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# Changes of glucose and cortisol during stress in fishes

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## Abstract

There is now extensive literature on the physiological and endocrine basis of stress in fish, largely constructed from studies of captive or cultured fish, and within this, largely examining the effects on teleosts fishes. The effects of stress resulting from aquaculture practices on fish and methods of minimizing such effects have received considerable attention through the years. Glucose is a carbohydrate that has a major role in the bioenergetics of animals, being transformed to chemical energy (ATP), which in turn can be expressed as mechanical energy. Cortisol is the principal glucocorticoid secreted by the interrenal tissue (steroidogenic cells) located in the head-kidney of teleost fish. This hormone is released by the activation of the hypothalamus-pituitary-interrenal axis (HPI axis).

**Keywords:** Salt stress, Glucose, cortisol, environment

## Introduction

There is now extensive literature on the physiological and endocrine basis of stress in fish, largely constructed from studies of captive or cultured fish, and within this, largely examining the effects on teleosts fishes (see reviews by Barton and Iwama, 1991; Pickering, 1998; Sumpter, 1997; Wendelaar Bonga, 1997; Barton, 2002) [5, 29, 38, 44, 2]. Because of the potential difficulties associated with accessing and suitably sampling fish in their natural environment there is considerably less information on stress and its physiological and endocrine effects, in natural settings (Sharples, 1992) [36].

## Stress

The effects of stress resulting from aquaculture practices on fish and methods of minimizing such effects have received considerable attention through the years (wedemeyer, 1972; Mazeaud *et al*., 1977; Nikinmaa *et al*., 1983; Barton and Iwama, 1991; Mazik *et al*., 1991; Cech *et al*., 1996.) [43, 19, 24, 5, 10]. Stress induced by common practices such as handling, crowding, transport, or poor water conditions can increase the incidence of diseases and mortality, and is therefore an important factor affecting the economics of aquaculture.

## Salt

Salt additives, particularly NaCl, can alleviate the severity of the stress response and improve survival during handling, transport and post-stress recovery in fish. The presence of salts apparently helps by reducing osmoregulatory dysfunction (Wedemeyer, 1972; Nikinmaa *et al*., 1983; Carmichael *et al*., 1984; Mazik *et al*., 1991; Barton and Zitzow, 1995) [43, 24, 7]. Although the addition of NaCl to the water is usually beneficial, it did not improve survival during stocking and harvest of juvenile striped bass *Morone saxatilis* and *Morone* hybrid bass *Morone chrysops*=*M. saxatilis* (Grizzle *et al*., 1985) [13]. It also did not reduce the effects of capture and transport on fingerling rainbow trout *Salmo gairdneri* (Barton and Peter, 1982) [1]. Therefore, the efficacy of salts to mitigate the effects of stress may vary between species and even stocks of the same species. It probably depends also on the severity of the stressor.

## Glucose

Glucose is a carbohydrate that has a major role in the bioenergetics of animals, being transformed to chemical energy (ATP), which in turn can be expressed as mechanical energy (Lucas 1996) [15]. In suboptimum or stressful conditions (internal or external) the chromaffin cells release catecholamine hormones, adrenaline and noradrenaline toward blood circulation (Reid *et al.* 1998) [34]. Those stress hormones in conjunction with cortisol mobilize and elevate glucose production in fish through glucogenesis and glycogenolysis pathways (Iwama *et al.* 1999) to cope with the energy demand produced by the stressor for the "fight of flight" reaction. This glucose production is mostly mediated by the action of cortisol which stimulates liver gluconeogenesis and also halts peripheral sugar uptake (Wedemeyer *et al.* 1990). Glucose is then released (from liver and muscle) toward blood circulation and enters into cells through the insulin action (Nelson & Cox 2005) [23]. Regardless of the wide use of glucose as an indicator of stress, some authors (Mommsen *et al.* 1999, Flodmark *et al.* 2001) [21] emphasized that care has to be taken when using plasma glucose as the only indicator. It has been reported that glucose content is a less precise indicator of stress than cortisol (Wedemeyer *et al.* 1990, Pottinger 1998). Mommsen *et al.* (1999) [21] were skeptical about the use of glucose as a stress indicator, whereas Simontacchi *et al.* (2008) [37] stated that glucose and cortisol "cannot be considered itself as reliable stress indicators". Factors that affect the intensity of response. Similar to cortisol, some factors can indirectly alter the response of glucose levels in blood. Vijayan & Moon (1994) suggests that "the rearing history including nutritional status may affect the stress response and glucose clearance rates". That affirmation is supported by other authors who concluded that blood glucose results have to be interpreted with care, taking into account extrinsic factors such as diet, life stage, time since last feeding and season of the year, etc., because they may affect liver glycogen stores (Nakano & Tomlinson 1967, Barton *et al.* 1988, McLeay 1977, Wedemeyer *et al.* 1990) [22]. Nutritional status is a factor that can have an effect in the glucose response. The intake of diets with different lipid and protein content resulted in different responses of blood glucose of the orangespotted Grouper (*Epinephelus coioides*) when it was exposed to cold stress (Cheng *et al.* 2006). The channel catfish under fasting conditions evidenced hyperglycemia after 30 days of experiment (22.8 versus 4.7 ng·ml-1 in the control group) (Peterson & Small 2004).

### Hematocrit values

Decreased hematocrit values with increasing salinity, as observed, have been also reported for chinook salmon *Oncorhynchus tshawytscha* fry (Morgan and Iwama, 1991).

### Stress responses in the natural environment

A primary question in the examination of environmental stress is whether animals in the wild typically experience stress over the normal range of activity and environmental conditions. The best field evidence is from studies on birds, and these suggest that probably they don't. Studies on free living populations show that quite harsh environmental conditions and the rigors of reproduction are not necessarily stressful if they are predictable (Wingfield, 1994). Increasing corticosteroid concentrations when conditions do become sufficiently adverse typically modulate behaviour (eg. a shift from nesting to foraging or even refuge seeking) both to ameliorate the stress but also to potentiate recovery and resumption of reproductive behaviour when conditions permit. Events capable of stimulating increases in plasma corticosterone levels include storms and extreme temperatures (Romero *et al*., 2000) [35]. However, even here the relationship may not be straightforward with Arctic passerines being able to cope with storm events during the breeding season but showing stress responses only during the more energetically demanding period of molting (Romero *et al*. 2000) [35]. This has lead to the view that events such as migration and reproduction are demanding but not necessarily stressful. Extreme events such as storms encapsulate an 'emergency life history stage' (ELHS) as a response to the unpredictable or extreme event. The ELHS is temporary and maximises survival chances through the associated stress response, only becoming maladaptive if the ELHS persists for too long (Wingfield and Ramenofsky, 1999) [47]. The phase of negative energy balance at which an ELHS is triggered is in turn thought likely to be a function of individual body condition. Cockrem *et al*. (2009) [11] suggest on the basis of observed correlation between behaviour and stress responsiveness, that low responders (which tend to have proactive 'personalities') do best under predictable environmental conditions, whereas high responders (which tend to have reactive personalities) are best adapted for responding to unpredictable events. This is similar to the view expressed by Øverli *et al*., (2002) [25] that a spread of stress responsiveness in fish populations allows for a range of adaptive or coping strategies in the face of environmental stress. In an examination of studies of 53 species (37 avian, 7 mammalian, 7, reptilian and 2 piscine) where some measure of fitness was correlated with basal corticosteroid concentrations, (Bonier *et al*., 2009) [8] concluded that there was not a consistent relationship between the two. This was despite a prediction based on the relationship in many species whereby basal corticosteroid levels, and fitness increase and decrease, respectively in the face of environmental challenge, that fitness would decline with increasing basal corticosteroid levels. This further emphasises that the use of corticosteroid levels as predictors of relative fitness requires careful validation in relation to the particular species, population and situation (Bonier *et al*., 2009) [8]. The level to which natural populations of fish experience stress is difficult to gauge for two reasons. Firstly, there is a very limited number of field studies where free ranging fish have been sampled in ways that allow correlation with pre-capture behaviour and activity, and secondly, the events that might equate to extreme conditions in terrestrial systems (storms and floods) largely preclude sampling, or observation of behaviour at these times. A possible exception to this might be stress associated with reduced flow rates and, or water levels in riverine systems. A series of field studies on the tropical spiny damselfish *Acanthochromis polyacanthus* does offer some insight of the corticosteroid dynamics in free living fish. Plasma cortisol levels in territorial adult fish captured underwater and sampled immediately, ranged between <1 and 42 ng mL-1, and there was no apparent relationship between cortisol level and the time a diver had been in close proximity to the territory (Pankhurst, 2001) [27]. Neither was there any relationship between baseline cortisol levels and plasma levels of testosterone (T) and 17β-estradiol in females, or T and 11-ketotestosterone (11KT) in males, despite laboratory experiments showing stress-suppression of sex steroids in both sexes. Earlier studies showed that there was some variation in plasma cortisol levels with behaviour in females but not males, with highest cortisol levels occurring when fish were paired but not yet protecting broods (both sexes of spiny damselfish tend the eggs and then juveniles for several months) (Pankhurst *et al*., 1999). Treatment of territorial adults with exogenous cortisol had no short-term effect on territorial or guarding behavior, but both control (saline-injected) and cortisol treated fish had become diver negative to the extent that recapture was not possible. Laboratory experiments indicated that the cortisol treatment probably elevated plasma levels to ~100 ng mL-1 (Pankhurst, 2001) [27]. Free ranging wild bluegill sunfish show elevated cortisol levels in males engaged in parental care (as noted earlier, up to 125 ng mL-1) but maintain regular cycles of plasma T and 11KT in association with spawning and egg protection (Magee *et al*., 2006) [16].

### Cortisol

Cortisol is the principal glucocorticoid secreted by the interrenal tissue (steroidogenic cells) located in the head-kidney of teleost fish (Iwama *et al.* 1999). This hormone is released by the activation of the hypothalamus-pituitary-interrenal axis (HPI axis) (Mommsen *et al.* 1999) [21]. When an organism undergoes stress conditions, the hypothalamus releases corticotropin-releasing factor (CRF) toward blood circulation. This polypeptide further stimulates secretion of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland (Fryer & Lederis 1986) which finally activates the release of cortisol by the interrenal tissue (Mommsen *et al.* 1999) [21]. Cholesterol is the precursor of cortisol; this sterol is transformed to pregnenolone by the action of the enzyme P450 side chain cleavage (P450SCC) in the inner mitochondrial membrane. Then pregnenolone is further converted into 11-deoxycortisol by steroidogenic enzymes and this product is finally converted to cortisol by enzyme 11b-hydroxylase (Miller 1998, Castillo *et al.* 2008) [20, 9]. The secretion of cortisol is slower than catecholamines, but its effects are more prolonged (Gamperl *et al.* 1994a, b; Waring *et al.* 1996), combining mineral and glucocorticoid actions to restore homeostasis (Wendelaar-Bonga 1997, Maule *et al.* 1993, Colombe *et al.* 2000) [44, 17, 12]. Cortisol activates glycogenolysis and gluconeogenesis processes in fish; but also causes that chromaffin cells increase the release of catecholamines which further increase glycogenolysis and modulate cardiovascular and respiratory function (Reid *et al.* 1992, Reid *et al.* 1998) [33, 34]. This whole process increases the substrate levels (glucose) to produce enough energy according with the demand.

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