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Cortisol profiles in sockeye salmon: Sample bias and baseline values at migration, maturation, spawning, and senescence

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ABSTRACT

Accurate and controlled methods to measure physiological stress are crucial to effectively monitor and assess the health of wildlife populations and evaluate resilience to external stressors. Glucocorticoids, particularly cortisol, are frequently used to measure stress in fish. While measurements of cortisol concentrations provide a powerful indicator of physiological stress, there are important considerations in accurately measuring and interpreting results. We assessed methods to capture and sample wild populations of salmonids and evaluated potential biases from sampling disturbance. We present results of a stress series and suggest approaches to mitigate bias associated with sampling disturbance. Studies on physiological stress in salmonids often focus on particular life stages (e.g. outward migration to marine waters, return migration to freshwater systems), or processes (e.g. fisheries interactions, spawning success), characterized by dramatic physiological challenges related to the developmental stage of the fish and the external environment. Such pressures influence baseline cortisol levels and complicate efforts to interpret the effects of additional external stressors. We present a profile for naturally occurring shifts in cortisol levels at migration, reproductive maturation, spawning, and senescence. This profile provides a crucial baseline for use as reference in evaluating physiological stress in Pacific salmon during crucial life stages. Our findings provide guidance for sampling wild salmonids and highlight the need for caution in interpreting cortisol in the context of physical challenges and physiological developments relevant to their complex life history.

1. Introduction

Despite extensive research on physiological stress in captive stocks of salmonids, relatively few studies have analyzed stress in wild stocks, due to difficulty sampling and controlling environmental effects. Extensive natural and anthropogenic stressors challenge wild salmon populations, including interactions with commercial and recreational fisheries, physical impediments to migration, pollutants, habitat loss, natural shifts in habitat and conditions, oscillations and variability in climate, and anthropogenic climate change. Throughout much of their historical range, salmon populations are listed as threatened or endangered (Ford et al., 2010). Means to accurately measure and effectively interpret blood chemistry to assess effects of stressors have direct utility toward effective management and monitoring of salmon populations (Cooke et al., 2012). We evaluate methods for capture and extraction of blood from wild fish, while minimizing sampling bias. We also provide baseline values for cortisol concentrations in sockeye salmon (*Oncorhynchus nerka*) at various stages of reproductive maturity to inform analyses of external stressors in the context of migration, reproductive maturation, spawning, and senescence.

Whether anthropogenic or naturally occurring, external stressors may have profound implications for the health and dynamics of fish stocks and the persistence, conservation, rebuilding, and management of wild populations (Pankhurst and Van Der Kraak, 1997). Both acute (Maule and VanderKooi, 1999) and chronic (Pickering and Pottinger, 1989) stressors have been shown to have detrimental effects on fish. Physiological stress may alter behavior (Schreck et al., 1997; Wingfield and Ramenofsky, 1999), lower immune responses (Balm, 1997; Maule et al., 1989), increase metabolic costs (Pankhurst and Van Der Kraak, 1997), and impair responses to physical challenges (Davis, 2006). Physiological stress may also inhibit sexual maturation (Carragher et al., 1989; Baker et al., 2013a) and reproductive function (reviewed in Pankhurst and Van Der Kraak, 1997; Schreck, 2010). Repeated exposure to acute stressors and prolonged exposure to chronic stressors disrupt endocrine processes (Sumpter et al., 1987) and may decrease gamete quality

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and reduce larval viability (Campbell et al., 1992; Kubokawa et al., 1999).

Cortisol is the major corticosteroid in salmonids (Billard and Gillet, 1981; Donaldson, 1981). The circulating level of cortisol is commonly used as an indicator of stress (Barton and Iwama, 1991; Wendelaar Bonga, 1997; Davis, 2010; Raby et al., 2012). Plasma level increases in cortisol are positively correlated with stress and do not reflect diurnal rhythms (Fagerlund et al., 1995). Cortisol therefore serves as a sensitive indicator of the severity of particular stressors. Yet caution is warranted in interpreting plasma cortisol concentrations as a metric for stress and condition in the context of secondary physiological processes and environmental challenges (Baker et al., 2013b). Many external stressors impact Pacific salmon at the juvenile stage, when fish migrate from freshwater to the ocean as smolts, or at the adult stage, when mature fish return to freshwater to spawn. Both life stages are also characterized by complex physiological processes that complicate interpretation of blood chemistry assays (Donaldson and Fagerlund, 1968; Mesa et al., 1998). At migration and spawning, salmon are subject to environmental stressors related to their transition from salt to fresh water habitat, the physical demands of migration, gonadal development, sexual maturation, competition for territory and mates, and the onset of senescence (Robertson and Wexler, 1960). Adult salmonids demonstrate sustained increases in cortisol in association with migration and sexual maturation (e.g. McBride et al., 1986; Carruth et al., 2000). In the case of extreme migrations, corticosteroid levels parallel those associated with chronic stress (Fagerlund, 1967; Fagerlund et al., 1995). Differentiating between natural and anthropogenic stressors and understanding the consequences of additional stressors at these critical life stages is imperative to effective approaches to restoration and sustainable management.

1.1. Purpose and approach

We investigated the following research questions: (1) what is an appropriate protocol to measure baseline levels of cortisol, given challenges to sampling wild populations in the field; (2) what are baseline levels of cortisol during the crucial stages of migration, reproductive maturation, spawning, and senescence. The former provides guidance to unbiased sampling of cortisol in wild Pacific salmon. The later provides a baseline for cortisol during migration and spawning and informs our understanding of external stressors in the context of complex physiological processes that occur at this life stage.

2. Material and methods

2.1. Cortisol assays

Cortisol concentrations in blood plasma were measured using a double antibody radioimmunoassay kit (DSL-2000) from Diagnostic System Laboratories, Inc., Webster, TX. Dilutions of plasma produced a curve parallel to the standard curve. Inter-assay variation was 6.4%. Intra-assay variation was 2.7%.

2.2. General sampling protocol

Staging equipment was prepared and processing stations established prior to initiation of each sampling sequence. Heparin ammonium salt (Sigma–Aldrich, 5000 IU mL^{-1}), an anticoagulant was applied to 15 ml sample collection tubes (150 μL heparin solution). Anesthetic baths of tricaine methanesulfonate (MS-222, 300 mg L^{-1}) buffered with sodium bicarbonate ($NaHCO_3$, 500 mg L^{-1}) were designed to induce rapid anesthesia but maintain vital functions until blood was collected.

Fish were sampled in-river or at the spawning stream (Fig. 1), using a beach seine. Fish were transferred to the lethal anesthetic bath until respiratory failure, and extracted when motionless. Blood was collected from the caudal vasculature into heparinized tubes. Clark et al. (2011) recently demonstrated that cortisol levels are not affected by lethal procedures (i.e. live versus recently sacrificed salmon) nor method of blood extraction (i.e. caudal puncture versus cannulated procedures). Blood samples were maintained on ice and, within hours, plasma was separated by centrifugation (1200–1500 rpm), transferred to 1.5 mL Eppendorf microcentrifuge vials, and stored at −80 °C until analyzed.

2.3. Sampling protocol for analyses of sampling bias (stress series)

To determine potential bias from sampling disturbance, sockeye salmon were sampled from a common school aggregated at the mouth of a spawning stream (Pick Creek, 59°33′02.40″N, 159°03′51.21″W). Sampling disturbance included the time from the initiation of the beach seine set to collection of the blood sample. All fish were sampled in a common set, but spent different amounts of time awaiting removal to lethal anesthesia bath, biopsy, and termination. Blood samples were collected at 2, 5, 10, and 20 min. Equal numbers of males and females were sampled at each interval.

2.4. Sampling protocol for analyses of cortisol at reproductive stages

To better understand cortisol levels in the context of reproductive maturation and spawning, we sampled sockeye salmon at multiple maturation stages. Fish were sampled at: (i) in-river migration (early-stage maturation, males and females $n = 42$); at the mouth of spawning stream (late-stage maturation, males and females $n = 44$); and in-stream (mature, spawning, and senescent stages, females only $n = 82$). Mean sampling time (initial disturbance until deposition in a lethal anesthetic bath) was 5.92 ± 1.79 (SD) min for in-river sampling (immature migrating stage) and 7.1 ± 1.4 min at the mouths of spawning streams (maturing stage). In-stream sampling (mature, spawning, senescent stages) occurred in <2 min.

2.5. Assessing maturation via coloration and egg development

To interpret the relationship between cortisol and reproductive maturation, we also assessed coloration in fish, which is related to the release of androgens (Idler et al., 1961). At maturity, sockeye salmon exhibit differential coloration from the immature ocean phase (Fig. 1), flushing carotenoid and lipophilic pigments from muscle to skin, shifting from silver coloration to deep red (Smirnov, 1958; Idler et al., 1961); Skin also thickens and scales are resorbed (Burgner, 1991). Fish were categorized by color and scale absorption (silver = none, blush = partial, red = complete). All females were also dissected to determine reproductive phase and egg development (Fig. 2).

3. Theory

To investigate sampling disturbance on plasma cortisol in wild salmon, we conducted a stress series to quantify sampling bias and determine time intervals that avoid bias. To develop baseline physiological profiles informative to analyses of external stressors at migration, maturation, spawning or senescence, we characterized cortisol levels at these stages in Bristol Bay, Alaska sockeye salmon.