The consequences of short-term cortisol elevation on individual physiology and growth rate in wild largemouth bass (*Micropterus salmoides*)

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Abstract: In this study, we explored the growth, survival, and potential population-level effects of short-term experimentally induced stress in largemouth bass (*Micropterus salmoides*). Cortisol implants [50 mg·(kg body mass)$^{-1}$] were used to increase circulating stress hormones in a group of wild fish in a research lake for ~6 d in June 2007. Through mark-and-recapture, we compared survival, growth, and plasma biochemistry of cortisol-treated, sham-treated, and control fish at liberty until October 2007. Cortisol-treated fish displayed persistent growth rate depression compared with other groups. However, neither plasma biochemistry nor mortality rates differed among treatments. In a complementary study, we found that the standard metabolic rates (SMR) of cortisol-treated fish were higher than control fish ~56 h following treatment. Bioenergetics modelling revealed that a transient elevation in SMR alone was insufficient to explain the observed growth depression. Finally, we constructed a simple population model to explore the potential consequences of growth depression. We found that a 10% reduction in population growth rate is conceivable when 39% of the population experiences a stress causing the growth rate depression documented in this study. Our study is novel in highlighting that individual and potentially population-level growth depression can result from a single stress event of short duration.

Résumé : Notre étude examine la croissance, la survie et les effets démographiques potentiels d'un stress à court terme provoqué expérimentalement chez l'achigan à grande bouche (*Micropterus salmoides*). Des implants de cortisol [50 mg·(kg de masse corporelle)$^{-1}$] ont servi à augmenter les hormones de stress en circulation chez un groupe de poissons sauvages dans un lac expérimental durant ~6 jours en juin 2007. Par marquage et recapture, nous avons comparé la survie, la croissance et la biochimie du plasma chez des poissons traités au cortisol, des poissons ayant reçu un traitement simulé et des poissons témoins jusqu'en octobre 2007. Par comparaison aux autres groupes, les poissons traités au cortisol accusent une dépression persistante de leur taux de croissance. Il n'y a cependant pas de différence dans la biochimie du plasma, ni dans la mortalité entre les traitements. Dans une étude complémentaire, nous avons observé que le taux métabolique standard (SMR) des poissons traités au cortisol était supérieur à celui des poissons témoins ~56 heures après le traitement. Un modèle bioénergétique montre qu'une élévation transitoire du SMR seule ne suffit pas à expliquer la dépression de la croissance observée. Nous avons enfin élaboré un modèle démographique simple pour examiner les conséquences de la dépression de la croissance. Il indique qu'il est concevable d'avoir une réduction de 10 % du taux de croissance de la population lorsque 39 % de la population encourt un stress provoquant une dépression du taux de croissance de l'ordre de celle observée dans notre étude. L'originalité de notre recherche est qu'elle souligne qu'une dépression de la croissance au niveau individuel et potentiellement à l'échelle de la population peut résulter d'un seul événement de stress de courte durée.

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Introduction

The endocrine stress response is an adaptive mechanism that promotes the survival and recovery of individuals during and after challenging events (Sapolsky et al. 2000; Greenberg et al. 2002). This response, characterized by the production and release of glucocorticoid hormones (Axelrod and Reisine 1984), is associated with a suite of secondary system-level and tertiary whole-animal changes that range from increases in carbohydrate catabolism to complex behavioural changes (Barton 2002). One of the primary adaptive roles of cortisol is the mobilization of energy reserves in response to stress (Van der Boon et al. 1991; Schreck et al. 1997; Wendelaar-Bonga 1997). Elevated cortisol is also associated with increases in aerobic and anaerobic metabolism (Morgan and Iwama 1996; De Boeck et al. 2001) and an increase in standard metabolic rate (Lankford et al. 2005). These changes serve to increase the energy immediately available for individuals to adequately respond to abiotic or biotic challenges (Barton 2002).

Elevated plasma cortisol is associated not only with the mobilization of energy reserves, but also with a reduction in feeding (Gregory and Wood 1999; Lankford et al. 2005). The relationship between foraging behaviour and stress has been explored in a wide variety of taxa using a range of stressful stimuli, and consistently links stress to a reduction in foraging (see reviews by Schreck et al. 1997; Carr 2002; Greenberg et al. 2002). For example, simulated trawling (Olla et al. 1997), environmental toxicants (McGeer et al. 2000), and salt stress (De Boeck et al. 2000) have been reported to reduce feeding behaviour in salmonids. In particular, food-searching behaviour is reduced in response to stress (Beitinger 1990), and in response to increased levels of predation (Gilliam and Fraser 1987; Abrahams and Sutterlin 1999). The proximate mechanism underlying the organismal changes in response to stress is uncertain, but evidence strongly supports the involvement of the hypothalamus–pituitary–interrenal axis (Bernier and Peter 2001), specifically cortisol and corticotropin-releasing factor (Bernier 2006). For example, administrations of exogenous cortisol (Gregory and Wood 1999) and corticotropin-releasing factor (De Pedro et al. 2003; Bernier and Peter 2001) have both been shown to decrease feeding in fishes in the laboratory. However, whether such mechanisms also operate in wild fish populations in situ has not yet been studied.

Although the cellular and organismal changes associated with a stress response are adaptive in the short term, these changes become maladaptive and are associated with decreases in somatic growth rate when circulating cortisol concentrations are chronically elevated (Gregory and Wood 1999; Edeline et al. 2009). For example, in goldfish (*Carassius auratus*), elevated cortisol has also been shown to reduce growth despite normal feeding behaviour (Bernier et al. 2004) indicating that there is a metabolic cost of stress. For wild fish, where animals rely on foraging success to recover energy stores lost during exposure to an acute stressor, and where anti-predator behaviour must be maintained to survive, reductions of individual growth rates may have important implications for survival and reproduction. For example, in males, larger individuals may be better competitors and attract more females (Foote 1988; Dunlop et al. 2007), and indeed larger male smallmouth bass (*Micropterus dolomieu*) are able to obtain more eggs to fertilize and guard than smaller males (Dunlop et al. 2007; Hanson and Cooke 2009). In females, fecundity is exponentially correlated with body size (Sargent and Gross 1986; Birkeland and Dayton 2005). Furthermore, fish size for both sexes can be correlated with overwinter survival in northern latitudes (Biro et al. 2004). Therefore, reductions in individual somatic growth rate can have a negative impact on reproduction and survival. While there have been laboratory studies that have confirmed costs to stress and extrapolated the results to wild animals (Edeline et al. 2009), it is unclear whether laboratory-assessed impacts of stress are relevant to wild fish populations.

In this study, we investigated the potential long-term (5 month) consequences of a transient (6 d) elevation of circulating cortisol in wild, free-swimming fish. We employed a mark-and-recapture approach to compare survival, growth rates, and plasma biochemical indices among largemouth bass (*Micropterus salmoides*) treated with an implant that raises circulating cortisol for ~6 d, sham-treated fish, and control fish at liberty over a 5 month period. Standard metabolic rate (SMR) measurements and bioenergetics modelling were employed to understand whether changes in metabolic rate could account for changes in growth rate. Finally, we constructed a simple population model to explore potential population-level consequences of short-term stress. The combination of experiments was aimed at exploring the long-term consequences of exposure to a transient cortisol elevation, identifying some of the potential regulating mechanisms, and exploring how these changes might affect wild fish populations.

Materials and methods

Long-term effects of transient plasma cortisol elevation in free-swimming wild fish

To study the long-term (5 month) costs of a short-term (6 d) cortisol elevation, we captured 207 mature (>250 mm) largemouth bass by rod-and-reel angling from Warner Lake, a small (8.2 ha surface area) private research lake in eastern Ontario (44°31′N, 76°22′W). To avoid any confounding effects of reproduction, initial treatment occurred between 24 and 28 June 2007, after the cessation of all spawning and parental care activities in this lake. Warner Lake is closed to immigration and emigration for fish, and fishing (including recreational angling) is prohibited aside from research purposes. All captured fish were landed within 20 s, and placed in a foam-lined trough that exposed the ventral side while keeping the gills submerged in fresh lake water. To establish baseline plasma biochemical parameters indicative of feeding and fasting (Congleton and Wagner 2006), approximately 1.5 mL of blood was withdrawn by caudal puncture into lithium-heparinized 3 mL vacutainer-style syringes (B.D., Franklin Lakes, New Jersey) from a random subset of animals. All fish were then scanned for a passive integrated transponder (PIT) tag (12.5 mm × 2.0 mm) using a PIT tag reader (Biomark, Boise, Idaho), and given a unique intracoelomic PIT tag if necessary. Warner Lake is a research lake, and fish have been PIT-tagged since 1993 for routine population monitoring. Of the 207 fish captured, 55 fish already carried PIT

tags. Fish were measured (total length, TL), and size-matched by TL into three treatment groups: cortisol, sham, or control (Table 1). Fish requiring new PIT tags were also distributed evenly among treatment groups. Cortisol-treated fish were injected with cortisol (hydrocortisone; Sigma H4001, Sigma-Aldrich Inc., St. Louis, Missouri) emulsified in coconut oil (*Cocos nucifera*; Sigma C1758, Sigma-Aldrich Inc.). Sham-treated fish were injected with pure coconut oil. Control fish were not injected. Cortisol was mixed with coconut oil at a constant ratio of 10 mg·mL^{-1}, and cortisol-treated fish were injected with the cortisol – coconut oil mixture based on body size at a dose of 0.005 mL·g^{1}, leading to a final dose of 0.05 mg·g^{-1} based on fish mass. Sham-treated fish were injected with pure coconut oil at the same dose of 0.005 mL·g^{1}. For all fish, body mass was estimated from a mass–TL relationship, (mass in g) $= (1.359 \cdot 10^{-4}) \cdot \text{TL in mm}^{2.996}$. This relationship ($R^2$ = 0.949) was previously developed from 68 mature largemouth bass captured between 2003 and 2006 in Warner Lake during the summer post-spawning period (i.e., June and July). All fish were released following treatment. Sexing largemouth bass externally is unreliable out of the breeding season, and so fish were not sexed, and were likely a mixture of males and females.

To monitor the fate and growth of the three treatment groups, between 20 and 25 August (i.e., 54 to 63 d following initial treatment), 206 mature largemouth bass were captured by rod-and-reel angling from Warner Lake, sampled for blood, measured (TL), and scanned with a PIT tag reader as described above. The process was repeated between 10 and 15 October (i.e., 105 to 114 d following initial treatment), when 151 mature largemouth bass were captured, sampled for blood, measured (TL), and scanned with a PIT tag reader.

All blood samples were held in water-ice slurries for no more than 1 h, until being centrifuged at 10 000*g* for 5 min (Compact II Centrifuge, Clay Adams, New Jersey). Plasma samples were flash-frozen in liquid nitrogen and stored at –80 °C until analysis.

Water temperatures were stable between 24 June and 25 August, ranging from 23 °C to 25 °C, with the highest temperatures occurring between 20 July and 10 August. After 25 August, water temperatures declined steadily through the fall, reaching 11 °C by 15 October.

Biochemical indices of fasting

To better appreciate the relevance of circulating plasma biochemical parameters assessed in the wild, 12 largemouth bass were captured by rod-and-reel angling from Lake Opinicon, a nearby public lake that is part of the Rideau River system in eastern Ontario (44°30′N, 76°20′W) from 15 to 19 June 2007. These fish were transported to the Queen's University Biological Station (on Lake Opinicon) in aerated coolers, where they were measured (TL), weighed, and size-matched by mass into two groups; fed and fasted fish (Table 1). Fish were anchor-tagged for individual identification, and then placed in two 750 L holding tanks with flow-through lake water, in mixed treatment groups (n = 3 from each treatment group in each tank; n = 6 fish per tank).

Starting on the day of capture, and every other day thereafter, fish in the fed treatment group were force-fed the equivalent of 2% of their body mass with a 1 g·mL^{-1} mixture

Table 1. Total length (TL in mm; mean ± SE; sample sizes are listed in parentheses) and mass (in g; mean ± SE; sample sizes are listed in parentheses) measurements for fish included in the study.

Study component	Cortisol	Sham	Control	Fed	Fasted	F statistic	t ratio	df	p value
Full group caught in June (TL)	326.7±47.8 (69)	331.6±50.1 (69)	325.4±44.0 (69)	—	—	0.33	—	204	0.72
Subset recaptured in August (TL)	337.4±12.9 (19)	336.2±13.8 (16)	322.7±9.0 (23)	—	—	0.58	—	55	0.58
Subset recaptured in October (TL)	307.5±6.5 (5)	339.4±19.1 (13)	331.3±16.4 (10)	—	—	0.67	—	25	0.51
Subset recaptured twice (TL)	298.5 (2)	317.0 (2)	299.7±5.6 (4)	—	—	3.12	—	4	0.15
Laboratory feeding study (TL)	—	—	—	310.0±17.6 (6)	296.7±23.3 (6)	—	0.21	10	0.66
Laboratory feeding study (mass)	—	—	—	381.7±69.8 (6)	373.3±65.6 (6)	—	0.09	10	0.93
Metabolic rate measurements (TL)	330.2±17.9 (6)	—	324.3±17.5 (6)	—	—	—	0.24	10	0.82
Metabolic rate measurements (mass)	494.5±72.7 (6)	—	473.3±72.3 (6)	—	—	—	0.20	10	0.84

Note: Shown are the TLs cortisol-treated, sham-treated, and control largemouth bass in June; initial TLs of fish recaptured only in August, only in October, and in both August and October; TLs and masses of fish used to obtain fasted and fed biochemical indices; and TLs and masses of fish used for metabolic rate measurements. Within each study, there were no statistical differences among the treatment groups (using analysis of variance, ANOVA; α = 0.05).

of blended trout pellets (Purina Aquamax Grower, Purina Mills Ltd., Missouri) and fresh lake water, using a 50 mL syringe attached to a piece of flexible plastic tubing that placed the food manually in the stomach. Fish in the fasted treatment group were handled in an identical fashion except that no food was injected. Fish were held singly in aerated coolers of fresh lake water for approximately 10 min post-feeding to ensure that no fish regurgitated the mixture. After 7 d (approximately 8 h following the fourth feeding), fish were caught individually from the group tanks and blood samples were withdrawn within 2 min. Plasma was separated and stored as described above. Water temperatures were maintained at 20.0 °C ± 0.9 (mean ± SD) throughout the experiment.

Biochemical analysis

As indicators of recent feeding (Congleton and Wagner 2006), plasma activity of aspartate transaminase and plasma concentrations of cholesterol, glucose, magnesium, total protein, and triglycerides were quantified using a Roche Hitachi 917 analyser (Roche, Basal, Switzerland). All techniques followed the procedural guidelines for standardization and quality assurance established by the Veterinary Laboratory Association Quality Assurance Program and the Canadian Food Inspection Agency External Proficiency Panel, and were carried out by Idexx Laboratories Inc. (Markham, Ontario). For the fish used in the Warner Lake long-term monitoring, pre-treatment (June) plasma samples were not available for all fish recaptured in August and October 2007, and a repeated measures approach was thus not possible. Therefore, if multiple plasma samples (i.e., at multiple capture periods) existed for an individual fish, a subset of plasma samples was analysed such that only a single plasma sample was analysed for each individual. From the June sampling period, 22 samples were analysed as pre-treatment values ($n = 8$ cortisol-treated, $n = 8$ sham-treated, $n = 6$ control). From the August sampling period, samples from 15 cortisol-treated, 15 sham-treated, and 14 control fish were analysed. From the October sampling period, samples from 7 cortisol-treated, 10 sham-treated, and 10 control fish were analysed. All plasma samples from the force-feeding experiment in the laboratory were analysed.

Metabolic effects of cortisol elevation

To determine whether metabolic rate was increased by the short-term elevation of circulating plasma cortisol, 12 largemouth bass were captured by rod-and-reel angling from Lake Opinicon from 21 to 23 June 2008. Fish were transported to the Queen's University Biological Station in aerated coolers, where they were measured (TL), weighed, and size-matched by mass into two groups; cortisol-treated and control (Table 1). Over the 3 d measurement period, four fish were captured per day, and two fish assigned to each treatment group per day. As described above, cortisol-treated fish were injected within 1 h of capture with 10 $mg \cdot mL^{-1}$ cortisol in a coconut oil vehicle at a dose of 0.005 $mL \cdot g^{-1}$, while control fish were not injected. In this case, we were interested in quantifying a change in metabolic rate as a result of our cortisol treatment, relative to a control animal. Since access to the respirometer was limited, and the objective of this portion of our study was to determine the short-term metabolic consequence of our cortisol treatment rather than to disentangle the specific effect of the cortisol elevation from the handling stress of injecting the implant, sham treatments were not employed for this aspect of the study (see DiBattista et al. 2005 for further rationale). All fish were housed singly in 40 L holding tanks with flow-through lake water for 48–52 h post-capture, both to ensure that metabolic measurements were carried out at peak circulating cortisol concentrations for the cortisol-treated fish (O'Connor et al. 2009), and to ensure that fish were in post-absorptive digestive state (Alsop and Wood 1997).

Metabolic measurements were carried out using the Loligo AutoResp intermittent flow-through respirometry equipment and software (Loligo Systems ApS, Tjele, Denmark) with an 11.4 L chamber (see Gingerich et al. 2009 for full description) from 23–25 June 2008. With this system, dissolved oxygen (DO) levels inside the closed system are measured every second for 15 min. The system is then opened for a 10 min flush period, and metabolic recordings for the next period begin following a 1 min lag after the system is closed. Blank tests were run for 2 to 3 h prior to placing a fish in the respirometry chamber, and oxygen consumption values were corrected accordingly. Fish (two cortisol-treated and two control fish per night) were then placed in the respirometry chamber between 2100 and 2300 hours for a 12 h period. The six lowest recordings were averaged to calculate the SMR for each fish (Steffensen et al. 1994). Mean temperature during the metabolic rate measurements was 23.8 ± 0.6 °C (mean ± SD).

Statistical analysis

For each individual study described above, analysis of variance (ANOVA) models were used to ensure that fish were appropriately size-matched. The χ^2 goodness-of-fit tests were used to test that the fish receiving new PIT tags were evenly distributed across treatment groups for the long-term monitoring aspect of the study.

To analyse mortality patterns of cortisol-implanted versus other treatment groups in the wild, recapture rates (ratios of fish recaptured once, recaptured twice, and never recaptured) were compared among treatment groups using χ^2 goodness-of-fit tests. Given low sample size for ratio estimators, a post-hoc power analysis was conducted to determine the power of this test.

Differences in mean growth rates among the treatment groups over the monitoring period were determined using an analysis of covariance (ANCOVA) with treatment group (cortisol, sham, control) as the independent variable, and initial TL as the covariate. The interaction term was included in the model. Growth rate across the monitoring period was the dependent variables, and was determined for each individual by subtracting the total length upon recapture from the initial total length, and dividing by the number of days between captures. For the fish captured more than once, only the final capture was used to calculate that individual's growth rate. Following a significant ANCOVA, post-hoc Tukey's honestly significant difference (HSD) tests on the treatment effect were used to determine where among the treatment groups the differences lay.

All biochemical parameters in the field were compared among cortisol-treated, sham-treated, and control wild large-

mouth bass in June prior to treatment, in August (54–63 d following treatment) and in October (105–114 d following treatment) using ANOVAs. Tukey's HSD post-hoc tests were employed following significant ANOVAs to determine group differences in mean biochemical parameters. In the complementary laboratory analysis, all plasma biochemical parameters were compared between the fed and fasted fish from the feeding experiment using Student's *t* tests. To ensure that all potentially important biochemical indices were identified, a liberal uncorrected $\alpha = 0.05$ was employed.

To determine whether cortisol treatment resulted in short-term changes in metabolic rate, treatment group were used as the dependent variables in an ANCOVA, with whole-animal SMR as the independent variable, and mass as a covariate. The interaction term was included in the model.

All residuals were tested for normal distribution using goodness-of-fit tests, and for homogeneity of variances using Levene's test, and assumptions were met. All statistical analyses were conducted using JMP 7 (SAS Institute Inc., Cary, North Carolina). All results are reported as mean ± SE, and $\alpha = 0.05$, unless otherwise specified.

Bioenergetics modelling

To explore the relative contribution of increase in SMR to growth rate depression in response to the cortisol injection, a series of simulations of fish growth were conducted using the largemouth bass model developed by Rice et al. (1983) using Fish Bioenergetics software (version 3.0, University of Wisconsin–Madison, Madison, Wisconsin). In a parallel study, field activity rates were found to be similar among the different treatment groups following the cessation of cortisol elevation (O'Connor et al. 2011), and therefore activity was assumed to be equal among treatment groups in all bioenergetic models. We also assumed piscivory (a reasonable assumption for largemouth bass of the sizes used in the present experiment; Heidinger 1975; Keast 1985), and therefore, that food would provide 4000 J of energy·g^{-1} (wet mass). Because the bioenergetics model requires a fish mass variable, mean initial and final masses for control and cortisol-treated fish in the long-term study were estimated using the mass–TL relationship described above. Taking into account the increase in SMR found in our study (see Results), we assumed that SMR remained increased for only the 6 d period of cortisol elevation, and used the bioenergetics model to determine whether an increase in SMR would alone be sufficient to cause the growth depression documented in this study between June and August. This 60 d period was selected rather than the entire 114 d monitoring period so that water temperature could be set as a constant 24 °C in the model.

Population modelling

To explore the potential demographic costs of endocrine stress in terms of individual growth depression, as assessed in the empirical portion of our study, we constructed a deterministic Leslie-matrix population model (Caswell 2001) and compared model runs with and without potential growth depression resulting from cortisol treatment. We also explored the fraction of the population that would need to experience a stress-induced growth depression to see reductions in the population growth rate. Population modelling was conducted as a theoretical experiment and was not indented to provide precise predictions for Warner Lake. The population model was first run with a standard growth model, and then contrasted to a model run in which somatic growth was depressed in fish for a single growing season by the empirical estimate of percentage growth depression measured in the field. We assumed that fish of all age classes (from age 1 to age 10) are influenced by the same percentage of growth depression for the single growing season. The approach followed the work by Edeline et al. (2009).

Largemouth bass length (TL, mm) at age a (years) was modelled using a von Bertalanffy growth model as

$$[1] \qquad L_a = L_\infty\{1 - \exp[-0.19(a + 0.024)]\}$$

The default parameter values for the growth model in eq. 1 were taken from the Ontario population of largemouth bass reported in Beamesderfer and North (1995). For simplicity, we assumed no differences in growth among males and females. To account for density-dependence in growth, a negative relationship between population abundance and individual growth estimated for smallmouth bass (Dunlop et al. 2007) was considered as

$$[2] \qquad L_\infty = L_{\infty,\max}/(1 + 0.37D^{0.29})$$

where D is the population density (the number of fish of age 1 or older per hectare). The value of $L_{\infty,\max}$ (mm) was determined so that $L_\infty = 560$ mm (an empirical value L_∞ for the Ontario population, Beamesderfer and North 1995) at an intermediate level of population abundance ($D = 3.5{\cdot}\text{ha}^{-1}$). We varied D between 0.5 and 10.0 to check the sensitivity of the model to the population density and found little effect on the outcome. Based on empirical data for maturation of largemouth bass reported by Carlander (1977), the relationship between fish length and proportion of sexually mature female fish at age class a was represented using a sigmoid function as

$$[3] \qquad p_a = 1/\{1 - 0.1366\exp[-(L_a - 208.2)]\}$$

We assumed that the sex ratio of the population is 50:50, and the number of recruits was determined by the number of eggs produced by females, since there is no information on density-dependence in spawning stock size-recruitment relationships reported for largemouth bass. To this end, size-dependent fecundity (egg number produced by a female of L_a) $f_{\mathrm{m},a}$ was defined according to Laarman and Schneider (1985) as

$$[4] \qquad \log_{10} f_{\mathrm{m},a} = -0.4254 + 3.2857\log_{10} L_a$$

where the subscript m represents mature fish. We assumed there is no sex difference in natural mortality rates but defined size-dependent natural mortality for immature and mature fish separately. Size-dependent annual survival for immature largemouth bass $s_{\mathrm{i},a}$ was based on the study of Gutreuter and Anderson (1985) as

$$[5] \qquad s_{\mathrm{i},a} = [1 - 1.2318\exp(-0.01933L_a)]^{12}$$

where the subscript "i" represents immature fish. A fixed annual survival rate (0.73) was assumed for immature fish of 200 mm or larger. Size-dependent annual survival for mature

fish was defined according to Dunlop et al. (2007) reporting in smallmouth bass. We took this relationship based on the finding that mortality rates of largemouth bass and smallmouth bass do not differ significantly (Beamesderfer and North 1995). Based on Dunlop et al. (2007) we defined an upper limit of annual survival rates (0.46) for very large fish based on their description of background mortality as

$$[6] \quad s_{m,a} = \exp(-0.00938L_a + 4.3572)$$

where the subscript "m" represents mature fish. Using the population model defined by eqs. 1–6 we computed the population's finite rate of increase λ as the dominant eigenvalue of the resulting Leslie matrix M (Caswell 2001). The form of the Leslie matrix we used was

$$[7] \quad M = \begin{pmatrix} s_0 f_1 & s_1 f_2 & \cdots & s_{a_{max}-2} f_{a_{max}-1} & s_{a_{max}-1} f_{a_{max}} \\ s_0 & 0 & \cdots & 0 & 0 \\ 0 & s_1 & & 0 & 0 \\ \vdots & & \ddots & \vdots & \vdots \\ 0 & 0 & \cdots & s_{a_{max}-2} & 0 \end{pmatrix}$$

The age-specific survival and fecundity (egg number per female at age a) in the matrix was represented as

$$[8] \quad s_a = p_m S_{m,a} + (1 - p_m) S_{i,a}$$

and

$$[9] \quad f_a = p_m f_{m,a}$$

The survival from eggs to age-1 fish, s_0, was determined by the method of Vaughan and Saila (1976), on the assumption that the population is at equilibrium without individual growth depression. We assumed $a_{max} = 11$, and sensitivity analyses showed that increasing a_{max} caused negligible changes in the population growth rate λ.

Results

Standardization among treatment groups

There were no differences in initial TLs among the three treatment groups from Warner Lake in June (Table 1). There were also no differences in initial TLs detected among the subsets of each treatment group recaptured only in August, only in October, or at both sampling periods (Table 1). There were no differences among the treatment groups in the proportion of fish given new PIT tags relative to fish already carrying PIT tags ($\lambda^2 = 2.53$, df = 2, $p = 0.28$). Similarly, there were no differences in mass or TL between the force-fed and fasted treatment groups in the complementary laboratory study examining biochemical indices of fasting (Table 1), and there were also no differences in mass or TL between the cortisol-treated and control fish used for metabolic rate measurements (Table 1).

Long-term effects of transient plasma cortisol elevation in wild largemouth bass

In total, 86 fish were recaptured once: 24 cortisol-treated fish, 29 sham-treated fish, and 33 control fish. Of these, 58 were recaptured once in August: 19 cortisol-treated, 16 sham-treated, and 23 control fish. Twenty-eight were recaptured once in October: 5 cortisol-treated, 13 sham-treated, and 10 control fish. An additional 8 fish were recaptured at both sampling periods: 2 cortisol-treated, 2 sham-treated, and 4 control fish (Table 2). While fewer cortisol-treated fish over the study period were recaptured than control and sham-treated fish, the ratios of fish recaptured once, recaptured twice, or never recaptured did not differ among the three groups ($\lambda^2 = 3.77$, df = 4, $p = 0.44$). This result indicates mortality rates did not differ significantly among the groups during the summer months. However, a power analysis revealed that with only 24 fish per treatment group recaptured across the monitoring period (the minimum number of fish per treatment group recaptured once in our study), the power of our study to detect biologically relevant levels of mortality (relative to the control group) was low (Table 3).

Mean growth rate (mean increase in TL per day for each fish) over the monitoring period was significantly lower for the cortisol-treated fish than for the control and sham-treated fish (model $F_{[5,84]} = 9.69$, $p < 0.001$; treatment group $F_{[2,2]} = 9.94$, $p < 0.001$; Fig. 1). As expected, initial TL was also important in predicting growth over the season (initial TL $F_{[1,1]} = 26.89$, $p < 0.001$). The interaction effect was not significant (interaction $F_{[2,2]} = 0.01$, $p = 0.98$). The mean growth rate for control fish was 0.15 ± 0.02 mm·d^{-1} (mean ± SE), while the growth rate for the sham-treated fish was 0.09 ± 0.06 mm·d^{-1}, and the mean growth rate for the cortisol-treated fish was 0.01 ± 0.02 mm·d^{-1}, which amounts to a 93% decrease in growth rate for the cortisol-treated group relative to the control group, and an 89% decrease in growth rate relative to the sham-control group. Note that for fish recaptured twice, only the data from October was used in the statistical analysis.

Biochemical indices of fasting

Among fish included in the long-term study from Warner Lake, no differences in biochemical variables were detected among the treatment groups in June, prior to treatment (Table 4). Chloride varied significantly among the treatment groups in August (54–63 d following treatment), with sham-treated fish exhibiting significantly higher plasma chloride concentrations than cortisol-treated fish (Table 5). Similarly, sham-treated bass exhibited significantly higher plasma glucose concentrations than cortisol-treated bass in October (105 to 114 d following treatment; Table 6). No other differences among treatment groups were detected (Tables 5 and 6).

Individuals that were force-fed exhibited significantly lower plasma sodium concentrations than bass that were fasted for 7 d, and significantly higher plasma concentrations of triglycerides (Table 7). No other differences were detected between fed and fasted fish in the laboratory (Table 7).

Short-term metabolic effects of cortisol elevation

Both cortisol treatment and mass significantly impacted mean SMR measured ~56 h after treatment with cortisol (model $F_{[3,8]} = 46.07$, $p < 0.01$; Fig. 2). Mass was the main effect (mass $F_{[1,1]} = 98.67$, $p < 0.01$), but treatment group was also a significant effect (treatment $F_{[1,1]} = 20.20$, $p < 0.01$), as was the interaction of these two factors (interaction $F_{[1,1]} = 12.54$, $p < 0.01$). Mean mass-corrected SMR for

Table 2. Number of fish recaptured once, twice, or never recaptured in the cortisol-treated, sham-treated, and control groups of fish.

Recaptured subset	Cortisol	Sham	Control
Fish recaptured once	24	29	33
Fish recaptured twice	2	2	4
Fish never recaptured	43	38	32

Note: There were 69 fish treated in each group in June. There are no differences among the treatment groups in the ratios of fish captured once, fish recaptured twice, and fish never recaptured among treatment groups (χ^2 tests; $\lambda^2 = 3.77$, df = 4, $p = 0.44$).

Table 3. Results of a post-hoc power analysis to determine the power of detecting a decline in recapture rate in the sham-treated or cortisol-treated group relative to the control group, using a conservative estimate of 24 fish recaptured once per treatment group across the monitoring period.

Theoretical % mortality (relative to the control group)	Power $(1 - \beta)$
5	0.05
10	0.10
20	0.18
30	0.46
50	0.86
90	0.99

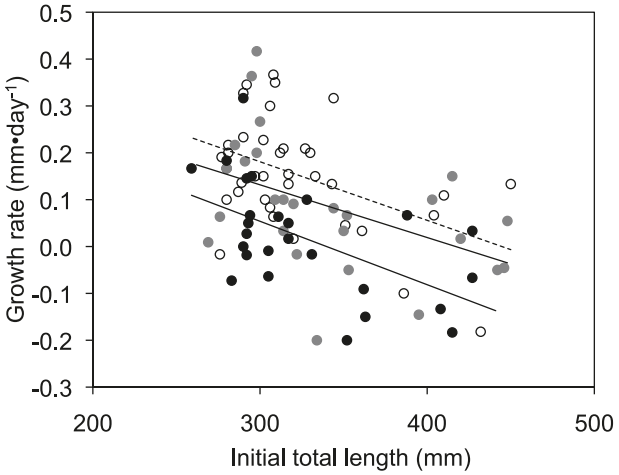
Note: Power analysis reveals that biologically relevant declines would be difficult to detect given the small sample size.

control fish was 79 ± 2 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$, compared with 93 ± 2 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ for cortisol-treated fish; this represented an 18% increase in SMR, on average, as a result of cortisol elevation.

Bioenergetics modelling

During the 60 d growth period between June and August, at a mean water temperature of 24 °C, the control fish grew from an initial mean mass of 459.1 g (calculated using the mass–TL relationship from the TL of 320.1 mm) to a mean final mass of 497.7 g (calculated from the TL of 329.2 mm). To achieve this level of growth, it was estimated in the bioenergetics model that individuals would have to have consumed on average 373.6 g of fish prey, with 33% of consumed food allocated to growth. Applying the same growth and consumption rates over the same period to the cortisol-treated fish revealed that these fish (with an initial mean mass of 529.2 g calculated from the TL of 333.5 mm) would have needed to consume on average 410.9 g of prey. Assuming piscivory, food would provide 4000 $J \cdot g^{-1}$. Taking into account the increase in SMR, and assuming that SMR remained increased for only the 6 d period of cortisol elevation, the model predicted that the cortisol-treated fish should have achieved a final mass of 568.7 g. From our growth data, we calculated from the mass–TL relationship that the control fish achieved a final mass of only 530.9 g (from the TL of 334.3 mm). Thus, the bioenergetics modelling revealed that either the metabolic rate of the cortisol-treated fish must have remained elevated beyond the 6 d period of cortisol elevation, or there must have been an additional mechanism causing the growth depression, such as a reduction in foraging, or sustained metabolic costs as a result of the transient stressor.

Fig. 1. Growth rate (change in total length (TL) in mm per day) plotted as a function of initial TL for cortisol-treated ($n = 26$), sham-treated ($n = 31$), and control ($n = 37$) largemouth bass. Cortisol-treated fish are represented by black-filled circles, sham-treated fish are represented by the grey-filled circles, and control fish are represented by the open circles. The mean growth rate for the cortisol-treated fish was lower than the mean growth rate for the sham-treated and control fish (Tukey's honestly significant difference (HSD) post-hoc test following a significant analysis of covariance (ANCOVA); model $F_{[5,84]} = 9.69$, $p < 0.001$; treatment group $F_{[2,2]} = 9.94$, $p < 0.001$). As expected, initial TL was also important in predicting growth over the season (initial TL $F_{[1,1]} = 26.89$, $p < 0.001$), but as there were no differences in the mean TL among the treatment groups at the onset of the experiment, this does not account for the documented difference in growth rates among the groups. The interaction term was not significant (interaction $F_{[2,2]} = 0.01$, $p = 0.98$).



The potential population-level consequences of stress-induced growth depression

Decreased somatic growth resulting from short-term cortisol implants affects age-specific survival and age-specific fecundity, which is predicted to result in a decrease in population growth rate λ of a prototypical largemouth bass population (Fig. 3*a*). For example, in our model, a 50% decline in somatic growth rate of all individuals of the population in a single growing season is predicted to result in a 14% decrease in population growth rate, while a 99% decline in somatic growth rate is predicted to result in a 37% decrease in population growth rate. In the present experiment, cortisol-treated fish showed an 89% depression in growth rate over the growing season compared with sham-treated fish (Fig. 1). When all the fish of the population would experience a similar level of stress, the decline of population growth rate as a result of such growth depression over a single growing season would be 23%. Since most stressors do not affect all individuals equally, we also modelled the percentage of the population that would need to experience growth depression to see biologically interesting reductions in population growth rate. We found that to experience a 5% and 10% reduction in population growth, 19% and 39% of the largemouth bass population, respectively, would need to experience stress causing the growth depression documented in the empirical component of this study (Fig. 3*b*).

Table 4. Plasma biochemistry (mean ± SE) of cortisol-treated (n = 8), sham-treated (n = 8), and control (n = 6) wild largemouth bass prior to treatment in June.

Parameter	Control	Sham	Cortisol	F statistic	Error df	p value
Aspartate transaminase ($U \cdot L^{-1}$)	40±12.2	46±10.6	38±10.6	0.14	19	0.87
Ca^{2+} ($mmol \cdot L^{-1}$)	3.4±0.08	3.5±0.07	3.5±0.07	0.67	19	0.52
Cl^{-} ($mmol \cdot L^{-1}$)	112±3.3	123±2.9	119±2.9	3.08	19	0.07
Cholesterol ($mmol \cdot L^{-1}$)	15±1.8	13±1.6	17±1.6	1.57	19	0.23
Glucose ($mmol \cdot L^{-1}$)	2.5±0.10	2.6±0.09	2.4±0.09	1.25	19	0.31
Mg^{2+} ($mmol \cdot L^{-1}$)	1.2±0.05	1.3±0.04	1.2±1.04	1.79	19	0.19
P^{3-} ($mmol \cdot L^{-1}$)	2.4±0.19	2.6±0.16	2.4±0.16	0.41	19	0.67
K^{+} ($mmol \cdot L^{-1}$)	3.0±0.3	3.4±0.2	3.5±0.2	0.82	19	0.45
Na^{+} ($mmol \cdot L^{-1}$)	167±3.8	178±3.3	174±3.3	2.45	19	0.11
Total protein ($g \cdot L^{-1}$)	40±2.0	42±1.7	42±1.7	0.24	19	0.79
Triglycerides ($mmol \cdot L^{-1}$)	3.9±0.63	2.8±0.55	2.6±0.55	1.22	19	0.32

Note: There are no statistical differences in plasma biochemistry among the groups (one-way analysis of variance, ANOVA; α = 0.05).

Table 5. Plasma biochemistry (mean ± SE) of cortisol-treated (n = 15), sham-treated (n = 15), and control (n = 14) wild largemouth bass 54–63 d after treatment, in August.

Parameter	Control	Sham	Cortisol	F-statistic	Error df	p value
Aspartate transaminase ($U \cdot L^{-1}$)	37±6.4	56±6.4	37±6.7	2.97	41	0.06
Ca^{2+} ($mmol \cdot L^{-1}$)	3.2±0.06	3.3±0.06	3.3±0.06	0.36	41	0.70
Cl^{-} ($mmol \cdot L^{-1}$)	**114±2.9**ab	**120±2.9**a	**109±3.0**b	**3.57**	**41**	**0.04**
Cholesterol ($mmol \cdot L^{-1}$)	12±0.8	12±0.8	12±0.8	0.02	41	0.98
Glucose ($mmol \cdot L^{-1}$)	2.4±0.12	2.7±0.12	2.6±0.12	1.67	41	0.20
Mg^{2+} ($mmol \cdot L^{-1}$)	2.3±0.11	2.5±0.11	2.4±0.11	0.63	41	0.54
P^{3-} ($mmol \cdot L^{-1}$)	2.3±0.11	2.5±0.11	2.4±0.11	0.63	41	0.54
K^{+} ($mmol \cdot L^{-1}$)	2.6±0.31	2.6±0.31	2.4±0.32	0.10	41	0.90
Na^{+} ($mmol \cdot L^{-1}$)	166±1.8	170±1.8	167±1.9	1.41	41	0.26
Total protein ($g \cdot L^{-1}$)	39±1.0	39±1.0	41±1.1	1.96	41	0.15
Triglycerides ($mmol \cdot L^{-1}$)	2.7±0.40	1.9±0.40	1.9±0.42	1.19	41	0.31

Note: Bold text indicates variables for which statistically significant differences were detected; treatment groups that differed are indicated with different letters (one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post-hoc tests; α = 0.05).

Table 6. Plasma biochemistry (mean ± SE) of cortisol-treated (n = 7), sham-treated (n = 10), and control (n = 10) wild largemouth bass 104–115 d after treatment (October).

Parameter	Control	Sham	Cortisol	F-statistic	Error df	p value
Aspartate transaminase ($U \cdot L^{-1}$)	30±11.9	57±11.9	40±14.2	1.26	24	0.30
Ca^{2+} ($mmol \cdot L^{-1}$)	3.5±0.12	3.6±0.12	3.3±0.14	1.51	24	0.24
Cl^{-} ($mmol \cdot L^{-1}$)	114±4.7	100±4.7	125±5.7	3.04	24	0.07
Cholesterol ($mmol \cdot L^{-1}$)	13±1.4	14±1.4	12±1.7	0.67	24	0.52
Glucose ($mmol \cdot L^{-1}$)	**2.2±0.19**ab	**2.8±0.19**a	**2.0±0.23**b	**4.55**	24	**0.02**
Mg^{2+} ($mmol \cdot L^{-1}$)	1.4±0.05	1.4±0.05	1.3±0.06	0.24	24	0.79
P^{3-} ($mmol \cdot L^{-1}$)	2.4±0.12	2.3±0.12	2.4±0.14	0.09	24	0.91
K^{+} ($mmol \cdot L^{-1}$)	2.1±0.37	2.0±0.37	2.6±0.45	0.66	24	0.52
Na^{+} ($mmol \cdot L^{-1}$)	173±1.9	170±1.9	171±2.3	0.56	24	0.58
Total protein ($g \cdot L^{-1}$)	39±1.4	40±1.4	38±1.7	0.66	24	0.53
Triglycerides ($mmol \cdot L^{-1}$)	2.5±0.71	2.1±0.71	3.6±0.84	1.00	24	0.38

Note: Bold text indicates variables for which statistically significant differences were detected; treatment groups that differed are indicated with different letters (one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post-hoc tests; α = 0.05).

Discussion

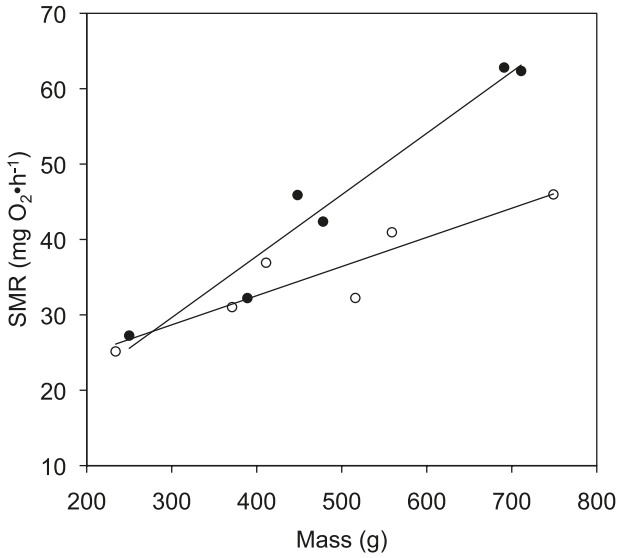
We found that a short-term cortisol implant resulted in changes in the long-term growth rate of wild largemouth bass in a natural lake. In the field, we found few long-term changes in plasma biochemistry, and no changes in plasma biochemistry that were consistent with complete fasting, as indicated by the complementary laboratory study. In exploring other potential mechanisms of the stress-induced growth rate depression, we found in the laboratory that metabolic rate was elevated during the period of cortisol elevation. However, bioenergetics modelling suggested that this increased metabolic rate was not sufficient to explain the observed growth depression in the field if metabolic rate

Table 7. Plasma biochemistry (mean ± SE) of fed and fasted wild largemouth bass in the laboratory.

Parameter	Fed	Fasted	*t* ratio	df	*p* value
Aspartate transaminase ($U \cdot L^{-1}$)	37±19	57±19	0.73	8	0.48
Ca^{2+} ($mmol \cdot L^{-1}$)	3.2±0.1	3.2±0.1	0.09	8	0.92
Cl^{-} ($mmol \cdot L^{-1}$)	75±5	88±5	1.89	8	0.09
Cholesterol ($mmol \cdot L^{-1}$)	12±1	15±1	1.74	8	0.11
Glucose ($mmol \cdot L^{-1}$)	9.9±1.0	6.8±1.0	–2.13	8	0.06
Mg^{2+} ($mmol \cdot L^{-1}$)	1.3±0.0	1.3±0.0	–0.66	8	0.53
P^{3-} ($mmol \cdot L^{-1}$)	1.6±0.1	1.9±0.1	1.79	8	0.11
K^{+} ($mmol \cdot L^{-1}$)	2.8±0.3	3.2±0.3	0.85	8	0.42
Na^{+} ($mmol \cdot L^{-1}$)	**145±2**	**153±2**	**2.86**	**8**	**0.02**
Total protein ($g \cdot L^{-1}$)	49±2	47±2	–0.77	8	0.46
Triglycerides ($mmol \cdot L^{-1}$)	**2.6±0.4**	**0.6±0.4**	**–3.59**	**8**	**<0.01**

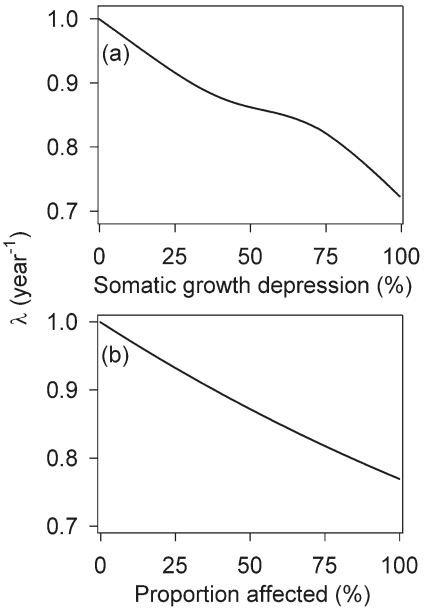
Note: Bold text indicates statistical differences using Student's *t* tests ($\alpha = 0.05$).

Fig. 2. Whole-animal standard metabolic rates depicted as a function of mass and treatment group (control and cortisol-treated fish; $n = 6$ in each group). Cortisol-treated fish are represented by filled circles, while control fish are represented by open circles. Mass, treatment group, and the interaction effect were all significant (model $F_{[3,8]} = 46.07$, $p < 0.001$; mass $F_{[1,1]} = 98.67$, $p < 0.001$; treatment $F_{[1,1]} = 20.20$, $p < 0.01$; interaction $F_{[1,1]} = 12.54$, $p < 0.01$; $\alpha = 0.05$).



elevation is maintained only while circulating cortisol is elevated. Therefore, growth depression is likely to be explained by other mechanisms such as energy loss resulting from coping with the stressor (i.e., metabolic costs are sustained beyond the elevation of plasma cortisol), or a reduction in foraging that was not captured using our measured biochemical indices. While we found no differences in recapture rates among the various treatment groups, suggesting no differences among groups in mortality across the summer, the power of our study was low, and results were thus inconclusive. However, even without differences in mortality rate among the groups, growth depression resulting from short-term stress was found to potentially alter long-term population growth rate as indicated by a complementary theoretical population modelling exercise. For a reduction in population growth rate to occur, a substantial fraction of the largemouth bass population would need to experience a stress event causing individual growth depression.

Fig. 3. The demographic cost of endocrine stress estimated by Leslie matrix projection modelling. (*a*) Estimated population rates of increase (λ) plotted against hypothetical somatic growth depression, with all individuals of the population being assumed to experience the same level of growth depression. (*b*) Estimated population rates of increase (λ) plotted against the proportion of population experiencing an 89% somatic growth depression for a single growing season, which corresponds to the empirical estimate.



Mechanisms of long-term growth suppression

The results of our study demonstrate that a cortisol hormone implant constitutes a challenge from which fish are unable to fully recover during a single growing season. We also found that the elevation of circulating cortisol was associated with an increase in SMR. This result was consistent with previous studies on laboratory rainbow trout (*Oncorhynchus mykiss*), where elevations of plasma cortisol have been associated with an increase in SMR (Morgan and Iwama 1996). Furthermore, this is consistent with previous studies examining chronic stressors, where both social stress in rainbow trout (Sloman et al. 2000) and chronic stress in green sturgeon (*Acipenser medirostris*) have been shown to increase SMR (Lankford et al. 2005), and is consistent with the prevalent notion that elevated cortisol in fish is associated with an

increase in catabolic activity and energy use (De Boeck et al. 2001). However, our bioenergetics modelling suggested that a short-term increase in SMR (i.e., an increase during only the 6 d period of cortisol elevation) would not be sufficient to account for the observed long-term differences in growth rate among treatments without some concurrent additional mechanism such as a reduction in feeding.

Stress in largemouth bass reduces feed intake (Siepker et al. 2006), and administration of exogenous cortisol hormone implants has also been shown to reduce appetite in rainbow trout (Gregory and Wood 1999). We did look at fasting and its biochemical correlates, but the most noteworthy finding was that there were very few changes either between the fed and fasted fish in the laboratory or among the cortisol-treated, sham-treated, and control fish over the 114 d monitoring period in the wild. These data suggested that wild fish defend physiological homeostasis in the measured plasma parameters despite fasting. However, stability of biochemical profiles does not exclude the possibility that feed intake of cortisol-treated fish was reduced relative to control fish, potentially explaining the growth depression observed in the field. Further studies that incorporate different response metrics of body condition and feeding history, and more detailed behavioural studies are necessary to understand the potential long-term changes in foraging behaviour as a result of elevated circulating plasma cortisol in largemouth bass and other fish in the wild.

The combination of results from the present study revealed that transient elevations of cortisol carry both immediate energetic costs and long-term growth costs. In addition to the possibilities investigated in the present study of cortisol-induced changes in feeding behaviour and metabolic rate, other factors may have contributed to the long-term impairment of growth by transient cortisol elevation. Both stress and experimental elevations of plasma cortisol are associated with reductions in immune function and increases in disease susceptibility (Wendelaar-Bonga 1997; Barton 2002; Greenberg et al. 2002), and it is possible that compensating for the long-term challenges associated with disease constitutes a persistent energetic cost that could also help to explain the differences in growth detected among the treatment groups in our study. Stress and experimental elevations of plasma cortisol are also associated with complex behavioural changes, including changes in social dominance (Barton 2002; Greenberg et al. 2002). Previous research looking at behavioural metrics found rapid resubmission of normal behavioural patterns after a single stress event in wild fish (e.g., resumption of normal behaviour in northern pike, *Esox lucius*, following a catch-and-release angling event; Klefoth et al. 2008; Arlinghaus et al. 2009). Similarly, previous research in Warner Lake indicates that largemouth bass treated with cortisol have similar activity levels when compared with sham-treated and control fish (O'Connor et al. 2010). However, cortisol-treated fish in the current study may have been altering their behaving on a fine scale in a way that also increased their energetic expenditure beyond the cessation of the cortisol hormone implant. Further research that examines the long-term metabolic costs of short-term cortisol elevation is necessary to fully understand the energetic costs of a short-term stressor in wild, free-swimming animals. Particularly useful would be research that incorporates detailed behavioural data to more thoroughly explore the feeding behaviour and activity rates of fish that have been exposed to a transient stressor or to transient cortisol elevation.

Potential consequences of individual physiological stress for the population

The results of the present study did not provide any evidence of differing mortality rates among the treatment groups. However, the power to detect biologically meaningful differences in mortality rates among the groups was low. Yet the long-term energetic costs, particularly the persistent growth costs, might have implications for the long-term survival and reproductive output of fish, and even population growth rates, without an immediate increase in mortality. Mortality rates in this species are size dependent, and female fecundity and female preferences scales with male size (Dunlop et al. 2007). Our population modelling was used to explore potential scenarios of how individual growth depression might affect population growth rate, and suggested that somatic growth depression caused by endocrine stress could result in a substantial decrease in population growth. This theoretical modelling exercise suggests that the experience of sublethal stress in wild fish has the potential to reduce long-term survival and lifetime reproductive out, and affect population growth rate, as has been shown in laboratory populations (Edeline et al. 2009). It is important to note that our population model indicated that a substantial fraction (39%) of the population would need to be exposed to a single stress event to cause a 10% reduction in population growth rate. Such wide-scale stress could conceivably occur as a result of environmental challenges such as oxygen depletion, or anthropogenic activities such as intense boating activity, or catch-and-release angling. It is important to also consider that in the model, growth was assumed to return to normal pre-stress levels after the single season of growth suppression. In reality, compensatory growth might occur (see Ali et al. 2003), and future studies monitoring fish over a longer period of time would be useful in determining when and to what degree this occurs.

Study limitations

The cortisol administered in our study generated supraphysiological concentrations of circulating cortisol (~2000 $ng \cdot mL^{-1}$) as reported elsewhere in detail (O'Connor et al. 2009). In the current study, the field setting precluded analysis of pilot samples prior to initiating the full experiment. The result was that the dose selected, based on literature for salmonid fish (Gamperl et al. 1994), elicited plasma cortisol concentrations in largemouth bass that were an order of magnitude higher than endogenous levels during a challenge (~300 $ng \cdot mL^{-1}$). However, despite the pharmacological dose, the increase in SMR with cortisol treatment was comparable in magnitude with previous studies where chronic stressors initiated the physiological response (Sloman et al. 2000; Lankford et al. 2005). Following a cortisol injection of the same dose in another study, largemouth bass exhibited a typical glucose response to elevated plasma cortisol (O'Connor et al. 2009), and no immediate mortality was observed in cortisol-treated fish (O'Connor et al. 2009, 2011). While acknowledging the methodological limitation of a supraphysiological dose, our examination of the long-term effects of cortisol elevation in

a wild free-swimming fish provides unique insight into the physiological mechanisms that drive whole-animal consequences of stress. The current study provides a first step in elucidating the nature of long-term consequences of stress in a wild fish population, and may inspire future research aimed at understanding the magnitude of effect caused by a single stressor of interest.

We used total length (TL) as a measure of fish size, and the increases in TL per day over the monitoring period were used as a measure of fish growth. The use of TL as a single measurement of growth is limited, because it does not take into account any measure of body condition (i.e., relative mass or girth per TL), which can also be affected by cortisol treatment (e.g., Barton et al. 1987). Furthermore, in our study there was no specific assessment of measurement error associated with taking measurements of total length (i.e., measuring the same fish multiple times during a single sampling period), or with other sources of variation (e.g., males versus females, or single versus double recapture). Given that decreases in TL were documented across the monitoring period in some individuals, it is clear that there was a level of error associated with taking TL measurements. However, we have no reason to suppose that sources of measurement error differentially affected a specific treatment group, and therefore we are confident that the differences among treatment groups reflect true differences, and not an anomaly caused by measurement error. Nonetheless, future studies should certainly include measurements of both TL and body mass and condition, as well as assessments of measurement error. Measurements of body condition would also yield more detailed insight into the potential for compensatory growth in a cortisol-treated fish (Ali et al. 2003).

A final limitation of our study relates to the population modelling exercise. Although our model illustrated the potential population-level effects of a single stress event affecting a portion of the target population, there are limitations to this model. We only used one process of density-dependence (i.e., density-dependence in growth), and we did not include density-dependent fecundity and mortality in the model because no data was available for largemouth bass. We also did not explicitly include size-dependent mate choice of males by females, because again, the data was not available for this species. Our model nevertheless served as a test of the impact of growth depression by holding the general population model constant and varying only the growth submodel. Thus, our predictions of population level effects of growth depression should hold qualitatively, although we cannot claim precise predictive power. Another limitation arises from modelling a single fish population closed to immigration, emigration, and fishing, and ignoring interspecies interactions. Interspecific interactions might accelerate or suppress the magnitude of the reduction of population growth rate in the target population. Finally, as the experimental data provided no information about the difference in growth depression between males and females, we assumed that both males and females experienced the same level of growth rate depression. The reduction of population growth rate shown in the present study essentially resulted from a reduction of lifetime reproductive success in females through decreased fecundity and increased mortality due to individual growth depression. Male largemouth bass provide nest-guarding parental care, and if the quality of the care depends on males' size (see Hanson and Cooke 2009), the reduction in male size might result in a further reduction of population growth rate.

To conclude, despite the limitations mentioned above, our study is the first to show growth rate depression after a single stress event in a wild free-swimming fish. Bioenergetics modelling suggests that mechanisms such as long-term metabolic costs or reduced feeding would account for this growth rate depression. Simple population modelling exercises suggest that if a substantial proportion of the population experiences such a stress event, the population-level growth rate will be affected. Across a range of taxa, there have been few studies that have studied or documented instances in which stress at the level of the individual cascades to influence population-level processes (Calow and Forbes 1998). Understanding the relationships between sublethal stressors and ecologically relevant measures such as individual or even population-level growth rates is critical to further understand the "ecology of stress" in wild fish, particularly given the level of environmental change and disturbance occurring in aquatic ecosystems.

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