number of (*i*) smallmouth bass transits among upper, middle, and lower sections and (*ii*) attacks on salmon prey.

Statistical analysis

From the 600 one-minute focal follows of juvenile salmon, the following average metrics were calculated for each trial and treatment: (*i*) percent time swimming, (*ii*) number of foraging attempts, (*iii*) shoaling score, (*iv*) vertical position score, and (*v*) number of aggressive encounters (Table 1). Given the expectation of some correlation in behavioral responses, ordination using principal components analysis (PCA) was used to summarize patterns of multivariate variation among variables and paired with permutational tests of significance (PERMANOVA on standardized data with 9999 permutations; Anderson 2001) and tests of homogeneity of multivariate dispersions (PERMDISP; Anderson 2006) between each treatment combination. PERMANOVA tests for significant differences between treatments in position of the multivariate centroid and PERMDISP for variability in behavioral response within treatments or dispersion around the centroid. Although permutational approaches offer greater freedom from assumptions of normality and heteroscedascity, the

behavioral data set was nonetheless examined for and met assumptions of univariate and multivariate normality.

Mean values of plasma glucose, plasma cortisol, and SGR were calculated for each trial and treatment. Behavior scores from principal components 1 and 2 (PC1, PC2) were also extracted for further analysis. Mortality, predator attacks, and predator activity were summarized by trial and treatment for predator treatments. For each of these variables, treatment effects relative to the cool reference group were analyzed using separate linear mixed models, which included trial as a random effect. Use of the mixed model allowed us to control for variation between experimental blocks (trials) as well as compare the direction and magnitude of treatment effects relative to a reference treatment through analysis of regression coefficients and associated *t* statistics (Faraway 2006; Crawley 2007). All variables were examined for and met assumptions of normality and heteroscedascity; all statistical analyses were conducted in the R Programming Environment using the vegan and nlme packages (The R Project for Statistical Computing, http://www.r-project.org/).

Results

Effect of temperature and predation on behavior and physiology

Behavioral responses of juvenile salmon differed among temperature, predation, and combined treatment groups, with 66.9% of the variation explained by the first two principal components (Fig. 2). PC1 (43.5% of variation explained, $p < 0.01$) distinguished increased shoaling activity, increased vertical position

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Table 2. Pairwise comparisons of behaviors between treatment groups in multivariate space.

Treatment	C	CP	W	WP
C	—	**<0.01**	0.76	**0.04**
CP	0.61	—	0.15	0.43
W	0.15	0.5	—	**0.04**
WP	**0.04**	0.11	0.25	—

Note: Treatment groups are as follows: C, cool; CP, cool + predator; W, warm; WP, warm + predator. Pseudo p values for permutational MANOVA (PERMANOVA) are above the diagonal, and permuted p values for tests of multivariate dispersion (PERMDISP) are below the diagonal. Significant ($p < 0.05$) comparisons are in bold.

(proximity to the surface), and reduced time swimming in predator treatments compared with no-predator treatments. This effect was greater in the warm + predator treatment, indicating that fish in the warmer water increased the strength of these antipredator behaviors. Aggression and feeding were associated with PC2, with fish in both predator treatments feeding less and increasing aggression; however, this axis did not explain a significant amount of variation in the behavioral data set (23.4%, $p = 0.76$). According to multivariate testing for differences in behavior among treatments (PERMANOVA test), both predator treatments were significantly different from the cool treatment, in addition to the warm versus warm + predator treatment (Table 2). Differences in variability of behavioral response, described by dispersion around the centroid (PERMDISP test), were more minimal, with only the warm + predator treatment significantly more variable than the reference treatment (Table 2; Fig. 2).

Linear mixed model analysis of the first two principal components (using the cool treatment as the reference) supported the behavioral patterns seen in ordination space. We found a significant treatment effect for PC1 (Table 3), which primarily described antipredator responses of shoaling, proximity to the surface, and reduced time swimming. The significant difference in the warm + predator treatment (regression coefficient = 2.29, $t_{12} = 2.99$; $p = 0.01$) reflects the greater response in these behaviors relative to the cool reference treatment. Analysis of PC2 (aggression and feeding) resulted in no significant differences between reference and other treatments (Table 3; $t_{12} < 1.07$; $p > 0.30$).

Juvenile salmon exhibited elevated plasma glucose in warm temperature and both predator treatments compared with the cool reference treatment, but differed in magnitude and significance of response (Table 3). The largest and statistically significant increase occurred in the cool + predator treatment (regression coefficient = 9.6, $t_{12} = 2.87$; $p = 0.01$), whereas glucose was similarly, but less, elevated in warm and warm + predator treatments (regression coefficients = 6.4 and 5.4, respectively, $t_{12} < 1.92$; $p > 0.08$). Plasma cortisol was more variable, but showed similar patterns as plasma glucose with increases in all treatments relative to the reference treatment in mixed model analysis (Table 3). Elevations were significant in both predator treatments (regression coefficients > 23.9, $t_{12} >$ 2.24; $p < 0.045$), with the largest increase in the cool + predator treatment.

Effect of temperature and predation on growth and mortality

Short-term trends in growth of juvenile salmon were positive in the cool reference treatment, trending toward neutral and negative SGR in predation, temperature, and combined treatments (Table 3). Relative to the cool reference treatment, the largest and statistically significant reduction in growth occurred in the warm + predator treatment (regression coefficient = −6.1, $t_{12} = 2.39$; $p = 0.03$), indicating that fish grew the slowest in response to the combined stressors.

Direct predation from smallmouth bass was generally low, with a mean of 11% (range: 0%–25%) across all trials (Table 4), equating to an average consumption rate of 1.15 juvenile Chinook salmon·predator^{-1}·day^{-1}. Warmer temperatures did not significantly affect predator consumption (Table 4; regression coefficient = 1.0, $t_5 = 0.27$; $p = 0.80$), nor did increased temperatures result in any significant differences in bass activity (number of attacks witnessed or transits observed during behavioral surveys; Table 4; regression coefficients = −0.1 and 0.6, respectively, $t_5 < 0.07$; $p > 0.94$).

Discussion

Within the dual context of global climate change and threats posed by invasive species in aquatic ecosystems, we demonstrated neutral, additive, and synergistic effects of increased temperature and predation on juvenile Chinook salmon. Our study enhances the current understanding of how climate-induced stream warming may pose a direct threat to the recovery of many endangered species (including salmon listed under the Endangered Species Act) and highlights the management challenges likely to emerge in association with the spread of warmwater invaders. Further, by investigating the effects of multiple stressors across different response types, our study sheds light on how behavior and physiology is related to whole-organism performance at the levels of growth and mortality.

We found no effect of increased temperature on direct predation of juvenile Chinook salmon, contrary to the expectation that predation would increase in the warmer experimental treatment because of increased metabolic demand nearer the thermal preferences (approximately 30 °C) for smallmouth bass (Johnson and Kelsch 1998). Predation on Chinook salmon during the 48 h experimental periods was low to moderate, with a maximum of 25% (or five juvenile salmon) consumed in any trial. However, in our study we used smallmouth bass larger than the most abundant size class (150–199 mm), which has also been shown to demonstrate the highest levels of predation on juvenile salmon (Fritts and Pearsons 2006). Our salmon prey, hatchery-raised spring-run Chinook, were larger than many populations vulnerable to bass predation in the wild, such as outmigrating fall Chinook salmon (Fritts and Pearsons 2004). Further, predation rates were considerably higher in preliminary trials where prey fish were smaller, and bass were (presumably) less habituated to laboratory feeding and were also not solitary (L. Kuehne, unpublished data). These observations and results suggest that future thermal predation studies may benefit from testing specific predator–prey size and density ratios of interest, as well as incorporating field-based experiments where predators would be less influenced by laboratory habituation or stress. In more realistic predation scenarios (e.g., multiple predators in

Table 3. Results of linear mixed model analysis of treatment effects on juvenile salmon responses.

Variable (unit)	Cool	Cool+Predator			Warm			Warm+Predator		
		RC	t	p	RC	t	p	RC	t	p
Behavior PC1 (no units)	NA	+0.88	1.15	0.27	−0.05	0.06	0.95	**+2.29**	2.99	0.01
Behavior PC2 (no units)	NA	−0.7	1.07	0.30	+0.57	0.88	0.40	−0.61	0.93	0.37
Glucose (mg·dL^{-1})	76.9±5.4	**+9.6**	2.87	0.01	+6.4	1.92	0.08	+5.4	1.62	0.13
Cortisol (ng·mL^{-1})	57.3±36.4	**+29.6**	2.77	0.02	+10.6	0.99	0.34	**+23.9**	2.24	0.045
Growth (%·day^{-1})	2.2±3.8	−2.9	1.12	0.28	−2.4	0.92	0.38	**−6.1**	2.39	0.03

Note: The mean value and standard deviation for each metric (when applicable) are reported for the cool reference treatment only. Direction and strength of the treatment effects relative to the cool reference group is indicated by the regression coefficient (RC); significant ($p < 0.05$) effects are highlighted in bold. Models included trial as a random effect to control for variation between experimental blocks.

Table 4. Results of linear mixed model analysis of temperature effect on predator responses.

Variable (unit)	Cool+ Predator	Warm+Predator		
		RC	t	p
Consumption (%)	11.0±9.0	+1.0	0.27	0.80
Attacks (sum)	2.7±4.4	−0.1	0.07	0.94
Predator transits (sum)	51.0±18.2	+0.6	0.06	0.95

Note: The mean value and standard deviation for each metric are reported for the cool + predator treatment only. Direction and strength of the relative treatment effect in the warm + predator treatment is indicated by the regression coefficient (RC). Models included trial as a random effect to control for variation between experimental blocks.

natural environments over longer time periods), thermal–metabolic predictions of increased consumption with warmer temperatures might be manifested.

Our mortality results are consistent with previous research examining the ability of juvenile salmon to compensate for short- to medium-term stressors in terms of vulnerability to predation. Conducted within the context of acute stressors experienced during outmigration through hydropower systems, Mesa (1994) and Mesa et al. (2002) examined effects of acute temperature increases and sequential handling events on predator avoidance ability and physiology of juvenile Chinook salmon. Both studies, conducted over similar periods as our experiment (24–48 h), found no difference or rapid recovery in predator avoidance ability, although significant alterations occurred in short-term (cortisol, glucose, lactate) and longer-term (liver hsp70) physiological homeostasis. Even given differences in experimental design, we believe our results are consistent with this body of work to support a model of short-term compensation to stressors that avoids increased vulnerability to predation (Barton 2002). However, long-term responses to stress include reduced growth and development and delayed mortality of aquatic organisms (Marine and Cech 2004; McCauley et al. 2011), and therefore these should be considered.

Our salmon prey exhibited appropriate behavioral responses to predators (regardless of temperature) by increasing shoaling, shifting to a higher vertical position, reducing activity, and reducing foraging. However, these behaviors differed significantly from reference conditions only when predation was combined with warmer temperatures. The few studies that have investigated temperature-driven behavior of fishes indicate increased activity rates, to some extent attributed to in-

creased foraging effort (Quigley and Hinch 2006; Biro et al. 2009). Although we also saw slight activity increases in response to elevated temperature in isolation, when combined with predation this resulted in an opposing trend of stronger (and overall more variable) antipredator behaviors. Interestingly this did not appear to result in an additive reduction in foraging. Interpretation of behavioral responses to stress is made difficult by the fact that animal behavior is often the result of competing motivations, which are poorly understood (Rushen 2000). This may be particularly true of our study in seminatural conditions where "motivation" in terms of predator threat, natural food supply, and initial size of juvenile salmon varied over time and across trials. However, the synergistic effect of temperature and predation on behavioral responses is notable and suggests an attempt to compensate for increased vulnerability to predation at some level or to offset the additive growth costs of the combined stressors.

As with behavior, trends in primary (plasma cortisol) and secondary (plasma glucose) physiological stress indicators were reasonable and consistent with other studies. Although cortisol is notoriously difficult to sample in seminatural and natural conditions because of rapid response time, the range of plasma cortisol values we report are similar to those documented for this same species within 30 min of a single disturbance event (i.e., movement of holding tank; Mesa 1994). This suggests that our rapid sampling largely avoided extreme cortisol elevations that can occur in response to sampling procedures and that the fish in our study were exhibiting moderate as opposed to acute stress responses to increased temperature and predation. Fish in both predator treatments had elevated plasma cortisol, but with no evidence of additive effects of the two stressors. This pattern is mirrored in the response of plasma glucose; while elevated in all treatments relative to the cool reference group, this relationship was significant only in the cool + predator treatment. As a secondary stress response, plasma glucose is less likely to be influenced by sampling procedures when conducted rapidly, and our values are consistent with those reported by Mesa (1994) in response to a single disturbance event. Based on studies examining physiological responses to acute stressors, we expected a possible additive effect (Barton et al. 1986; Mesa et al. 2002; Quigley and Hinch 2006). Our failure to find this in the context of moderate or chronic stressors, and to see evidence instead for an antagonistic or diminished effect is, however, not without support. Although metabolic mechanisms and feedbacks are complex, strong evidence indicates that sustained stress can down-regulate the hypothalamic–

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pituitary–interrenal axis, which is responsible for cortisol response, reducing response to subsequent stressors (Barton 2002). Plasma glucose (and other secondary stress responses) have also been shown to exhibit reduced response under conditions of chronic stress, including low quality or insufficient diet (Barton et al. 1987; Mesa et al. 1998; Schreck 2000). As expected with higher metabolic rates driven by an increase in temperature, we saw elevated plasma glucose and cortisol in the warm temperature treatment. We hypothesize that the lack of additive predator effect under these conditions reflects reduced physiological capacity to respond to additional stressors. Taken together, the trends in our physiological data suggest that temperature and predation, in combination, can result in suppressed physiological responses to stress over relatively short periods.

Lastly, our results showed an additive effect of the combined stressors on the growth of juvenile Chinook salmon, evidenced by a significant negative effect in the warm + predator treatment. We have also documented significant behavioral and physiological responses corresponding to these patterns in growth, such as reduced feeding in predator treatments coupled with (*i*) more conservative antipredator behaviors and (*ii*) increased metabolic demands in the warmer treatment. The specific mechanisms of behavioral and physiological coupling that mediate growth in different contexts (Stoks et al. 2005; Steiner 2007), while very important to understand, are beyond the scope of this work; our intention was to investigate impacts of multiple stressors at an ecologically meaningful scale that still allowed careful measurement of biological response. Our ability to estimate growth within this experimental design had the potential to be confounded by size-selective predation by smallmouth bass, which has been documented in the wild (Fritts and Pearsons 2004). However, not only were predation rates low (in some trials equal to zero), but if size-selection was occurring and the smallest individuals were removed by predators, our estimates of reduced growth (based on a final group mean) would be conservative. Further, although our growth estimates may be subject to size biases, which are often inevitable in group sampling, the systematic nature of these biases gives us confidence in the relative trends between treatments. Given that individual size and growth rate is often the best predictor of survival and reproductive success of juvenile salmonids (Zabel and Achord 2004; Reed et al. 2010; Duffy and Beauchamp 2011), the additive negative impact of the two stressors indicates the strong incentives that these (and other) animals have to avoid suboptimal habitats resulting in reduced growth potential.

Schreck (2000) reviewed the cumulative and long-term effects of stress in fish and noted the lack of empirical knowledge on multiple stressors. He proposed that fish living near the tolerance limit of one stressor will exhibit magnified responses to a second stressor, but that these will be primarily manifested at the level of performance (e.g., behavior, development, or reproduction). Our results are consistent with this prediction; at temperatures near the upper range of thermal tolerance for juvenile Chinook salmon, we saw magnified behavioral responses to a second stressor (predation) and magnified costs in growth. Our results do not indicate that these costs extended to increased mortality due to predation in the short term, but it is likely that over longer periods fish exposed to stressful environmental conditions such as these

would be more likely to succumb to size-selective predation, increased vulnerability to disease, or impaired development (Schreck 2000; Marine and Cech 2004; Cairns et al. 2005).

We believe that our study results, which assess multiple responses of juvenile Chinook salmon to two ubiquitous stressors, demonstrate the relative importance of lethal and sublethal effects to species facing rapid environmental change. Focus in conservation biology is often on direct mortality or decline in population abundance due to acute stressors or biotic interactions; however, the insidious (and challenging to quantify) effects of cumulative stressors at the level of behavior, physiology, and development may be of equal or greater importance in the long-term viability of populations. We suggest that studies that examine a comprehensive suite of responses will result in more accurate estimates of consequences of multiple stressors on whole organism performance, including animal behavior and distribution at landscape scales.

Acknowledgements

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