

Daily Cycles in Serum Cortisol Levels in the Bluegill (*Lepomis macrochirus*): Effect of Temperature

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Introduction

Daily fluctuations in serum corticosteroid hormone levels have been demonstrated in several species of teleosts (channel catfish, *Ictalurus punctatus*, Boehlke et al. (1); Gulf killifish, *Fundulus grandis*, Garcia and Meier (4); carp, *Cyprinus carpio*, Redgate (6); goldfish, *Carassius auratus*, Singley and Chavin (7); Peter et al. (5)). However, Delahunty et al. (2) found that the response to photoperiod varied with season as they found photoperiod had no effect on serum corticoids during July. Strange et al. (8) found no evidence of diurnal cycles in corticoid levels in chinook salmon. Also data presented by Donaldson and Dye (3) are not indicative of the presence of a diurnal cycle in corticoids of sockeye salmon. It could well be, as suggested by Strange et al. (8), that between fish variation in corticoids of salmonids precludes detection of a rhythm.

While sudden changes in water temperature has been shown to affect cortisol in teleosts (8), the effect of different acclimation temperatures on diurnal cortisol cycles has not been established. Peter et al. (5) studied daily cycles in serum cortisol levels in goldfish in response to both photoperiod and temperature. The lack of any common pattern in the cortisol cycles under either cold (12°C) or warm (21°C) temperatures led these workers to suggest that temperature is not an important synchronizer.

The objective of this study was to determine if serum cortisol in bluegill, *Lepomis macrochirus*, exhibits a diurnal cycle and what effect acclimation temperature might have on such a cycle.

Materials and Methods

Young bluegill (sexually immature) were captured with minnow traps from St. Mary's Pond. The fish were transported to Indiana University at South Bend's animal holding facilities and placed in large aquaria (150 gallon). Fish were acclimated to a 12 hour light 12 hour dark photoperiod for at least two weeks prior to acclimation to experimental temperatures. The fish were then allowed to acclimate to either 20°C or 5°C water temperatures for at least two weeks prior to the experiments. Fish were fed Purina trout chow daily until 24 hours before sampling.

Blood samples were taken every 2 hours for a 48 hour period. Fish were netted from the aquarium with as little disturbance as possible to the remaining fish and placed into a tricane methane sulfonate anesthetic solution (1:1000). Blood samples were taken from fish while under deep anesthesia by severing the caudal peduncle and collecting the blood in heparinized capillary tubes.

Serum cortisol was determined on pooled serum (10-15 animals) by a modification of the fluorometric method described by Wedemeyer and Yasutake (11). Cor-

tisol from 100-200 μ 1 of serum was extracted into dichloromethane and extensively purified. The extracted cortisol was treated with a sulfuric acid-ethanol (3:1) mixture and the cortisol-sulfuric acid-ethanol solution's fluorescence measured in a fluorimeter with excitation at 470 nm and emission at 550 nm. The same volumes were used for blanks, standards and unknowns to avoid the need for dilution factors in the calculations.

Results and Discussion

The effects of exposure to different environmental temperatures (5° and 20°C) on serum cortisol fluctuations are reported in Figure 1. Significant daily fluctuations in serum cortisol levels occurred in both experimental groups, fish acclimated to 20°C having two daily peaks 12 hours apart while fish acclimated to 5°C had four daily peaks ranging from 4 to 8 hours apart. Several other investigators have reported two daily fluctuations in serum cortisol (2, 4, 7) however, Peter et al. (5) found as many as three daily peaks in goldfish but were unable to show any common pattern in cortisol cycles in either cold (12°) or warm (21°) acclimated fish. The reason for the 4 daily peaks in the cold (5°C) acclimated fish (Figure 1) is difficult to assess. Strange (8) studied serum cortisol levels in channel catfish subjected to the stress of confinement at three different acclimation temperatures and found basal concentrations of serum cortisol were partially temperature dependent. However, his data does not suggest a circadian rhythm at any temperature. We do note however, that samples were taken at various time intervals ranging from 3 to 6 hours apart in any one day. Thus, a fluctuation in serum cortisol could have occurred between samples. Peter et al. (5) found no common pattern in the cortisol cycles of goldfish under either cold (12°C) or warm (21°) acclimated fish which lead these workers to suggest that temperature is not an important synchronizer. The adaptive significance of increased cortisol fluctuations in cold acclimated bluegill is not clear although it could be a result of increased stress.

The fish acclimated to the higher environmental temperature had lower basal serum cortisol levels than the fish acclimated to the lower environmental temperature (Figure 1). Terkatin-Shimony et al. (10) found cortisol levels to be higher in *Tilapia aurea* acclimated to 17°C than those acclimated to 28°C. It could well be that the differences in serum cortisol levels at these temperatures could be attributed to differences in cortisol clearance rates. Terkatin-Shimony et al. (10) found the $T_{1/2}$ of cortisol in *T. aurea* acclimated to 17°C to be 208 minutes while the $T_{1/2}$ of cortisol in those acclimated to 28°C to be 132 minutes. Thus, if secretion rates of cortisol do not change, the difference in serum cortisol, may be due to differences in clearance rates at the two different acclimation temperatures.

Bluegill acclimated to the lower temperature (5°C) were found to feed quite sparingly and in this respect were in a situation somewhat similar to migrating eels and salmonids. Terkatin-Shimony et al. (10) observed the same behavior in *T. aurea* acclimated to lower temperature and suggested that high cortisol levels in fasting fish probably reflect a physiological stress during which an increased secretion of corticosteroids would be expected, related to the mobilization and metabolism of protein and fats and their conversion to carbohydrates. Peter et al. (5) however, found goldfish had low serum cortisol levels under cold (12°) conditions but under warm (21°C) conditions, they had high levels and large fluctuations. Since it is assumed that the goldfish fed normally at 12°C, this study cannot be directly compared with our results. That high levels of serum cortisol in cold acclimated fish could be attributed to either the high activity of interrenal tissue in fasting fish or to the slow rate of cortisol clearance cannot be excluded. However, the reason for

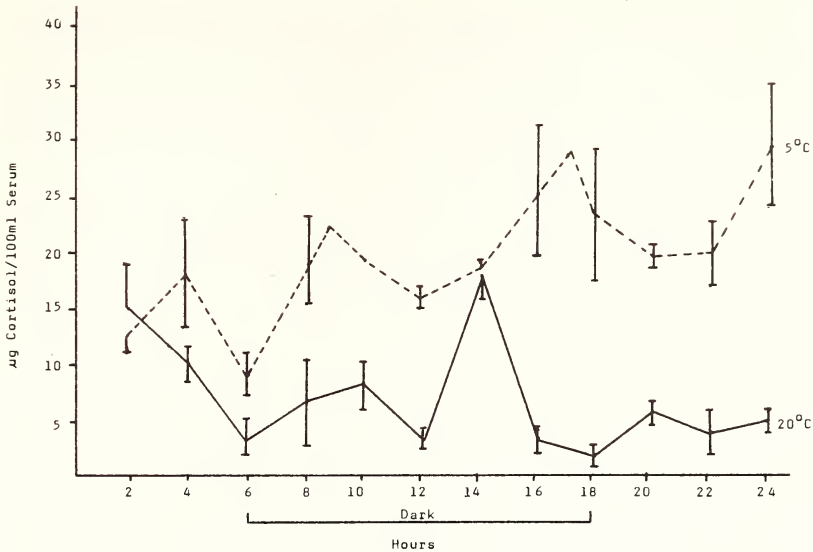


FIGURE I. Daily fluctuations in serum cortisol levels under different environmental conditions.

the different pattern in daily cycles in serum cortisol in cold acclimated fish is not known and further work is necessary to confirm the stability of these patterns over long periods.

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