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Consequences of acute stress and cortisol manipulation on the physiology, behavior, and reproductive outcome of female Pacific salmon on spawning grounds

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ABSTRACT

Life-history theory predicts that stress responses should be muted to maximize reproductive fitness. Yet, the relationship between stress and reproduction for semelparous salmon is unusual because successfully spawning individuals have elevated plasma cortisol levels. To tease apart the effects of high baseline cortisol levels and stress-induced elevation of cortisol titers, we determined how varying degrees of cortisol elevation (i.e., acute and chronic) affected behavior, reproductive physiology, and reproductive success of adult female pink salmon (*Oncorhynchus gorbuscha*) relative to different states of ovulation (i.e., ripe and unripe). Exhaustive exercise and air exposure were applied as acute stressors to manipulate plasma cortisol in salmon either confined to a behavioral arena or free-swimming in a spawning channel. Cortisol (eliciting a cortisol elevation to levels similar to those in post-spawn female salmon) and metyrapone (a corticosteroid synthesis inhibitor) implants were also used to chemically manipulate plasma cortisol. Cortisol implants elevated plasma cortisol, and impaired reproductive success; cortisol-treated fish released fewer eggs and died sooner than fish in other treatment groups. In contrast, acute stressors elevated plasma cortisol and the metyrapone implant suppressed plasma cortisol, but neither treatment significantly altered reproductive success, behavior, or physiology. Our results suggest that acute stressors do not influence behavior or reproductive outcome when experienced upon arrival at spawning grounds. Thus, certain critical aspects of salmonid reproduction can become refractory to various stressful conditions on spawning grounds. However, there is a limit to the ability of these fish to tolerate elevated cortisol levels as revealed by experimental elevation of cortisol.

Introduction

Considerable evidence supports the notion that stress can impair the reproductive outcome of a wide range of vertebrates, including birds (Silverin, 1997; Wingfield, 1988), reptiles (DeNardo and Sinervo, 1994a, 1994b), mammals (Negro-Vilar, 1993; Boonstra et al., 1998), and fish (Pickering et al., 1987; Schreck et al., 2001). The acute stress response and associated elevation of glucocorticoids is believed to be adaptive, while chronic elevation of glucocorticoids can have various negative tertiary effects, including impaired immune function and fitness whenever resources are directed towards an emergency response (Barton and Iwama, 1991; Wingfield et al., 1998; Barton, 2002; Wingfield, 2003) and animals attempt to regain allostasis (Wingfield, 2003; Schreck, 2010). Yet, much of the existing work on chronic stress/glucocorticoid elevation is focused on the long-term consequences for animals during non-reproductive periods rather than immediately before or during reproduction. For example, many toxicological studies demonstrate direct long-term reproductive impairments (e.g., suppression of reproductive hormones) associated with emergency resource reallocation to maintenance and survival (e.g., reviewed in Van Der Kraak et al., 1998; see also Jardine et al., 1996; Janz et al., 1997; Bowron et al., 2009). Furthermore, most of these studies consider iteroparous species (i.e. repeat breeders), which have the life-history option of delaying a reproductive event when challenged.

In contrast, semelparous species usually cannot delay the reproductive event because they invest in reproduction only once in a lifetime. For semelparous fishes such as Pacific salmonids (*Oncorhynchus*

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spp.), some argue that the spawning date is genetically fixed, which implies that it cannot be altered by external stressors (Quinn et al., 2000). Curiously, virtually nothing is known about whether exposing semelparous Pacific salmonids to stress on spawning grounds influences their behavior and reproductive success. Yet, these fish routinely encounter many stressors that trigger a cortisol response as they approach their spawning date, suggesting that the acute stress response remains active during the reproductive period. For example, plasma cortisol rises when fish encounter hydraulic challenges and elevated water temperature during the spawning migration (Hinch et al., 2006; Mathes et al., 2010). Furthermore, a progressive increase in baseline plasma cortisol levels of unknown etiology occurs as salmon swim to the spawning grounds (Robertson and Wexler, 1959; McBride et al., 1986; Tierney et al., 2009; Hruska et al., 2010). Plasma cortisol concentrations rise from ~25 ng ml^{-1} in pink salmon (*O. gorbuscha*) at river entry (McBride et al., 1986), to ~350 ng ml^{-1} on arrival at the spawning ground (female sockeye salmon [*O. nerka*]; Hruska et al., 2010), and ~1287 ng ml^{-1} when the fish become moribund (female sockeye salmon; Hruska et al., 2010). Thus, an acute stressor can elevate plasma cortisol against a background of progressively increasing plasma cortisol levels during the spawning migration.

A stressed state should generally be incompatible with reproduction and, based on life-history theory, one could postulate that the cortisol stress response of semelparous salmon should be muted, or physiologically irrelevant, during this period (Wingfield and Sapolsky, 2003) to mitigate any potential negative effects of cortisol elevation above the (high) baseline levels on spawning grounds. Thus, we postulate that reproductive drive in a semelparous salmon species will outweigh any cortisol-mediated mating inhibition. Acute, stress-related increases in plasma cortisol suppress the normal increases in plasma sex hormone concentrations for Pacific salmon during early phases of upriver migration (Dye et al., 1986). However, increases in plasma cortisol during migration are regarded as adaptive and necessary for salmon to be able to return to their natal streams and spawn (Carruth et al., 2002). Complicating matters is the fact that spawning Pacific salmon also undergo senescence, which alters many physiological processes, including hormone regulation (Morbey et al., 2005; Hruska et al., 2007, 2010). To address these issues, we experimentally determined how short-term changes in and experimental manipulation of plasma cortisol influenced the reproductive physiology, behavior, and spawning outcome of wild female pink salmon (*O. gorbuscha*). We administered cortisol implants and predicted that plasma cortisol elevation, lasting between 2 and 5 days, would negatively affect reproductive behavior (e.g., less time spent guarding eggs or fighting for a mate), physiology (i.e., suppression of reproductive hormones), and outcome (i.e., number of eggs released). We also predicted that the response to acute stressors (i.e., exhaustive exercise or air exposure) would be muted in semelparous salmon and would not alter these same responses. Conversely, an intraperitoneal (IP) implant of metyrapone, which blocks the last step of glucocorticoid synthesis, was expected to lower plasma cortisol levels (Doyon et al., 2006) and retard reproduction and senescence. To our knowledge, hormone manipulations of this type had not before been performed on senescing Pacific salmon.

Materials and methods

Metyrapone validation

All fish were handled in accordance with the guidelines of the Canadian Council on Animal Care (Carleton University, B09-12; University of Ottawa, BL-228). A pilot laboratory experiment was carried out to determine the effectiveness of metyrapone (2-methyl-1, 2-di-3-pyridyl-1-propanone; Sigma 85625, Sigma-Aldrich) at blocking cortisol synthesis when delivered in a cocoa butter implant. Metyrapone successfully blocks cortisol synthesis in fish in the short-term (<24 h: e.g., Hopkins et al., 1995; Milligan, 2003; Rodela et al., 2009), but has rarely been used with a cocoa butter carrier (but see Doyon et al., 2006). Rainbow trout (*O. mykiss*), a congeneric of pink salmon, weighing approximately 150 g were anesthetized with benzocaine (0.05 mg ml^{-1} water; p-aminobenzoic acid ethyl ester; Sigma E1501, Sigma-Aldrich) and given an IP injection of metyrapone mixed in heated liquid cocoa butter (200 mg kg^{-1} fish in l ml cocoa butter kg^{-1} fish); upon injection into the fish, the cocoa butter rapidly cools to a thick paste, providing a slow-release metyrapone implant. After 1 and 5 days, fish were subjected to 1 min of air exposure as an acute stressor, and a blood sample was withdrawn by caudal puncture 30 min later for assessment of plasma cortisol levels. The expectation was that this 30-min delay would be sufficient for the maximum or near maximum rise in plasma cortisol level to be manifested (Gilmour et al., 2005).

Weaver Creek spawning channel

All field experiments were conducted at the Weaver Creek spawning channel located in British Columbia, Canada (see Hruska et al., 2010 for detailed information). Each experiment involved groups of naive fish (i.e., fish were not reused among experiments). The artificial channel, 2.93 km long and 6.1 m wide, is composed of a cobble (1.2–7.6 cm) substrate and has a consistent water depth of 25–30 cm. Fish densities and flow conditions were monitored throughout the spawning period and manually operated gates were used to regulate fish movements into the spawning channel (Hruska et al., 2010). Experiments were timed to coincide with peak pink salmon spawning activity in early October 2009.

Reproductive physiology on arrival

On arrival at the spawning channel in early October, female pink salmon were individually removed from the raceway via dip net and immediately placed in a trough supplied with flow-through water from the raceway. Fish were categorized as either “unripe” (N = 52, unovulated, where eggs are still confined to intact ovaries) or “ripe” (N = 60, ovulated, where eggs have been released into the body cavity and gentle abdominal pressure near the vent easily expels eggs) and a blood sample was collected via caudal puncture (2 ml blood sample; collected using 3 ml vacutainer and 1.5 in., 18 ga needle, lithium heparin; Becton Dickson, NJ) within 30 s (Cooke et al., 2006). Within 3 min the fish were released back into the spawning channel. Blood samples were stored in an ice-water slurry and centrifuged (5 min at 10,000 *g*) within 45 min, after which the plasma was frozen in liquid nitrogen immediately. Samples were subsequently stored at −80 °C until further analysis.

In addition, subsets of ripe (N = 6) and unripe (N = 12) salmon were given an intraperitoneal (IP) injection of either cortisol (hydrocortisone 21-hemisuccinate; Sigma H4881, Sigma-Aldrich; 110 mg kg^{-1} fish in 50 ml melted cocoa butter kg^{-1} fish; Dibattista et al., 2005) to elevate cortisol levels for a short period (i.e., 2 to 5 days), or metyrapone (200 mg kg^{-1} fish; 1 ml cocoa butter kg^{-1} fish) to block glucocorticoid synthesis (Mommsen et al., 1999), before being placed in individual, opaque, experimental chambers (~50 l) situated on the bank of the channel and equipped with flow-through water. Fish were left undisturbed for approximately 24 h, after which they were individually removed and blood was sampled immediately via caudal puncture.

Longevity and reproductive status study

On October 6th and 7th 2009, 120 unripe pink salmon that had voluntarily entered the raceway were marked with unique individual Peterson disk tags placed in the dorsal musculature. The tags could be read on free-swimming fish with binoculars, which allowed the fish