

# Rates of cortisol increase and decrease in channel catfish and sunshine bass exposed to an acute confinement stressor

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## Abstract

Channel catfish and sunshine bass were exposed to a low-water stress event and allowed to recover in fresh water or a solution of metomidate (dl-1-(1-phenylethyl)-5-(metoxycarbonyl) imidazole hydrochloride), which inhibits the synthesis of cortisol. Change in time of plasma cortisol was used as an index of cortisol secretion and clearance. Plasma cortisol and glucose increased during the exposure to low-water stress in both fish, but the changes of both plasma components were more dramatic in sunshine bass. Exposure to metomidate during recovery resulted in a short-term increase in plasma glucose but differences between controls and metomidate-exposed fish were relatively minor thereafter. Cortisol began to decrease in catfish immediately after the removal of the stress but continued to increase for 15 min in sunshine bass recovering in fresh water and for 5 min in bass recovering in metomidate. Catfish recovering in fresh water had a cortisol elimination rate of  $-1.28$  ng/mL/min compared with  $-2.45$  ng/mL/min for fish recovering in metomidate ( $P > 0.05$ ) while sunshine bass recovering in fresh water had an elimination rate of  $-6.96$  ng/mL/min compared with  $-4.50$  ng/mL/min for fish recovering in metomidate ( $P > 0.05$ ). These data indicate that the rapid decrease of plasma cortisol after removal of the stressor is due to an almost immediate decrease of secretion, tissue uptake and a rapid renal loss due to the absence of a plasma binding protein. Published by Elsevier Inc.

**Keywords:** Cortisol; Stress; Sunshine bass; Channel catfish; Metomidate

## 1. Introduction

Many aquaculture practices induce a physiological stress response in fish. The response is characterized by an increase in plasma cortisol and glucose which is initiated during the first several minutes following the beginning of the stress and continues as long as the stress remains imposed. The rate of increase and the magnitude of the final cortisol concentration are related to the temperature, severity of the stressor, and the species (Davis et al., 1984; Davis and Parker, 1986). Plasma cortisol concentrations fall quickly after the removal of the stress and

often reach pre-stress concentrations after 2 h (Davis et al., 1984). Cortisol, under certain conditions, has both a gluconeogenic (Freeman and Idler, 1973) and an immunosuppressive activity (Schreck, 1996). Recent evidence, however, suggests that plasma cortisol may have both positive and negative effects on immune function and disease resistance (Maule et al., 1989; Dhabhar and McEwen, 2001). Which of these seemingly paradoxical effects dominates may be due to the length of time cortisol secretion is maintained and plasma levels remain high. The magnitude and duration of the cortisol response is known to be related to the severity of the imposed stress (Carmichael et al., 1984). Different effects of cortisol might result from acute versus chronic elevation of cortisol. The mechanisms for the decrease of plasma cortisol following stress removal may be important in lessening the negative effects of stress-induced cortisol secretion.

The decrease in plasma cortisol after removal of the stress is a function of decreased cortisol secretion, tissue uptake, and

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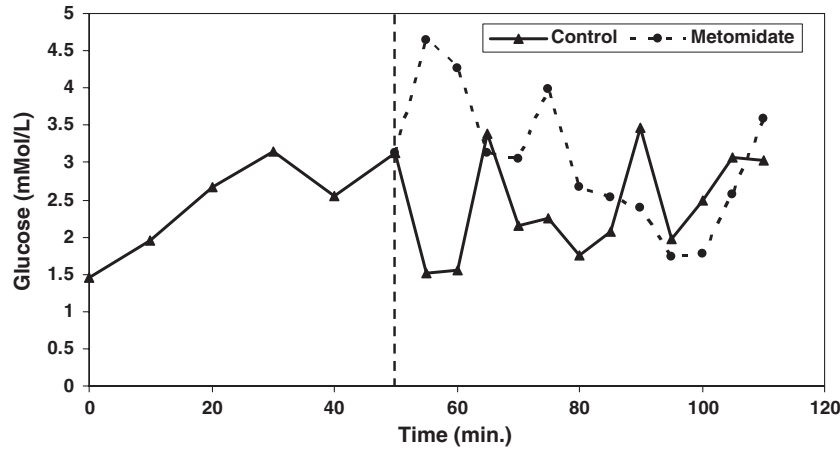


Fig. 1. The change in plasma glucose concentration in channel catfish exposed to a 50-min low-water stress and then allowed to recover in fresh water (●), or 1.5 mg/L metomidate (▲). The perpendicular dashed line indicates the time at which the stress was removed. Each point represents the mean of 6 fish. Pooled standard error=0.64 mM.

excretion. It has been difficult to distinguish the relative contribution of these three mechanisms. An anesthetic, metomidate hydrochloride, has been shown to inhibit cortisol synthesis and secretion in stressed fish (Thomas and Robertson, 1991; Iverson et al., 2003; Small, 2003). This compound inhibits the synthesis and release of cortisol from the interrenal tissue and can be used to determine the elimination of plasma cortisol without the complication of continued cortisol secretion. The following experiments were carried out to examine the rate of cortisol increase due to confinement stress and the rate of cortisol decrease in the presence and absence of metomidate after removal of the stress in an effort to better understand the dynamics of cortisol secretion and recovery during and following an acute stress event. These experiments were carried out in important aquaculture species, channel catfish (*Ictalurus punctatus*) and sunshine bass, a hybrid produced by crossing a white bass (*Morone chrysops*) female with a striped bass (*Morone saxatilis*) male.

## 2. Materials and methods

Yearling channel catfish (*I. punctatus*) and sunshine bass (*M. chrysops* × *saxatilis*) were reared under optimal culture conditions, respectively, and subjected to a standard confinement stress and a period of recovery. Recovery occurred in the presence or absence of the anesthetic metomidate (DL-1-(1-phenylethyl)-5-(methoxycarbonyl) imidazole hydrochloride; Janssen Pharmaceutica, Belgium), which inhibits synthesis and release of the hormone cortisol. Stress challenges for both types of fish, although similar, were temporally different in an attempt to maximize the stress response and ensure enough time for clearance of stress induced cortisol from circulation.

Channel catfish (54.4 g ± 10.6; mean mass ± SEM) were acclimated for 10 d in 75-L aquaria in a flow-through system held at 26 ± 1 °C. Six fish were held in each of 30 aquaria. The stress was accomplished by changing the volume of the water in the each aquarium from 75-L to 5-L by replacing the tall

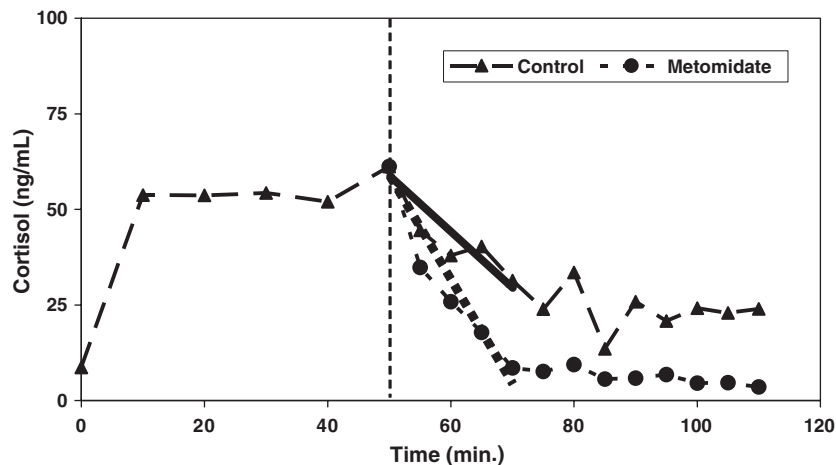


Fig. 2. The change in plasma cortisol in channel catfish exposed to a 50 min low-water stress and then allowed to recover in fresh water (●), or 1.5 mg/L metomidate (▲). The perpendicular dashed line indicates the time at which the stress was removed. The dark solid line shows the regression over the 20 min period used for calculation of the elimination rate for cortisol in the controls and the dashed line shows the regression for the 20 min period used to calculate the elimination of cortisol in the metomidate group. Each point represents the mean of 6 fish. Pooled standard error=3.0 ng/mL.

Table 1  
The initial concentration, maximum concentration, rate of increase, and rate of elimination of plasma cortisol in channel catfish and sunshine bass during a low-water stress event and recovery in fresh water (Control) or metomidate (1.5 mg/L)

Units	Initial (ng/ml)	Maximum peak value		Rate of increase (ng/ml/min)	$R^2$	Rate of elimination		$R^2$	$R^2$
		Control (ng/ml)	Metomidate (ng/ml)			Control (ng/ml/min)	Metomidate (ng/ml/min)		
Channel catfish	8.7±2.3	61.2±10.0	61.2±10.0	4.51	1.00	-1.28	0.21	-2.45	0.61
Sunshine bass	11.4±5.0	189.6±6.2	150.9±6.2	6.94	0.82	-6.96	0.60	-4.50	0.56

Values represent mean±SEM ( $n=6$ ). Plasma cortisol elimination is calculated for the 20 min period following the maximum cortisol concentration reached.

standpipe with a short one. The volume was reduced in about 5 min and held at the lower level for 50 min. The low-water level was selected so that the fish were submerged to approximately eye level. Three fish were sampled every 10 min for 50 min during the low-water stressor. After 50 min, six groups of three fish were transferred to six aquaria with 75-L of untreated fresh water, and a second set of three fish were transferred to each of six aquaria with 75-L of water treated with a sedating dose of 1.5 mg/L metomidate. Three fish were sampled from treated and untreated groups every 5 min for 60 min during recovery. Sampling was done rapidly by three individuals to minimize changes during the sampling period. The complete protocol was repeated twice so that each data point represents a mean of six fish.

Sunshine bass (149.9 g±43.3; mean mass±SEM) were acclimated for 7 d in 60-L aquaria in a flow-through system held at 23±1 °C. Six fish were held in each of 30 tanks. The stress was accomplished by changing the volume of the aquarium from 60 to 5 L by reducing the length of the standpipe. The volume was reduced in about 5 min and held at the lower level for 10 min. The water level was selected so that the fish were submerged, but were unable to maintain their posture in the tank. After 15 min, six groups of fish were transferred each to six 60-L aquariums filled with untreated fresh water and a second group was transferred to a tanks containing a sedating dose of 1.5 mg/L metomidate. Three fish were sampled every 3 min for the first 15 min during the stress and every five min for 60 min during recovery. Sampling was carried out rapidly by three individuals to avoid changes during the sampling period. The complete

protocol was repeated twice so that each data point represents a mean of six fish.

Blood was collected in heparinized syringes from the caudal vessel in the hemal arch from unanesthetized fish. Each fish was sampled only one time. Plasma was separated by centrifugation and stored frozen for later analysis. Plasma cortisol in channel catfish was determined by time-resolved fluoroimmunoassay which has been validated for channel catfish (Small and Davis, 2002). Assay sensitivity in catfish plasma was 1.2 ng/mL. Plasma cortisol in sunshine bass was determined by radioimmunoassay using the BioChem Immunosystems Cortisol Bridge kit (No. 14394, Polymedco, Inc., Cortlandt Manor, NY, USA) which has been validated for sunshine bass (Davis and Griffin, 2004). Assay sensitivity in sunshine bass plasma was 2.7 ng/mL. Intra- and inter-assay coefficients of variation for both assays are reported to be less than 10%. Accuracy of both assays, calculated as the percent of exogenous cortisol recovered from spiked fish plasma has been reported to be greater than 95%. Plasma glucose concentrations were determined by the glucose oxidase procedure (Sigma Diagnostics, No. 510A, St. Louis, MO, USA). The coefficient of variation for analyses over 11 consecutive days of a normal human serum pool was 3.2% as reported by the manufacturer.

Experimental data were subjected to analysis of variance (ANOVA) GLM procedures followed by Tukey separation when indicated at  $P<0.05$  using the SAS software system version 8.00 (SAS Institute, Cary, NC, USA). No statistical comparisons were done between species due to the different husbandry conditions

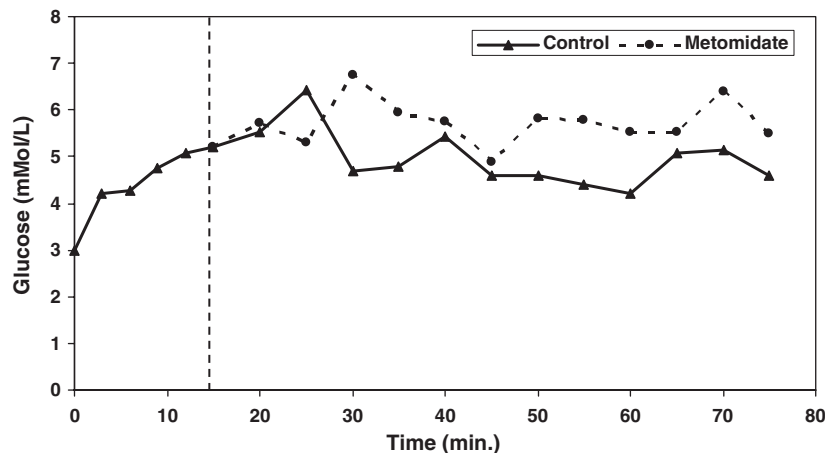


Fig. 3. The change in plasma glucose concentration in sunshine bass exposed to a 15-min low-water stress and then allowed to recover in fresh water (●), or 1.5 mg/L metomidate (▲). Each point represents the mean of 6 fish. Pooled standard error=0.81 mM.

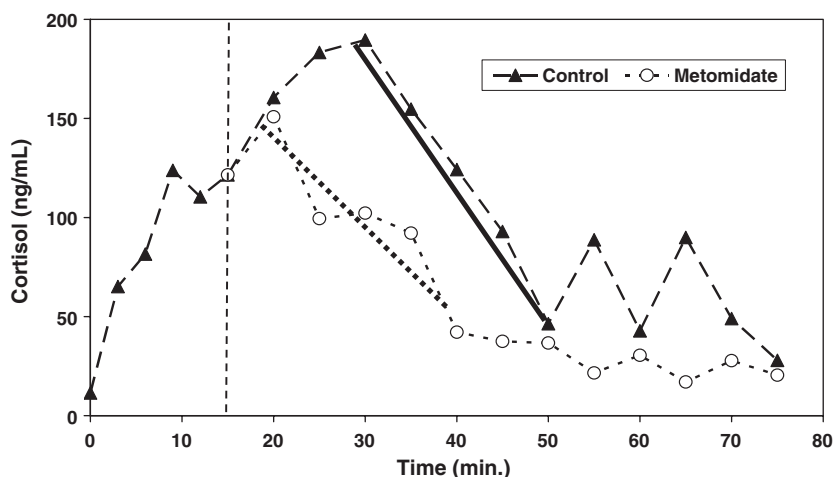


Fig. 4. The change in plasma cortisol in sunshine bass exposed to a 15 min low-water stress and then allowed to recover in fresh water (○), or 1.5 mg/L metomidate (▲). The dark solid line shows the regression over the 20 min time period used for calculation of the rate of elimination for cortisol in the controls and the dashed line shows the regression for the 20 min period used to calculate the rate of elimination of cortisol in the metomidate group. Each point represents the mean of 6 fish. Pooled standard error=17.0 ng/mL.

(e.g. temperatures) and the different temporal design of the confinement stress.

The rate of plasma cortisol increase for catfish was calculated as the slope of the linear regression line of plasma cortisol only for the first 10 min of the 50 min low-water stress and for the slope of the entire 15 min low-water stress in sunshine bass. The data were expressed as ng/mL/min. The rate of plasma cortisol decrease was calculated as the slope of the linear regression line of the plasma concentration during the 20 min period following the highest concentration reached and expressed as ng/mL/min. This measurement was used to avoid confusion with metabolic clearance rates used in other studies, which take into consideration the weight of the fish (for review see Mommensen et al., 1999).

### 3. Results

#### 3.1. Channel catfish

Plasma glucose in channel catfish increased from about 1.44 to about 3.11 mMol/L by the end of the 50 min stressor (Fig. 1). However, most of the change occurred in the first 30 min. Fish recovering in metomidate markedly increased plasma glucose during the first 10 min of recovery; however, after 15 min of recovery, plasma glucose for control and metomidate-treated fish were similar.

Plasma cortisol increased from about 9 to about 61 ng/mL during the 50 min of confinement stress; however, the concentration had already increased to about 54 ng/mL in the first 10 min (Fig. 2). Plasma cortisol concentrations in both control and metomidate-treated fish began to fall immediately after stress removal; however, plasma cortisol decreased in metomidate-treated fish faster and reached initial levels by 20 min after removal of the stress. Concentrations in control fish were significantly higher ( $P < 0.05$ ) than the metomidate-treated fish from 15 min after the beginning of recovery (except for the 85-min sample) until the end of the experiment. The cortisol

rate of decrease for the channel catfish recovering in metomidate was not significantly lower ( $P > 0.05$ ) than that in control fish during the 20 min period immediately following recovery (Table 1).

#### 3.2. Sunshine bass

Plasma glucose concentrations increased from 2.98 to 5.19 mMol/L during the 15 min confinement stress. The concentrations in controls continued to increase during the first 10 min after removal of the stressor, then began to decline, but did not reach the basal concentrations during the 60 min recovery period. Fish recovering in metomidate showed no consistent decline of glucose during the entire 60 min after removal of the stressor. However, glucose concentrations in metomidate-treated fish were significantly higher ( $P < 0.05$ ) than controls in the 15 and 45 min samples during recovery (Fig. 3).

Plasma cortisol concentration increased from 11 to 120 ng/mL during the 15 min confinement stress (Fig. 4). Cortisol secretion continued for 15 min after stress removal and reached 189 ng/mL before falling to concentrations about twice that of the initial concentration. Cortisol in fish recovering in metomidate also continued to increase for 5 min, followed by a steady decrease and reached concentrations similar to the initial levels about 40 min after recovery. The rate for cortisol decrease for the sunshine bass recovering in metomidate was not significantly different than that in control fish (Table 1). Both groups had reached plasma cortisol concentrations similar to the initial levels by 60 min of recovery.

### 4. Discussion

Metomidate had little impact on the post-stress concentrations of plasma glucose in sunshine bass and only a transient effect in channel catfish for about 10 min after transfer to water containing the anesthetic. This effect was more pronounced and occurred quicker in channel catfish than in sunshine bass. Further, channel catfish recovering in metomidate appeared to

have lower plasma cortisol levels at the end of the experiment than control fish.

Cortisol secretion in sunshine bass was more dynamic than in channel catfish. Peak concentrations were higher and the increase continued for a longer period of time. In sunshine bass, cortisol concentrations increased throughout the stressor, continued to increase for 5 min after recovery in metomidate, and for 15 min after recovery in fresh water, whereas cortisol concentrations in catfish reached a plateau after the first 10 min, and began decreasing immediately after removal of the stressor. Despite the difference in the timing of cortisol clearance following stress removal, fish of both species recovering in metomidate had cortisol elimination rates similar to fish in the respective control group. Cortisol elimination from the plasma reflects the net effect of cortisol production and plasma clearance of the hormone. Plasma clearance involves tissue uptake and excretion and has been studied in other species by exogenous cortisol administration and radiolabeling, as a bolus or constant infusion, and has been expressed as mL/kg/h (Owen and Idler, 1972; Donaldson and Fagerlund, 1968; Redding et al., 1984; Patiño et al., 1985). Plasma cortisol profiles may not reflect tissue responses because hormone taken up by the target tissues may continue to activate the signaling pathways. Further, many environmental factors including temperature, salinity, nutritional state, and maturational state influence metabolic clearance of cortisol (Donaldson and Fagerlund, 1968; Leloup-Hatey, 1974; Vijayan and Moon, 1994; Redding et al., 1984). The different techniques, species, and conditions used in those studies and the different reporting units make comparison with the data reported here inappropriate.

Controlling plasma cortisol responses during stress is considered desirable during aquaculture operations such as handling. The efficacy of many anesthetics in reducing the stress response to handling has been demonstrated in a number of teleost species (Strange and Schreck, 1978; Tomasso et al., 1980; Iverson et al., 2003). Metomidate was the only compound used which inhibited the stress induced increase in cortisol in sunshine bass exposed to sedating concentrations of seven commonly used fish anesthetics (Davis and Griffin, 2004). Further, exposure to a sedating concentration in the absence of a stressor of all anesthetics used, except metomidate, resulted in a significant increase of plasma cortisol. In assessing the effects of various anesthetics on cortisol secretion in channel catfish, Small (2003) also found metomidate to be effective at suppressing stress induced cortisol increase.

Immobilizing doses of many anesthetics may reduce the cortisol stress response by affecting the perception of the stressor (Schreck, 1981) while sedating concentrations may not have sufficiently suppressed this perception. This does not appear to be the case when sedating with metomidate. Metomidate is the methyl derivative of etomidate and both are non-barbiturate hypnotics, which have been shown to reduce cortisol concentrations in mammals (Preziosi and Vacca, 1982; Fraser et al., 1984) and fish (Davis et al., 1982; Thomas and Robertson, 1991; Olsen et al., 1995; Small, 2003). Etomidate inhibits the mitochondrial cytochrome *P*450-dependent enzymes that catalyze the synthesis of cortisol in mammals (Wagner et al., 1984; Vanden Bossche

et al., 1984) and metomidate is thought to have the same action. Other compounds have also been used to decrease endogenous cortisol secretion in fish including dexamethasone, metyrapone (SU 4885), and RU 486 (Baulieu, 1997). Metyrapone inhibits 11-hydroxylase and other cytochrome *P*450-dependent monooxygenases and prevents the de novo synthesis of cortisol from 11-deoxycortisol. This compound has been used to block cortisol synthesis and stress-induced increases of plasma cortisol in fishes (Hopkins et al., 1995; Milligan, 1997), but has not been used to estimate cortisol clearance due to other effects of this drug on cortisol metabolism (for review see Mommsen et al., 1999). RU 486 (RU 38486), Mifepristone, is an antiglucocorticosteroid that exerts its effect at the receptor level and results in an increase in ACTH in mammals (Baulieu, 1997). Dexamethasone decreases plasma cortisol by blocking release of ACTH (Pickering et al., 1987). Due to the mode of action of the latter two compounds, neither is appropriate to estimate cortisol clearance.

Plasma reduction of cortisol in this study represents a combination of tissue uptake and loss by excretion in the presence (control) and absence (metomidate) of continued cortisol synthesis and secretion. In both cases and for both species, cortisol reduction was very rapid. Rapid cortisol clearance is likely due to the lack of a cortisol binding globulin (CBP) to protect it from degradation (for review see Mommsen et al., 1999). Most fish do not possess a specific CBP. The few reports of protein bound corticosteroids in fish plasma do not demonstrate a specific binding protein, but do suggest some non-specific binding under special cases such as during vitellogenesis (Caldwell et al., 1991; Barry and Unwin, 2001). In the present study, no distinction can be made between cortisol lost by target tissue uptake and that lost by excretion. The mechanisms of tissue uptake and secretion are not well defined in teleost fish. Entry of cortisol into target cells is thought to be by passive diffusion; however, recent studies have presented evidence suggesting a low-affinity carrier protein may be involved (Vijayan et al., 1997). Cortisol disposal occurs by way of the hepato-biliary system (Idler and Truscott, 1972; Wilson et al., 1998).

The degree of stress in this study is rather mild for these species and should be considered an acute stress response. The rapid cortisol clearance observed here emphasizes the importance of considering the quantitative and temporal aspects of the stress response to cortisol when evaluating the physiological impact of a cortisol inducing event. The significance of the event to the animal represents the amount of time the animal is exposed to high cortisol levels and, since cortisol can be eliminated so quickly after the end of the stressor, acute responses may have little lasting effect on the fish.

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