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Effects of predator exposure on baseline and stress-induced glucocorticoid hormone concentrations in pumpkinseed *Lepomis gibbosus*

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Abstract

We compared baseline and maximal cortisol concentrations between predator exposure and prey blood samples in pumpkinseed *Lepomis gibbosus*, captured using a standardised fishing event underneath osprey *Pandion haliaetus* nests and away from osprey nests. We did not detect differences in cortisol or glucose between sites. These findings suggest that predictable sources of predation risk may not confer stress-related costs in teleosts.

KEYWORDS
cortisol, *Lepomis gibbosus*, predator, prey, risk, stress

Predator-prey interactions have become an increasingly important area of research for ecologists (Boonstra, 2013; Hawlena & Schmitz, 2010; Laundré *et al*., 2010; Lima & Dill, 1990). In teleosts specifically, the stress response is moderated in part by the hypothalamic–pituitary–interrenal (HPI) axis which is involved in the production and secretion of cortisol, the primary corticosteroid hormone in fishes (Mommsen *et al*., 1999; Schreck & Tort, 2016). Exposure of prey items to a predator can be considered a stressor (*i.e*., activation of the HPI axis; Barcellos *et al*., 2007; Woodley & Peterson, 2003), which often induce both behavioural (Creel *et al*., 2007; Godin, 1997; Møller *et al*., 2016) and physiological effects (Cooke *et al*., 2003; Lawrence *et al*., 2018a; Sunardi *et al*., 2007). Yet, individuals can temper or even eliminate the risk and thus any long-term physiological consequences of stress by simply leaving the area (Creel & Winnie, 2005; Godin, 1997; Lima & Dill, 1990). Predation risk may also correspond with other fitness-related trade-offs including suboptimal foraging and missed mating opportunities (Godin, 1997; Lima & Dill, 1990). Thus, predation risk represents an important component dictating animal spatial ecology and resource use patterns as well as influencing decision making (Gallagher *et al*., 2017; Godin, 1997; Lima & Dill, 1990).

Avian predation has been determined to influence the physiological, behavioural and decision-making dynamics in teleosts (Allouche & Gaudin, 2001; Cooke *et al*., 2003; Gallagher *et al*., 2016; Gotceitas & Godin, 1991). For example, Dill and Fraser (1984) found that the presence of an avian predation threat greatly modified feeding behaviours in coho salmon *Oncorhynchus kisutch* (Walbaum 1792). Additionally, simulated attacks by a model osprey *Pandion haliaetus* induced a rapid physiological and metabolic response in largemouth bass *Micropterus salmoides* (Lacépède 1802) (Cooke *et al*., 2003). Similarly, a model osprey flying overhead resulted in significant behavioural modifications in nesting pumpkinseed *Lepomis gibbosus* (L. 1758), where the majority of predator-exposed paternal fish temporarily abandoned their nests to find refuge away from the threat (Gallagher *et al*., 2016). These insights, along with others (Hill & Heck, 2015; Pepino *et al*., 2015; Steinmetz *et al*., 2003), suggest that avian predators may exert

All experiments were conducted in accordance with the standards set by the Canadian Council of Animal Care (CCAC) under authorisation from Carleton University's Animal Care Committee (AUP B15-12).

top-down influences on fish populations. However, the effects of avian predation on the physiological stress response of fish remains poorly characterised. Here, we explored the physiological consequences of exposure to nesting osprey predators on wild *L. gibbosus* fish. Specifically, we evaluated circulating concentrations of baseline and stress-induced cortisol and glucose, between fish captured in areas of high and low osprey exposure on a lake in Ontario, Canada. We hypothesised that, due to the exposure of a live avian predator, a desensitisation of the stress response would occur in fish living close to osprey nests. If this were true, we would predict that concentrations of basal cortisol in circulation would be lower (or muted) for fish near osprey nests, relative to areas of lower osprey exposure.

Ospreys are highly mobile fish predators and fishing grounds can be located both away from their nests (Alerstam *et al*., 2006) and within meters of their nests (links to videos in Appendix S1a–c). From 14 to 24 May 2015, we located three active coastal osprey nests (each containing a breeding pair), which were selected alongside paired control sites within 1 km of each other, in Lake Opinicon, a mesophotic lake located in eastern Ontario (44° 33′ 56.0″ N, 76° 19′ 23.6″ W). Nesting sites were located at the top of eastern white pine trees *Pinus strobus* situated on small islands separated by <10 m from the outer perimeter of the lake (except for one site which was *c.* 100 m). Control sites mimicked the habitats from nesting sites (*i.e.*, littoral edges) but were situated outside the visual detection range of the nesting sites. The average distance between nest and control sites was 0.51 km and the three nest-control sites covered a 5.9 km northeast transect covering nearly the entire area of the lake. While not recorded, water temperatures generally range between 15 and 20°C and are fairly unstable during this time of year.

We focused our efforts on *L. gibbosus* as a prey item, as they can constitute a major portion of the osprey diet (Steeger *et al*., 1992). Studies of the spatial ecology of *L. gibbosus* involving telemetry (Fish & Savitz, 1983; Savitz *et al*., 1983) and mark–recapture (McCairns & Fox, 2004) have revealed that long-range movements are uncommon, home range size is relatively small and they have high levels of site fidelity. For adult *L. gibbosus* (*i.e.*, all fish used in this study) in Lake Opinicon there would unlikely be piscine predators of sufficient size to prey on the size class of fish we used here (*c.* 20 cm total length; Werner & Hall, 1988). Fish-eating birds, small mammals (*e.g.*, otters, mink), and humans (*i.e.*, harvesting for food by fishing) would be the principal predators of adult *L. gibbosus* and we recognise that we could not have controlled this aspect of the study.

Adult *L. gibbosus* were caught at all sites using standardised fishing gear: 2 m long medium action fishing rods and reels with 2.7 kg breakstrength monofilament fishing line and size 6 non-offset small J-hooks baited with live worms. Once hooked, the following experimental protocol was executed for each fish: an initial blood sample (baseline) of *c.* 0.25 ml was collected by caudal puncture between 3 and 6 min after hooking using 1 ml pre-heparinized syringes with 25 G, 3.8 cm needles (BD; www.bd.com). A study by Lawrence *et al*. (2018b) indicated that baseline cortisol titres can be obtained if *L. gibbosus* are sampled within 8 min of hooking. However, variation in plasma cortisol titres begins to manifest in the sample as one approaches this threshold. As every attempt was made to capture the sample within this timeframe, our samples probably reflect values that are likely to be indicative of a baseline state. In support of this, our baseline cortisol values ranged between 1.28 and 49.9 ng ml^{-1}, which is similar to resting pre-stress levels found in other teleosts, including centrarchids (Barton & Iwama, 1991). If blood was not readily available, fish were immediately released overboard. Fish were then subjected to an additional period of 3–5 min of air exposure (inclusive of time needed to take the initial blood sample), a protocol that reliably induces physiological stress in *L. gibbosus* (Cook *et al*., 2012; Gingerich *et al*., 2007). All fish were then held in a 20 l cooler with fresh lake water for 45 min from when they were landed, allowing elevated glucocorticoid hormone concentrations to be achieved (following Cook *et al*., 2012). Fish were then removed and a second blood sample was collected as above. For both baseline and stressed blood samples, blood glucose levels were measured immediately using a small aliquot (0.05 ml) of whole blood with a hand-held portable glucose meter previously validated for use in fish (Accu-check glucose meter, Roche Diagnostics; www.accu-chek.com; Stoot *et al*., 2014). Remaining blood samples were then kept on ice (>2 h) until further processing onshore. Onshore, blood was centrifuged (3 min at 2000 *g*; Mandel Mini Centrifuge, Mandel Scientific www.mandel.ca) with the resulting plasma fraction being decanted, flash frozen and stored (−80°C) for later analysis of plasma cortisol titres. Plasma cortisol concentrations were measured using a commercial radioimmunoassay kit (RIA; ImmuChem Cortisol Coated Tube RIA Kit, MP Biomedicals; www.mpbio.com) which has been validated for use in teleosts (Gamperl *et al*., 1994). This entire approach yielded paired blood glucose values and plasma cortisol concentrations (baseline, maximum stress-induced) per fish at osprey nest sites and control sites. We used generalised linear models (Gaussian distribution) to investigate the effects of exposure to osprey predators on the baseline and maximal blood glucose and plasma cortisol concentrations, as well as the responsiveness of each parameter (maximal *v.* baseline), with body size (fork length, L_F, cm) as a covariate. We only analysed fish where paired baseline and maximal values for either parameter were obtained. To evaluate whether size significantly differed across sampling sites or treatments, we used Kruskall-Wallis tests. To evaluate whether baseline *v.* maximal cortisol and glucose levels were significantly different, we used a Kruskall-Wallis test. All analyses were conducted in R (www.r-project.org) and significance was declared at $P < 0.05$.

Seventy-one fish were caught and sampled across six sites (three under active osprey nests; three control sites), yielding 142 initial–final linked samples for each of glucose and plasma cortisol. Mean (±SD) fish fork length was $L_F = 18.1 \pm 2.7$ cm, suggesting sampled individuals were all adults (Gallagher *et al*., 2016). Fish size did not significantly differ amongst sampling sites or between treatments (Kruskal-Wallis; $P > 0.05$ in each case). Baseline glucose levels were not affected by exposure to osprey nests ($t = 1.95$, $P > 0.05$; Figure 1a), nor fish size ($t = 0.39$, $P > 0.05$), but it was affected by sampling site ($t = -2.50$, $P < 0.05$). Maximal glucose levels were similarly unaffected by exposure to osprey nests ($t = 0.90$, $P > 0.05$; Figure 1b), sampling site ($t = 0.24$, $P > 0.05$), and fish size ($t = 1.52$, $P > 0.05$).

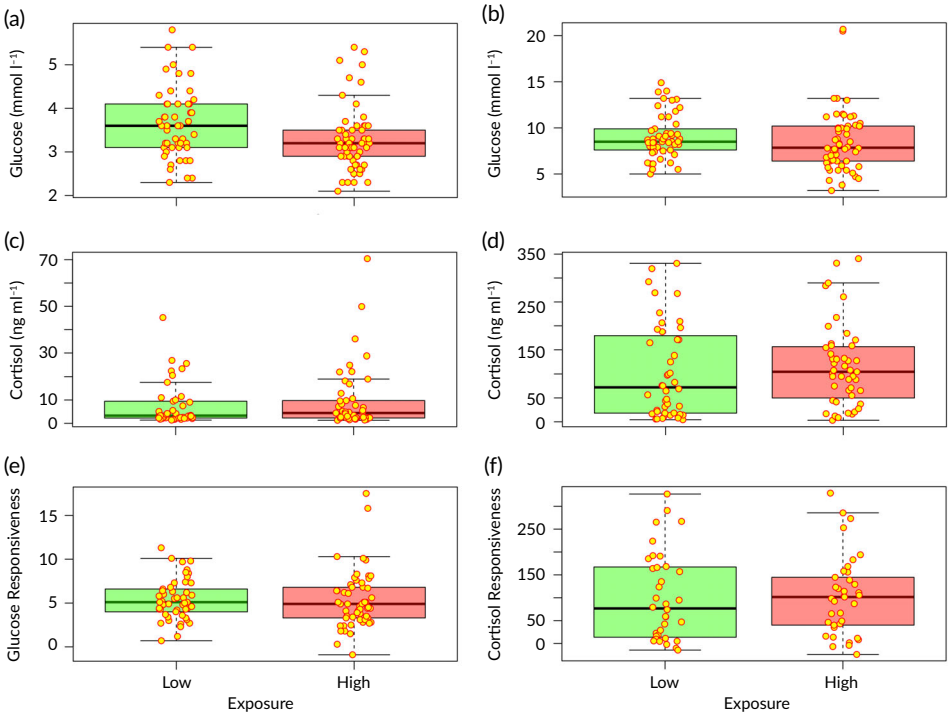

FIGURE 1 Boxplots (——, median; □, 25–75th percentiles; T, 95% range) showing the effects of varying predator exposure (osprey nest – high exposure *v.* no osprey nest – low exposure) on *Lepomis gibbosus*: (a) baseline glucose and (b) maximal glucose (mmol l^{-1}), (c) baseline cortisol and (d) maximal cortisol (ng ml^{-1}), (e) glucose and (f) cortisol responsiveness (maximal *v.* baseline)

Baseline plasma cortisol concentrations were not affected by exposure to osprey nests (t = 0.68, P > 0.05; Figure 1c), sampling site (t = −0.28, P > 0.05), nor fish size (t = −0.24, P > 0.05). Maximal plasma cortisol concentrations were not affected by exposure to osprey nests (t = 0.50, P > 0.05; Figure 1d) nor sampling site (t = 0.31, P > 0.05), although fish size did show a significant effect (t = −2.45, P > 0.05). Plasma cortisol concentrations increased significantly from baseline to maximal values across all fish (Kruskal-Wallis P > 0.05), however the cortisol responsiveness (maximum *v.* baseline) was not affected by exposure to osprey nests (t = 0.41, P > 0.05; Figure 1e) nor sampling sites (t = −3.43, P > 0.05), yet it was affected by fish size (t = −2.38, P < 0.05). Glucose levels increased significantly from baseline to maximal values across all fish (Kruskal-Wallis P < 0.05), although the glucose responsiveness (maximum *v.* baseline) was not affected by exposure to osprey nests (t = 0.40, P > 0.05; Figure 1f), sampling sites (t = 0.97, P > 0.05), nor fish size (t = 1.51, P > 0.05).

We had predicted that occupying an area near an active predator would constitute a chronic stressor resulting in an altered stress physiological profile. However, this was not the case as stress-related metrics of osprey-exposed and control fish were comparable. This result may be explained by the control-of-risk hypothesis, which states that predictable sources of predation risk are unlikely to result in stress-related costs (Creel, 2018). Instead, fish living near osprey nests may change their behaviour to mitigate risk, which could include altered foraging dynamics, differential refuge use and altered water-column profiles (Milinskim 1993; Godinm 1997). While the fine-scale behavioural–spatial dynamics were not assessed here, our work may add support to the general lack of consensus on a standard endocrine profile for chronically stressed wild animals particularly in the context of predator–prey interactions (Boonstra, 2013; Dickens & Romero, 2013). However, we are cautious in this interpretation as the relative risk of predation to *L. gibbosus* inhabiting regions near the osprey nest was not quantified here.

The influences of predation, including avian predation threats, on the physiology of fish is somewhat understood. Previous work has demonstrated that predatory threats resulting from bird foraging, including osprey, can induce a stress-like response at both physiological and behavioural levels of scale in teleost fishes (Cooke *et al*., 2003; Dill & Fraser, 1984; Gallagher *et al*., 2016; Gotcietas & Godin, 1991). We expected that the exposure to predators would modulate the responsiveness of the HPI axis in some manner. This would be advantageous as it prevents the negative consequences of continued stress axis induction during chronic stressor exposure (Romero *et al*., 2009). This effect has been widely characterised in teleost fishes. For example, killifish *Brachyrhaphis episcopi* (Steindachner 1878) individuals captured from higher predation risk areas exhibited a lower maximal cortisol response in relation to a simulated stressor when compared with their low-predation risk counterparts, which suggests an attenuation of the stress response under chronic predation risk (Archard *et al*., 2012). Similarly, *B. episcopi* caught from low predation risk areas exhibited higher breathing rates when exposed to predation risk stressors, when compared to their high-risk counterparts (Brown *et al*., 2005). This contrasts our findings and our predictions and initially suggests that there are probably no adverse physiological effects associated with *L. gibbosus* inhabiting waters near an avian predator's nest.

The lack of physiological response in our *L. gibbosus* may also be the result of the osprey not appearing in a threatening manner (*i.e.*, low-perceived predation risk). Movement from the predator, in a manner that is typical of an impending predation event (*e.g.*, diving, flying

directly overhead, *etc.*), may signal fish to mount an anti-predator behavioural (Gallagher *et al.*, 2016) and physiological responses (Cooke *et al.*, 2003; Sunardi *et al.*, 2007; Sundström *et al.*, 2005). Thus, if the osprey is simply nest-tending while in the vicinity, then anti-predator activities by *L. gibbosus* may not necessarily manifest with the osprey not serving as a chronic stressor in this particular context.

Contextual effects such as sex and or season are also factors to consider in interpreting *L. gibbosus* responsiveness. Our fish were collected just prior to their spawning period, which could conceivably affect the operation of the HPI axis (Carruth *et al.*, 2000; Leatherland *et al.*, 2010) and affect our results in a way outside of our control (*e.g.*, higher baseline cortisol titres in pre-spawning fish). As well, other factors such as sex (Cook *et al.*, 2012), inherent individual-level variation in HPI-axis responsiveness (*i.e.*, high *v.* low responders; Øverli *et al.*, 2002) and other physiological traits (*e.g.*, body condition, parasite burden, feeding status, *etc.*) may have influenced the baseline glucose and cortisol levels observed in this study. As this study did not take lethal samples, the sex of individual fish could not be determined and we recognise this as a shortcoming. We did, however, detect a subtle difference in baseline glucose levels across sampling sites, independent of treatment type and size effects on maximal plasma cortisol concentrations and the cortisol responsiveness, suggesting that there may be some level of intraspecific variation here.

Physiological stress is viewed as a fundamental link between predation and the function of entire ecosystems (Hawlena & Schmitz, 2010). Our preliminary work suggests that, in the particular context, living close to predators may not actually confer physiological changes associated with perceived chronic stressors in *L. gibbosus*. However, given the lack of responsiveness, further work is needed to address the role of predators in constituting a chronic stressor in a wild setting, while accounting for how intraspecific variation in the population drives these effects. Presumably, these processes act on a continuum through which the strength of interaction may be a driving influence by which prey species respond to predation threats.

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CONFLICTS OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

A.J.G. and S.J.C. generated the idea for the manuscript. A.J.G., M.J.L., S.J.S., A.D.W., S.J.C. generated the data. A.J.G. and M.J.L. analysed the data. All authors prepared the manuscript and approved of its submission.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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