ABSTRACT

Title of Thesis: IMPLICATIONS OF DIVERGENT CORTISOL STRESS RESPONSIVENESS IN MALE STRIPED BASS (*MORONE SAXATILIS*)

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The aim of this study was to identify whether differences exist in reproductive and growth performance of male striped bass selected for high cortisol stress responsiveness (HCR) and low cortisol stress responsiveness (LCR), when exposed to standardized stress challenges before and during the spawning season. HCR (n=10) and LCR (n=10) were identified out of a population of 67 three-year-old striped bass males. Although no significant differences in weight, length, or coefficient of condition were found between HCR and LCR, HCR had a significantly greater specific growth rate when compared to LCR. Circulating levels of testosterone and 11-ketotestosterone were significantly higher in HCR than in LCR before and during the spawning season. HCR

also had more fish spermiating across all sample dates compared to LCR. These results suggest that striped bass selected for high cortisol stress responsiveness have better reproductive and growth performance than fish selected for lower cortisol stress responsiveness.

IMPLICATIONS OF DIVERGENT CORTISOL STRESS RESPONSIVENESS IN MALE STRIPED BASS (*MORONE SAXATILIS*)

by

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LIST OF SCIENTIFIC NAMES

Common Name	Scientific Name
Atlantic cod	*Gadus morhua*
Atlantic salmon	*Salmo salar*
Black bream	*Acanthopagrus butcheri*
Brook trout	*Salmo gairdneri*
Brown trout	*Salmo truta*
Common carp	*Cyprinus carpio*
Domestic fowl	*Gallus domesticus*
European eel	*Anguilla anguilla*
Gilthead seabream	*Sparus aurata*
Goldfish	*Carassius auratus*
Japanese quail	*Coturnix coturnix*
New Zealand snapper	*Pagrus auratus*
Rainbow trout	*Oncorhynchus mykiss*
Striped bass	*Morone saxatilis*
Tilapia	*Oreochromis mossambicus*
Turkey	*Meleagris gallopavo*
White bass	*Morone chrysops*

LIST OF ABBREVIATIONS

Name	Abbreviation
Adrenocorticotropic hormone	ACTH
Corticotropin-releasing hormone	CRH
Dexamethasone	DEX
Gonadotropin	GtH
Gonadotropin-I	GTH-I
Gonadotropin-II	GTH-II
Gonadotropin-releasing hormone	GnRH
Gonadotropin-releasing hormone analogue	GnRHa
High cortisol responders	HCR
Low cortisol responders	LCR
Parts per thousand	ppt
Standard error of the mean	SEM
Testosterone	T
11-Ketotestosterone	11-KT
17β-estradiol	E_2

INTRODUCTION

As world fisheries harvests begin to reach their maximum sustainable yield of 90 to 100 million metric tons, the demand for fisheries products continues to increase, not because of an increase in per capita demand, but rather due to an increasing population (FAO, 2002). In order to meet the increasing demand for fisheries products, aquaculture production has continued to grow at an average rate of more than 9% per year since 1970 (FAO, 2002). Aquaculture is now responsible for more than 20% of worldwide fisheries production (Jennings et al., 2001), but continued research is needed to optimize conditions for growth of the industry and to genetically select organisms for increased productivity. Unlike more traditional forms of agriculture, in which a few animals are responsible for the majority of production, aquaculture includes a diverse group of nearly 200 species (FAO, 2002).

In developed nations, culture is focused on carnivorous finfish species that comprise more than seventy percent of aquaculture production (FAO, 2002). In the United States, one of the fastest growing sectors of the aquaculture industry is the production of the striped bass (*Morone saxatilis*) and its hybrids (Harrell, 1997). Currently, *Morone* culture emphasizes the rearing of the sunshine bass (white bass, *Morone chrysops* ♀ X striped bass *Morone saxatilis* ♂) (Hodson et al., 1999).

Harrell and Webster (1997) listed the traits that producers thought were most influential on production, in order from highest to lowest priority (regardless of whether the traits had a genetic component), and at the top of the list was stress

tolerance. The focus of my research was to examine the influence that selecting for high or low cortisol responsiveness to stress has on growth and reproductive performance in domesticated male striped bass broodstock. Although cortisol stress responsiveness has been shown to be a heritable trait in some fish species like the Atlantic salmon (*Salmo salar)* (Fevolden et al., 1991), rainbow trout (*Oncorhynchus mykiss*) (Fevolden et al., 1991, Pottinger et al., 1994), and the common carp (*Cyprinus carpio*) (Tanck et al., 2001), it's heritability has not yet been evaluated in striped bass.

Understanding the repercussions of selecting broodfish for cortisol responsiveness could aid in determining whether further studies should be conducted to determine if cortisol responsiveness is heritable in striped bass and if it should be used as a selection marker. Such selection could not only aid in the domestication of striped bass, but may also enhance the hybrid striped bass industry by identifying superior striped bass males to be crossed with female white bass. Recent advances in cryopreservation techniques of striped bass sperm makes the feasibility of preserving genetic material from selected individuals feasible (He and Woods, 2003a,b).

Recent studies in our laboratory have demonstrated that a significant heterogeneity exists in the cortisol response to stress in male striped bass (Wang et al., 2003). The consequences of selecting striped bass for high and low cortisol stress responsiveness are unknown. The aim of this study was to examine differences between male striped bass broodstock selected for high and low cortisol stress responsiveness with an emphasis on growth and reproductive function.

The results of studies examining differences in growth parameters between fish selected for high and low cortisol stress responsiveness are ambiguous. Since

chronically elevated levels of cortisol are detrimental to vertebrate animals, it would be logical to assume that a fish selected for low cortisol responsiveness would be better suited to live in a stressful culture environment as compared to a fish selected for high cortisol stress responsiveness, but work done in this area does not support this hypothesis. Pottinger and Carrick (1999a) showed that female rainbow trout selected for high cortisol responsiveness had significantly greater weights, lengths, and coefficient of condition when exposed to an acute stressor on a monthly basis when compared to female rainbow trout selected for low cortisol responsiveness. However, mortality rates were greater in lines of rainbow trout selected for high cortisol stress responsiveness when compared to those selected for low responsiveness when the fish were exposed to a combined confinement and salt stress (Fevolden et al., 2003). Weil et al., (2001) found that rainbow trout selected for low, three-hour-post-stress cortisol levels were significantly heavier than fish selected for high three-hour, post-stress cortisol levels, suggesting that the rate at which plasma cortisol levels decrease after a stress is more important than peak cortisol response levels.

The metabolic effects associated with the stress response are more pronounced in lines of rainbow trout selected for low cortisol stress responsiveness when compared to lines selected for high cortisol responsiveness (Trenzado et al., 2003). When exposed to a combined tank transfer, and confinement stress, low cortisol responding lines of rainbow trout had significantly greater plasma glucose and plasma lactate levels and significantly lower levels of liver glycogen when compared to high cortisol responding lines of rainbow trout (Trenzado et al., 2003). These results suggest that cortisol may not be the mediator of the metabolic divergence seen

between high and low cortisol responding rainbow trout strains (Trenzado et al., 2003).

Exposure to acute or chronic stressors adversely affects reproductive performance (Schreck et al., 2001). The impact of stress on reproduction can vary greatly between species (Barton, 2002). Stress has been shown to cause a decrease in circulating plasma androgen levels in mature male brown trout (*Salmo truta*) (Pickering et al., 1987), in New Zealand snapper (*Pagrus auratus*) (Cleary et al., 2000), and in black bream (*Acanthopagrus butcheri*) (Haddy and Pankhurst, 1999). Stress has also been associated with decreased egg size and early ovulation in rainbow trout (Contreras-Sanchez et al., 1998).

Cortisol is the primary glucocorticoid released in teleost fish in response to stress, and is believed to be responsible for many of the negative reproductive consequences associated with the stress response (Pankhurst and Van der Kraak, 1997). Using cortisol pellet implantation to raise plasma cortisol levels to elevated but physiologically reasonable levels in sexually mature male brown tout, Carragher et al., (1989) noted a decrease in plasma testosterone levels, smaller gonads, and decreased pituitary gonadotropin, though plasma levels of gonadotropin and 11-ketotestesterone were not significantly different when compared to sham implanted male brown trout. In sexually mature female brown trout, cortisol pellet implantation caused a decrease in plasma levels of vitellogenin, 17β-estradiol, and a decrease in the amount of gonadotropin in the pituitary, though plasma levels of gonadotropin were not different when compared to sham implanted fish (Carragher et al., 1989). Studies using cortisol infused feeds have shown that cortisol has a significant

inhibitory effect on the circulating levels of 11-ketotestosterone and a significant reduction in the gonadosomatic index in pubertal male common carp when compared to control fish (Consten et al., 2002).

Whether selection for cortisol stress responsiveness will affect reproductive function in cultured fish is still unclear. Pottinger and Carrick (2000) examined reproductive performance parameters in rainbow trout selected for high and low cortisol stress responsiveness by measuring sperm counts in males, and plasma 17-β estradiol, gonadosomatic index, total number of eggs, egg weight / body weight, and egg volume in females, as well as mortality rates of eggs from high cortisol responding broodstock compared to eggs from low responding broodstock. No significant differences were found for any of the reproductive performance parameters examined between high cortisol responding rainbow trout and low responding rainbow trout, except for egg mortality rates (Pottinger and Carrick, 2000). Eggs produced from high cortisol responding rainbow trout had significantly higher mortality rates than eggs produced from low cortisol responding rainbow trout at 70 days post fertilization, but because egg mortality rates did not exceed 12% Pottinger and Carrick (2000) concluded that selecting rainbow trout for cortisol stress responsiveness does not affect reproductive function under non-stressful conditions. Whether selecting for cortisol stress responsiveness would have an impact on reproductive function if the fish were stressed during the spawning season is not clear from this research (Pottinger and Carrick, 2000).

The goal of my research was to assess the effect of selecting striped bass males for high and low cortisol stress responsiveness. The specific objectives were to:

1. Determine a baseline cortisol response within the population without the fish being exposed to the standardized net challenge (once at the beginning of cortisol responsiveness testing and once at the end).
2. Identify fish that had consistently high cortisol stress responsiveness, or consistently low stress responsiveness when exposed to a standardized stressor.
3. Compare plasma cortisol, testosterone and 11-ketotestosterone levels between groups of fish selected for high cortisol stress responsiveness, or low stress responsiveness during gonadal maturation.
4. Compare plasma cortisol, testosterone and 11-ketotestosterone levels and sperm quality between groups of fish selected for high cortisol stress responsiveness, or low stress responsiveness during a simulated spawning season.

LITERATURE REVIEW

The stress response

The stress response has been categorized into primary, secondary, tertiary, and quaternary responses (Wedemeyer, 1996). The physiological changes that occur as a direct result of the stressor are considered primary stress responses. When fish perceive a stressor, the hypothalamus stimulates the chromaffin cells located in the walls of the cardinal vein, in the head kidney region, to release catecholamines (epinephrine and norepinephrine) (Reid et al., 1998). These hormones increase heart rate and increase blood glucose levels through glycogenolysis, gluconeogenesis, and through the inhibition glycolysis (Hazon and Balmet, 1998). Catecholamines are also responsible for an increase in the oxygen transport capacity of the blood (Wendelaar Bonga, 1997).

In fish, catecholamines also increase gill permeability and ion exchange rates (Hazon and Balmet, 1998). In addition to the release of catecholamines, the hypothalamic-pituitary-interrenal-axis (HPI) is stimulated (Schreck et al., 2001). The hypothalamus releases corticotropin-releasing hormone (CRH). CRH then binds to receptor cells in the anterior pituitary, causing the release of adrenocorticotropic hormone (ACTH), which in turn stimulates the release of corticosteroids from the interrenal cells located in the head kidney. The primary corticosteroid in fish is cortisol, which plays important roles in the mobilization of energy stores, and

osmoregulation (Hazon and Balmet, 1998). Cortisol is believed to have a negative feedback effect on the secretion of both CRH and ACTH (Sumpter, 1997).

In addition to the release of catecholamines and cortisol, other factors are released into the blood in response to stress including, prolactin, somatolactin, growth hormone, and urotensin-1, all of which have been described as playing a role in osmoregulation in fish (Wendelaar Bonga, 1997). It is unclear whether these hormones play a role in the stress response or if fluctuating levels of these hormones are linked to physiological responses to osmoregulatory dysfunction, and not a direct result of the stress response (Wendelaar Bonga, 1997).

Physiological changes that occur as an indirect result of the perception of stress are considered secondary stress responses. An increase in plasma glucose is believed to be caused both by increased levels of corticosteroids and catecholamines (McDonald et al., 1997) and is considered a secondary stress response (Mazeud et al., 1977). Epinephrine also increases gill permeability, which leads to a loss of plasma ions and hemodilution when fish are in a freshwater environment, and hemoconcentration when fish are in a salt-water environment (McDonald et al. 1997). The change in plasma ion concentration is also considered a secondary stress response (Mazeud et al. 1977).

The stress response can be divided further to include the tertiary responses or whole body effects, including decreases in growth rate and reproductive function, and further still into quaternary effects, or those that affect the entire population and affect larval recruitment and ecosystem dynamics (Wedemeyer, 1996).

Because the rise in plasma cortisol takes several minutes, it is possible to anesthetize two or three fish at the same time and measure their response to an induced stressor, and not their response to being netted and anesthetized (Wedemeyer et al., 1990). Cortisol is stable when the plasma is kept in the freezer, which also makes its measurement practical (Wedemeyer et al., 1990).

Although an increase in catecholamines is directly related to the stress response, and methods are available to measure catecholamine concentrations in blood plasma, the simple act of netting the fish can cause the release of catecholamines, which makes it extremely difficult to measure baseline levels. Catecholamines are also released in very small amounts, which fluctuate rapidly, so even slight stresses can cause large variation. Normal frozen storage of plasma will not prevent catecholamine degradation making the measurements inaccurate and causing a major problem if many fish are to be sampled at the same time (Wedemeyer et al., 1990).

Reproduction in teleosts

Striped bass are an anandromous fish species, meaning that they live in a salt or brackish water environment, and they migrate into freshwater rivers to spawn. Spawning rituals can appear violent to the onlooker and have been dubbed “rock Fights”, as several males chase one female as she prepares to ovulate (Cooper, 1983). Striped bass are also iteroparous fish (Sullivan et al., 1997), meaning they will spawn for several consecutive years after they become sexually mature (Bond, 1996). In the wild, striped bass females reach maturity between three and seven years of age, while

male striped bass are believed to mature by age three (Sullivan et al., 1997). In captivity, female striped bass reach sexual maturity faster than wild fish, reaching maturity between the ages of three and four (Sullivan et al., 1997). Individual female striped bass often have to go through one full gametogenic cycle before a large quantity of viable gametes will be produced (Sullivan et al., 1997). Captive striped bass males can become mature at age one, two, or three. depending on environmental and genetic factors (Hassin et al., 2000).

Reproduction in striped bass as well as other teleosts is regulated by the hypothalamus-pituitary-gonadal axis. Several forms of gonadotropin-releasing hormones (GnRH) have been discovered in striped bass and other species, including salmon GnRH (sGnRH), chicken GnRH-II (cGnRH-II), and sea bream GnRH (sbGnRH) (Chow et al., 1998). Presently a total of 12 GnRHs have been discovered across vertebrate taxa, but their functions remain unclear, though different forms of GnRH in the same species may have separate functions (Carolsfeld et al., 2000). GnRH stimulates the release of two forms of gonadotropin from the pituitary (GTH-I and GTH-II) (Van Der Kraak et al., 1998) depending upon the maturational stage of the fish. In some species, primarily the cyprinids, there are compounds that actively inhibit GTH II release, including dopamine (Pankhurst and Van Der Kraak, 1997). Querat (1995) investigated the structural, evolutionary, and functional similarities of GTH-II to luteinizing hormone (LH) and GTH-I to follicle stimulating hormone (FSH), and concluded that their similarities may merit the change in nomenclature of GTH-II to LH, and GTH-I to FSH. Currently, both descriptions of these hormones are in use by scientists, but for the sake of clarity, GTH-I and GTH-II will be used to

describe the gonadotropins in fish for the rest of this paper. In addition to GnRH, it is believed that other substances can elicit the secretion of GTH-I and GTH-II, including other hormones and growth factors (Van Der Kraak et al., 1998). GTH-I circulates in high levels during growth and development of the gonad, while GTH-II is secreted closer to the spawning period and initiates final maturation of gametes leading up to ovulation in females and spermiation in males (Van Der Kraak et al., 1998).

In females, gonadotropins (primarily GTH I) stimulate the synthesis and release of 17β-estradiol (E_2), which stimulates the liver to synthesize and release vitellogenin, an egg yolk precursor that supplies an energy source for developing embryos, and is absorbed into the oocytes (Pankhurst and Van Der Kraak, 1997). The progestogens 17,20β,21-trihydroxy-4-pregnen-3-one (17,20β,21-P) and 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P) are involved in final oocyte maturation in female striped bass, through direct action on the oocyte membrane (Mylonas and Zohar, 2001).

In male fish, reproductive development involves the formation of spermatozoa in the testis, which is called spermatogenesis. Spermatogenesis can be broken down into spermatocytogenesis, or the series of mitotic divisions of the spermatogonia to form primary spermatocytes. Primary spermatocytes then undergo meiosis to produce secondary spermatocytes. Secondary spermatocytes then undergo another meiotic division forming spermatids. Spermatids then go through a process called spermiogenesis, which results in flagellated spermatozoa (Schulz and Miura, 2002). The process in which the testis become hydrated, and is associated with the ability to express milt, is known as spermiation (Sullivan et al., 1997).

In mammals, the functional roles of LH (GTH-II in fish) and FSH (GTH-I in fish) have been described as stimulating leydig cells to produce androgens, and stimulating sertoli cells to activate germ cell development, respectively. However, in fish the functions of GTH-I and GTH-II overlap; both have been shown to stimulate androgen production (Schulz and Miura 2002). In male striped bass, implants of gonadotropin-releasing hormone analogue (GnRHa) initiate an increase in GTH-II levels, and a subsequent increase in milt production (Mylonas et al., 1998). Progestogens are believed to play an important role in inducing fish to spermiate, but in wild striped bass captured on their spawning grounds, high levels of GTH-II corresponded to increased milt volumes, while progestogen levels remained low (Mylonas et al., 1997). The role of GTH-I in male striped bass has not been elucidated, but it is believed to stimulate spermatogenesis (Mylonas et al., 1997).

11-ketotestosterone (11-KT) is an important fish specific androgen having a stimulatory effect on spermatogenesis (Schulz and Miura, 2002) and reproductive behavior (Pall et al., 2002). Testosterone (T) is also linked to reproductive behavior in some species and is the precursor of 11-KT (Schulz and Miura, 2002). A balance between 11-KT and T is important for spermatogenesis to occur in fish because there is evidence that 11-KT stimulates spermatogenesis, while T has an inhibitory effect on the release of gonadotropins from the pituitary (Schulz and Miura, 2002), though this relationship has not been demonstrated in striped bass. In striped bass a pre-spawning peak is seen in these androgens which is believed to be associated with the growth of the testes (Sullivan et al., 1997). T and 11-KT have been used as indicators of sexual maturation in striped bass males (Woods and Sullivan, 1993).

Environmental cues are essential for reproductive development to occur in male and female striped bass. The most important environmental factors are temperature and photoperiod (Sullivan et al., 1997). Changes in photoperiod affect circulating levels of GTH-II and GnRH through the increase or decrease in melatonin secretions from the pineal gland (Van Der Kraak et al. 1997). In striped bass exposed to mid Atlantic photothermal conditions, gonadal development begins in the fall and is believed to be in response to shortening day lengths (Sullivan et al., 1997). Peaks of gonadal steroids occur when day lengths are shortest in the winter months (Sullivan et al., 1997). Increases in water temperature in the spring are believed to induce final oocyte maturation in female striped bass (Sullivan et al., 1997). In rainbow trout, a similar relationship between environmental cues and sexual maturation exists. When groups of female rainbow trout were exposed to identical photoperiods, but varying water temperatures, sexual maturation followed similar patterns in both groups of fish, causing the author to conclude that the driving force behind reproductive development in female rainbow trout is photoperiod, while temperature plays a role in modulating reproductive development (Davies and Bromage, 2002). Understanding environmental controls on reproductive development is important for aquaculture, so that environmental conditions can be controlled and fry can be produced year round from fish that spawn seasonally (Davies and Bromage, 2002, Tate and Helfrich, 1998).

Stress and reproduction

One of the tertiary effects of stress is its negative impact on reproduction. CRH is believed to be responsible for the negative effects of stress on reproduction, but Wendelaar Bonga (1997) clarifies that the neuroendicrine mechanism for this relationship has yet to be described in fish. Using CRH-deficient knockout mice, Jeong et al., (1999) challenged the theory that CRH was the main hormone responsible for reproductive dysfunction caused by the stress response. The mice lacking the CRH gene still had suppressed reproductive function when exposed to a confinement stress. In fish, it is clear that CRH plays an integral role in the hypothalamic-pituitary-interrenal axis, but it is not known if this is the only way in which CRH plays a role in inhibiting reproductive function in fish (Wendelaar Bonga, 1997). In fish, the only mechanism through which CRH and ACTH have a negative effect on reproductive function is through their stimulatory effect on cortisol release (Sumpter, 1997).

Determining exactly how stress inhibits reproductive function in fish has proven to be a difficult task. Carragher and Sumpter (1990) showed that cortisol depressed ovarian secretions of T and E_2 *in vitro* in rainbow trout. These results could not be repeated by Pankhurst et al., (1995). *In vitro* studies on goldfish (*Carassius auratus*), common carp, and New Zealand snapper found that cortisol treatments of the ovary did not inhibit the release of T or E_2, and in some cases levels of T and E_2 were increased by cortisol treatments, indicating that cortisol does not inhibit steroidogenisis at the level of the ovary (Pankhurst et al., 1995). However, rainbow trout injected with cortisol had a significant decrease in T and E_2 levels, but levels of

gonadotropins were unchanged (Pankhurst and Van Der Kraak, 2000). The original hypothesis proposed by Pankhurst et al., (1995) after the first *in vitro* experiment, was that the inhibition of steroidoegenesis in response to cortisol happens somewhere above the level of the ovary. After the *in vivo* study showed that cortisol treatment decreased T and E_2 levels, but GtH levels remained unchanged, they hypothesized that cortisol somehow interfered with GtH signal transduction (Pankhurst and Van Der Kraak, 2000). It is apparent that cortisol mediates a decrease in E_2, T, and 11-KT levels, but the exact mechanism remains unclear.

Teitsma et al., (1998) investigated the close association between rainbow trout E_2 receptor (rtER) and rainbow trout glucocorticoid receptor (rtGR) to try to explain possible mechanisms for the inhibitory effect of cortisol on reproductive function. They found that rtGR are present in both GTH-II cells and dopamine-producing cells, showing a possible pathway for cortisol to inhibit GtH release. Dexamethasone (DEX), a super active synthetic analogue of cortisol, was shown to bind to rtER in hepatic cells, inhibiting the release of vitelogenin and showing another possible mechanism for cortisol's inhibitory effect on reproduction (Teitsma et al., 1998).

In male common carp, the secretion of GTH-II seems to be unaffected by cortisol, though T levels decreased in cortisol treated animals, and gonadosomatic indexes were higher in animals that did not receive the treatment (Consten et al., 2001).

When discussing the effects of stress on reproductive function in fish, it is important to consider the diversity of its effects that have been identified between species and their influence on reproduction (Schreck et al., 2001).

The heritability of the stress response

The ability to selectively breed terrestrial animals for stress responsiveness has been demonstrated in the Japanese quail (*Coturnix coturnix*) (Carsia et al., 1988), the turkey (*Meleagris gallopavo*) (Brown and Nestor 1973), and domestic fowl (*Gallus domesticus*) (Carsia and Weber, 1986). Because of the many negative physiologic effects associated with the stress response in fish and the high propensity towards stress in the aquaculture environment, the desire to selectively breed fish for attenuated stress responsiveness has inspired some interesting studies. First it was determined that some individual rainbow trout have consistently high cortisol stress responses and other individuals have consistently low cortisol stress responses to a standardized stress (Pottinger et al., 1992). It has also been shown that HCR broodstock produce HCR progeny, and LCR broodstock produce LCR progeny in Atlantic salmon (Fevolden et al., 1991), rainbow trout (Fevolden et al., 1991 and Pottinger et al., 1994) and in common carp (Tanck et al., 2001). In the species in which it has been studied, the heritability of the stress response has been moderate to high.

Heritability can be defined as the percentage of a phenotype that is due to genotype expression (Damron, 2003). Heritability is expressed as a number from 0 to 1, with a value of zero meaning that genetics do not play a role in the expression of a given phenotype, and a value of 1 meaning that expression of that phenotype is completely reliant on genetic factors. When calculating the heritability of the stress response in fish, the variance within a given progeny group is compared to the

variance across progeny groups, if the within progeny group variance is relatively low, then a large genetic component exists, and if the within progeny group variance is relatively high then there is less of a genetic component present (Tanck et al., 2001). In common carp, the heritability of increased plasma cortisol in response to a standardized acute stress was 0.60 (Tanck et al., 2001). In rainbow trout the heritability of the cortisol stress response was 0.41 in an experiment conducted by Pottinger and Carrick (1999b), and 0.50 in an experiment conducted by Fevolden et al., (2002). Pottinger and Carrick (1999b) also calculated separate heritabilities for male and female parents and found the heritability from male parents was 0.27, and from female parents was 0.41.

Although the cortisol stress response has a high heritability in the few species in which it has been calculated, the value in selecting fish for high or low cortisol stress responsiveness is less clear. There is no published information on the heritability of the cortisol stress response or any other trait in striped bass, but calculating heritabilities will be essential for effective striped bass selective breeding programs (Harrell, 1997).

MATERIALS AND METHODS

Experimental fish

Second generation captive male striped bass from the 1999 year class were moved from the Crane Aquaculture Facility located on the Gunpowder River east of Baltimore MD to a recirculating system on campus at the University of Maryland in February of 2002. Fish were held in an 8600-l fiberglass tank that was part of a larger recirculating system. High quality filtered water at a salinity of five parts per thousand (ppt) was circulated through the system. Dissolved oxygen was injected through an oxygen cone into the system as needed to maintain dissolved oxygen levels of 6 mg/l or greater. Ozone injection and foam fractionation was used to eliminate dissolved organic matter. The system pH was monitored on a daily basis and was held between 7.5 and 8.2 by adding sodium bicarbonate as needed. Nitrogenous waste concentrations were controlled through the use of biological filtration and continual gradual water changes (total ammonia nitrogen < 0.5 mg/l, total nitrite nitrogen < 0.3 mg/l, total nitrate nitrogen < 100 mg/l. Calcium concentrations were kept between 150 and 200 mg/l).

Experimental fish were fed a specially formulated striped bass broodfish diet (Ziegler Bros., Gardners, PA) *ad libitum* twice daily. Feed was withheld two days before to all samplings. Experimental fish were kept on a blocked photoperiod that consisted of 14h light and 10h of darkness from their arrival into the new facility until November of 2002, when the photoperiod was switched to 10h of light and 14h of darkness. The photoperiod was then returned to 14h light and 10h dark in February

2003. Fish were kept on a thermal regime that simulated the water temperature conditions of the mid-Atlantic region. On July 17, 2002 all fish (n = 67) in the experimental tank were weighed and measured (total length), and their PIT (passive integrated transponder, Avid®) tag numbers were recorded.

Determining high and low cortisol stress responsiveness

To determine which striped bass had a consistently higher relative cortisol stress response and which striped bass had a consistently lower relative cortisol stress response, the fish were exposed to standardized net challenges. A fish concentrator (a two part screen that closes to concentrate fish) was placed into the tank containing the population of 67 experimental fish. The fish were then sequentially exposed to a two-minute out of water net challenge. Fish were captured two at a time, and each held in a separate net. Fish were then placed into a 1600-l holding tank (four fish per tank) until one hour had elapsed. Fish were then anesthetized in a 300-l water bath containing MS-222 (tricaine methanesulfonate, Finquel®, Argent Laboratories, Redmond, WA) at a concentration of 150 ppm and buffered with sodium bicarbonate to a pH of 7.8. Fish were then bled (1.5 ml) from their caudal vasculature using 3 ml heparinized syringes fitted with 21-gauge needles. Blood samples were kept on ice until transfer into individual 1.5ml microfuge tubes and centrifuged in a refrigerated centrifuge (Labofuge 400 R, Hraeus Instruments 10,000 X g). Plasma was distributed into 250µl microfuge tubes and stored at –20º C.

Because plasma cortisol levels were only measured at one time point post stress (one hour), and the term response usually indicates a change, the argument can

be made that post stress cortisol levels were measured, which is not the same as measuring a response. Because it is assumed that circulating levels of cortisol are lower prior to stress compared to post stress then the increase at one hour can be thought of as a change. The two foremost authorities on selecting for stress responsiveness, Pottinger and Fevolden, both measure post stress cortisol levels at one point post stress and consider fish high or low stress responding based on such measurements. The "responsiveness" nomenclature will be used throughout this paper.

Sampling continued once every four weeks for four samples starting on August 7, 2002. A two-minute net challenge was used because previous research in our laboratory showed that male striped bass broodstock may have adapted to a one minute, out of water net challenge (Wang et al., 2003). At one hour post stress, mean plasma cortisol levels for each fish were determined across all four sample dates. Means were ranked. The fish with the ten highest mean cortisol values were designated high cortisol responders (HCR), and the fish with the ten lowest mean cortisol values were designated low cortisol responders (LCR). Fish were weighed and measured on all sample dates. Coefficient of condition ($K= 100{,}000\ W/L^3$) (Williams, 2000) and specific growth rate for weight and length ($SGRW = [(\ln W_2 - \ln W_1)/(t_2 - t_1)]100$ and $SGRL =[(\ln L_2 - \ln L_1)/(t_2 - t_1)]100$ where W_1, W_2, L_1, and L_2 are the weights and lengths at the beginning and end of a sample period and $t_1 - t_2$ is the length of time in days between samples) was measured and compared between responder groups.

Baseline determinations

Attempts to quantify "baseline" plasma levels of cortisol and glucose were made before and after the first four sample dates (July 17, and November 20). The fish concentrator was placed into the tank. Fish were removed from the tank without the two-minute standardized net challenge, and immediately anesthetized and bled as described above.

Plasma cortisol and androgen concentrations before to spawning

Stress sampling of the entire population (n =67), as described for the stress response determination sampling, continued at four week intervals from November 2002 through March 2002. In addition to measuring plasma levels of cortisol and glucose, T and 11-KT were also measured. Starting in December 2002, in addition to taking blood samples from all experimental fish on sample dates, the fish's abdomens were gently squeezed to see if spermiation was occurring.

Stress induction and sampling during the spawning season

On March 24, 2003 the 20 fish designated as HCR and LCR were moved from the 8600 l tank and randomly assigned to one of five 1600 l tanks at a stocking density of four fish per tank. Fish were stressed and sampled three times per week for five weeks starting on March 31, 2003. Every Monday all fish were anesthetized (MS 222), and blood samples were taken from the caudal vasculature using heparinized syringes and processed as described above. Every Wednesday all fish were

anesthetized (MS 222), and sperm samples (≈0.5ml) were collected into 1.5 ml microfuge tubes by gently, but firmly, pressing on the sides of the fish's abdomen. Every Friday fish were anesthetized (MS 222), bled from the caudal vasculature, and weighed and measured. In addition to measuring plasma levels of cortisol, T, 11-KT, and glucose, hematocrits were also run on all blood samples taken during the spawning season. Hematocrit was determined by filling and sealing 3 heparinized microhematocrit tubes (Clay Adams, New York) with whole blood, per fish sample. The tubes were centrifuged for 5 minutes in a hematocrit centrifuge (Autocrit Ultra3, Becton Dickinson, Sparks, MD). Hematocrit was determined using a standard hematocrit reader. Before the fish were anesthetized on each sample day, they were held out of water in a net for thirty seconds. The standardized net challenge employed three times per week ensured that all fish were sufficiently stressed during the spawning season sampling.

Sperm quality analysis

Sperm samples were stored on ice until samples from all four fish in one tank had been collected. Those four samples were then analyzed before moving on to the next tank. A toothpick was used to remove a sperm sample and place it on a Mackler counting chamber containing 10 μl of deionized ultrafiltered water (Fisher scientific) to initiate activation. Activation is the transformation that spermatozoa undergo when they change from a state of inactivity to a state of vigorous activity associated with the pursuit of an egg to fertilize. Sperm samples were analyzed using a phase contrast microscope (Zeiss model D-7082, 400X) linked to a video camera, which was

connected to a television with a videocassette recorder. Duration of activation was measured as the time from placing the sample on the counting chamber until forward progression of sperm cells stopped. Percent motile was measured as the number of cells in a field at the end of motility minus the number of cells that were in that field and not moving at the beginning of activation, divided by the total number of cells in the field. Each sperm sample was activated and measured three times. All activations were recorded for later review.

Spermatocrit was measured by injecting sperm samples into capillary tubes using a pipette. The tubes were centrifuged at 13000 rpm (1,000 X g) for ten minutes and then read using a hematocrit reader. Spermatocrits were run in triplicate for each sample. On the first sperm sample date, spermatocrits were centrifuged for five, ten, twenty, and thirty minutes and no significant decreases were found after ten minutes, so a ten minute centrifuge time was used for the rest of the study. A significant correlation was found between spermatocrit (spun for ten minutes) and sperm density in Atlantic cod (*Gadus morhua*) (Rakitin et al. 1999).

Stress sample time course on HCR and LCR

After the spawning season HCR and LCR were placed into an 8000 l tank until October 13, 2003. At 8:00 am on the 13th, all HCR and LCR fish were exposed to a two-minute net challenge and immediately anesthetized and bled from their caudal vasculature. Fish were handled in groups of three and placed into a 1600 l holding tank after the first sample was collected. Fish were then sequentially bled at 1, 3, 6, 12, 24, and 48 hours post stress. Approximately 0.5 ml of blood was extracted

at each sampling time. Each sample was centrifuged, and plasma concentrations of cortisol and glucose were measured. Hematocrits were also run on all blood samples as described above.

Blood plasma assay procedures

After blood samples were collected (≈1.5ml during response identification sampling, and ≈1ml during spawning season sampling), they were kept on ice until centrifuged in a refrigerated centrifuge (Labofuge 400 R, Hraeus Instruments 10,000 X g). Plasma was distributed into 250µl microfuge tubes and stored at –20º C. Just before assay, plasma samples were thawed on ice and centrifuged for three minutes at 13000 rpm. Plasma cortisol was measured using an ELISA test kit (DRG Diagnostics, Mountainside, NJ) (between assay CV= 12.3%) validated for striped bass by Wang et al. (2003). Plasma testosterone was measured using an ELISA test kit (DRG Diagnostics, Mountainside, NJ) between assay CV = 4.56%. 11-ketotestosterone was measured using an ELISA test kit (Cayman Chemical, Ann Arbor, MI). Serial dilutions of a male striped bass plasma pool and serial dilutions of ether extracts from the same pool were not different from each other for each assay, and both diluted parallel to their respective (T, 11-KT and cortisol) standard curve. Plasma glucose levels were measured using the microplate procedure and the hexokinase/glucose-6-phosphate dehydrogenase enzyme kit (Sigma Diagnostics, St. Louis, MO) in blank 96 well plates, and read with a 96 well plate reader.

Statistical analysis

Repeated measures ANOVA analyses were used to assess differences between high and low cortisol responders. Homogeneity of variances was checked, and if variances were homogeneous, the following covariance structures were tested: unstructured, compound symmetry, first order autoregressive spatial power, and toeplitz. If variances were heterogeneous, the following variance structures were tested: unstructured, heterogeneous compound symmetry, heterogeneous first order autoregressive, spatial power, and heterogeneous toeplitz. The covariance structure with the best goodness of fit statistics was used for the final means comparisons and tests of significance. Differences were considered significant at $p \leq 0.05$. Results are presented as means ± SEM unless otherwise noted. Spermiation count data was analyzed using a mixed model macro specially designed for binomial data. The binomial data was transformed to the logit scale and analyzed using a repeated measures ANOVA. Means and standard error of the means were then retransformed to the percent scale for data presentation and easy interpretation. All data was analyzed using SAS v8 Mixed model analysis (SAS Institute; Cary NC).

RESULTS

Determining high and low stress responsiveness

Selected HCR had significantly higher mean plasma cortisol levels on all four stress sample dates when compared to LCR ($p<0.05$) (Fig.1). HCR also had significantly higher mean plasma cortisol levels on these dates when compared to the mean plasma cortisol levels of the population (fish not selected for high or low cortisol stress response, n=47) (Fig. 1). LCR had significantly lower mean plasma cortisol levels when compared to the population ($p<0.05$) on all sample dates except the first date, August 7th ($p=0.079$).

The mean plasma glucose levels of HCR (177±10.2 mg/dl) were not significantly different from those of LCR (151±10.2 mg/dl) when averaged across these four sample dates ($p = 0.07$), but the p-value does suggest a possible trend. Mean plasma glucose levels of HCR were significantly higher ($p < 0.05$) than the mean plasma glucose levels of LCR and the population mean on the first sample date (Fig. 2). Mean plasma glucose levels of LCR were significantly lower ($p > 0.05$) than the mean of the population on the first sample date. No differences between LCR, HCR and the population were significant for any of the stress responsiveness determination samplings.

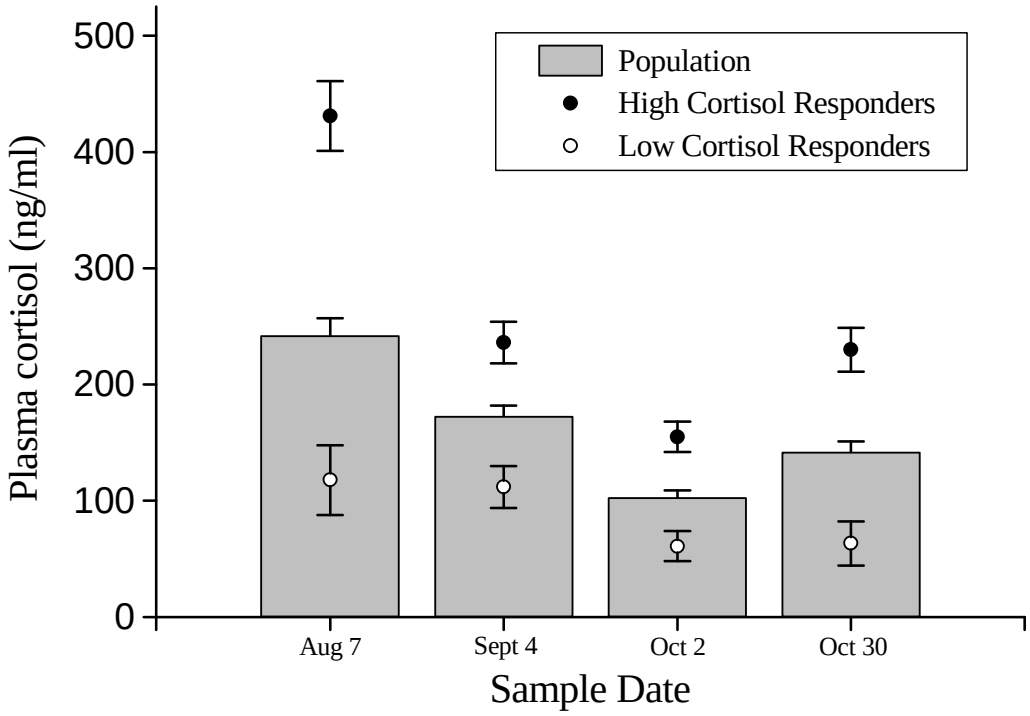

Figure 1. Mean plasma cortisol levels one hour after a two-minute net challenge for striped bass males selected for high cortisol responsiveness (HCR) (n=10), low cortisol responsiveness (LCR) (n=10) and the remaining unselected fish (population n=47). HCR mean plasma cortisol levels were significantly ($p<0.05$) greater than those of LCRs on all four sample dates. Vertical brackets represent the SEM.

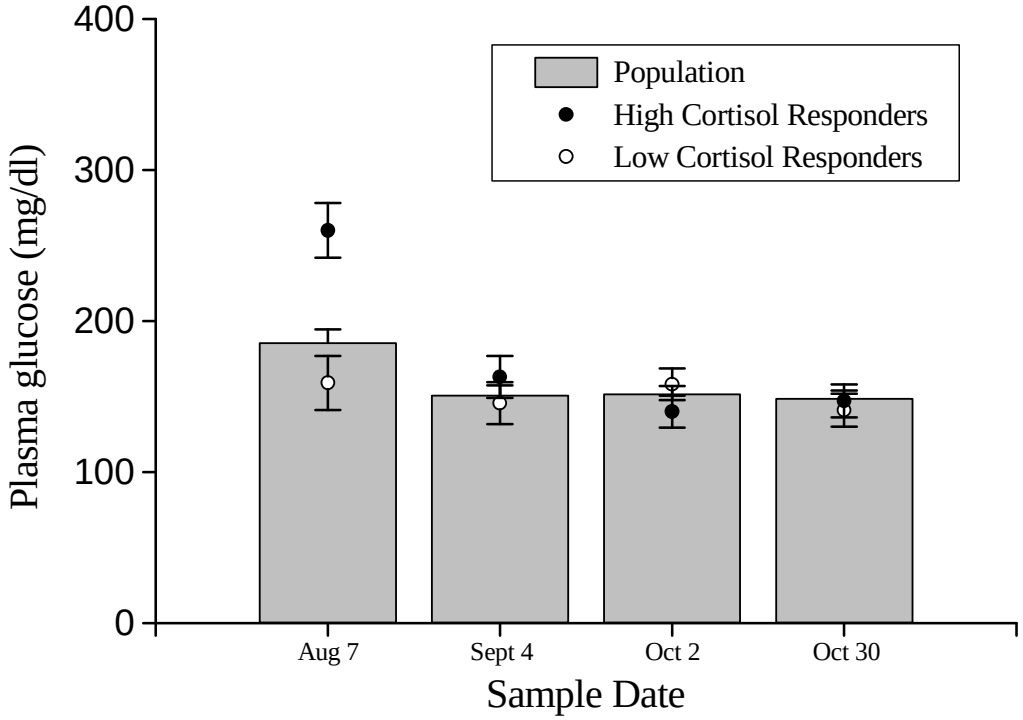

Figure 2. Mean plasma glucose levels one hour after a two-minute net challenge for striped bass males selected for high cortisol responsiveness (HCR) (n=10), low cortisol responsiveness (LCR) (n=10) and the remaining unselected fish (population n=47). HCR and LCR mean plasma glucose levels were significantly different on the first stress sample date ($p<0.05$), but the overall effect of cortisol responsiveness on plasma glucose levels was not significant ($p=0.07$). Vertical brackets represent the SEM.

Baseline determinations

The mean cortisol levels of HCR (210±22.2 ng/ml) were significantly greater than those of LCR (131±22.2 ng/ml) when averaged across both sample dates (July and November 2002) ($p = 0.013$). The difference between the mean plasma cortisol levels of HCR and LCR were not significant on the July sample date ($p = 0.09$), but the difference between LCR and the rest of the population was significant ($p = 0.01$) (Fig. 3). On the November sample date the difference between HCR and LCR was significant ($p = 0.02$), but the differences between the population and HCR and the population and LCR were not significant ($p>0.05$) (Fig. 3).

Mean plasma glucose levels of HCR (107 ± 5.8) were not significantly different from those of LCR (110 ± 5.8) when averaged across both non-stress challenge sample dates ($p = 0.77$). The effect of sample date on plasma glucose levels was significant ($p < 0.0001$) (Fig. 4).

Differences in growth

Differences in growth parameters between HCR, and LCR were analyzed from sample dates starting in August 2002 and continuing through November 2002. Growth estimates from December 2002 through April 2003 were not analyzed due to the confounding effects of testes hydration on body weight. No significant differences ($p > 0.05$) in mean weight, length, or coefficient of condition were found between HCR and LCR from the August through November sample dates (table 1). However, the difference in mean specific growth rate, calculated for weight, between HCR (0.25 ± 0.022)

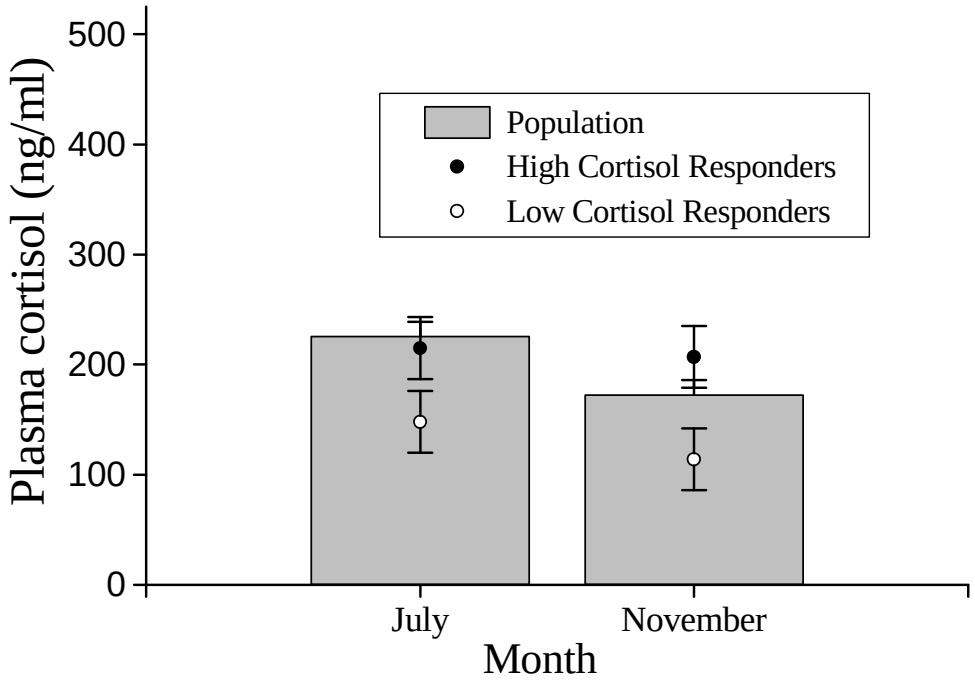

Figure 3. Mean plasma cortisol levels prior to and after monthly stress sampling. Fish were not exposed to a net challenge prior to sampling. Figure shows striped bass males selected for high cortisol responsiveness (HCR) (n=10), low cortisol responsiveness (LCR) (n=10) and the remaining unselected fish (population n=47). The overall difference between HCR and LCR mean plasma cortisol levels was significant ($p = 0.014$). Vertical brackets represent the SEM.

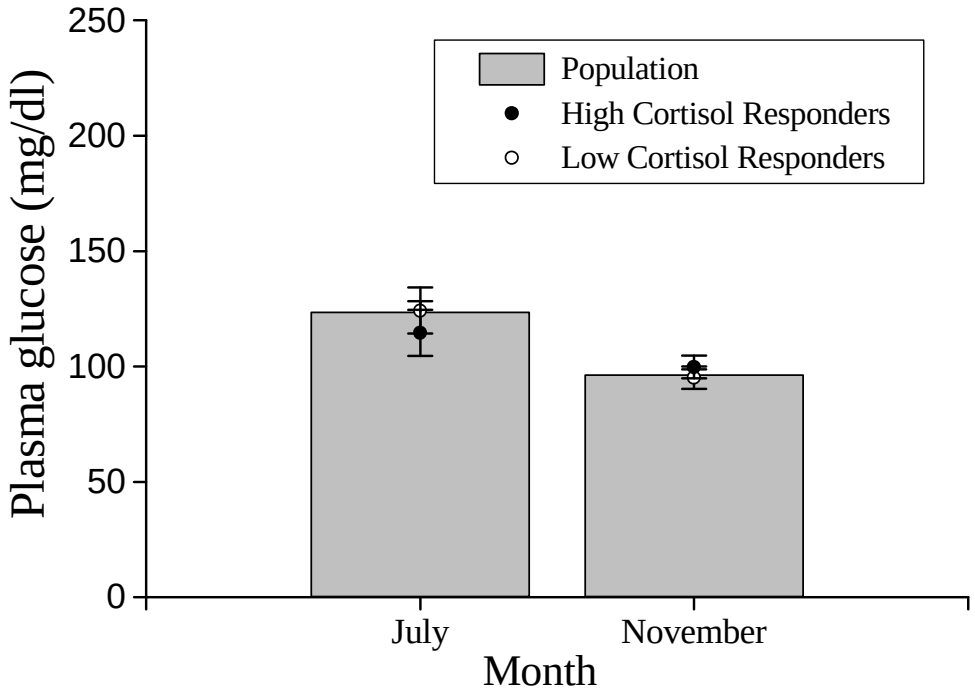

Figure 4. Mean plasma glucose levels prior to and after monthly stress sampling. Fish were not exposed to a net challenge prior to sampling. Figure shows striped bass males selected for high cortisol responsiveness (HCR) (n=10), low cortisol responsiveness (LCR) (n=10) and the remaining unselected fish (population n=47). The overall effect of cortisol responsiveness on mean plasma glucose levels was not significant ($p = 0.90$). The effect of sample date on plasma glucose levels was significant ($p < 0.0001$). Vertical brackets represent the SEM.

Table 1. Mean ± SEM Length, weight, and Coeficient of condition for high (HCR) and low cortisol responding (LCR) populations on monthly stress sample dateds before the spawning season. No significant differences between HCRs and LCRs were found for any parameter on any sample date, but the sample date effect was significant for all parameters.

	Length (cm)			Weight (kg)			Coefficient of condition		
Sample	HCR	Pop	LCR	HCR	Pop	LCR	HCR	Pop	LCR
Jul 17	56±1.1	55 0.5	57±1.1	2.2±0.16	2.2±0.07	2.4±0.16	1.3±0.04	1.3±0.02	1.3±0.04
Aug 17	57±1.2	56±0.5	57±1.2	2.4±0.16	2.3±0.08	2.5±0.16	1.3±0.04	1.3±0.02	1.3±0.04
Sep 4	57±1.1	56 0.5	57±1.1	2.4±0.17	2.4±0.08	2.5±0.17	1.3±0.04	1.3±0.02	1.3±0.04
Oct 2	58±1.2	57±0.5	58±1.2	2.6±0.18	2.5±0.08	2.7±0.18	1.3 0.04	1.4 0.02	1.4±0.04
Oct 30	59±1.2	58±0.6	59±1.2	2.9±0.20	2.8±0.09	2.9 0.20	1.4 0.04	1.4 ±0.02	1.4±0.04
Nov 20	60±1.2	59±0.7	59 1.2	3.1±0.22	3.0±0.10	3.1±0.22	1.4±0.04	1.5±0.02	1.4±0.04

and LCR (0.17 ± 0.022) was significantly different on two sample intervals, and also significant when averaged across the five sample intervals ($p < 0.007$) (Fig.5). Specific growth rates calculated for length were not significantly different between HCR (0.056 ± 0.008) and LCR (0.038 ± 0.007) when averaged across all sample intervals, but the p-value is suggestive of a possible trend ($p = 0.10$) (Fig. 6).

Plasma hormone levels before spawning season

Mean plasma T levels of HCR were significantly higher than LCR on all sample dates ($p < 0.05$) (Fig. 6). In addition to being significantly lower than HCR, mean plasma T levels of LCR were also significantly lower than population means. Mean plasma 11-KT levels of HCR were significantly greater than LCR on all sample dates as well ($p < 0.05$) (Fig. 7). Mean plasma 11-KT levels of LCR were significantly lower than HCR and population means ($p < 0.05$). Mean plasma T and 11-KT levels of HCR were not significantly different from those of the population, but a possible trend was seen in the overall differences (T $p = 0.12$, 11-KT $p = 0.08$). Mean plasma cortisol and glucose levels of LCR, HCR, and the rest of the population were not significantly different ($p > 0.05$) during this period (Fig. 8 & 9).

Population fluctuations in plasma cortisol and androgen levels

Mean plasma cortisol levels for the entire population of striped bass males (n=67) were highest following the first stress sample (Fig. 10). Plasma cortisol levels then dropped significantly from the first through the third stress samples ($p < 0.05$). A significant increase in plasma cortisol levels was then seen from the third to fourth,

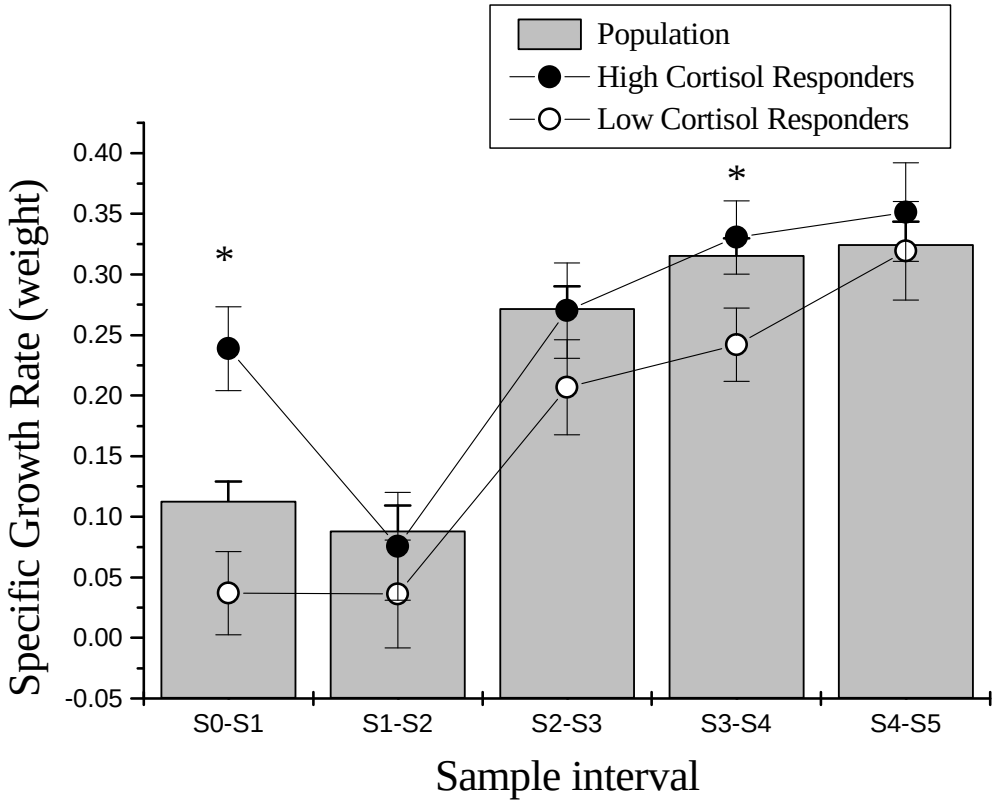

Figure 5. Mean specific growth rates (weight) for striped bass males selected for high cortisol responsiveness (HCR) (n=10), low cortisol responsiveness (LCR) (n=10) and the remaining unselected fish (population n=47) for samples taken from July to November, 2002. HCR had significantly greater specific growth rates than those of LCR on two sample intervals (*), and the differences between HCR and LCR were significant when averaged across all sample intervals. Vertical brackets represent the SEM.

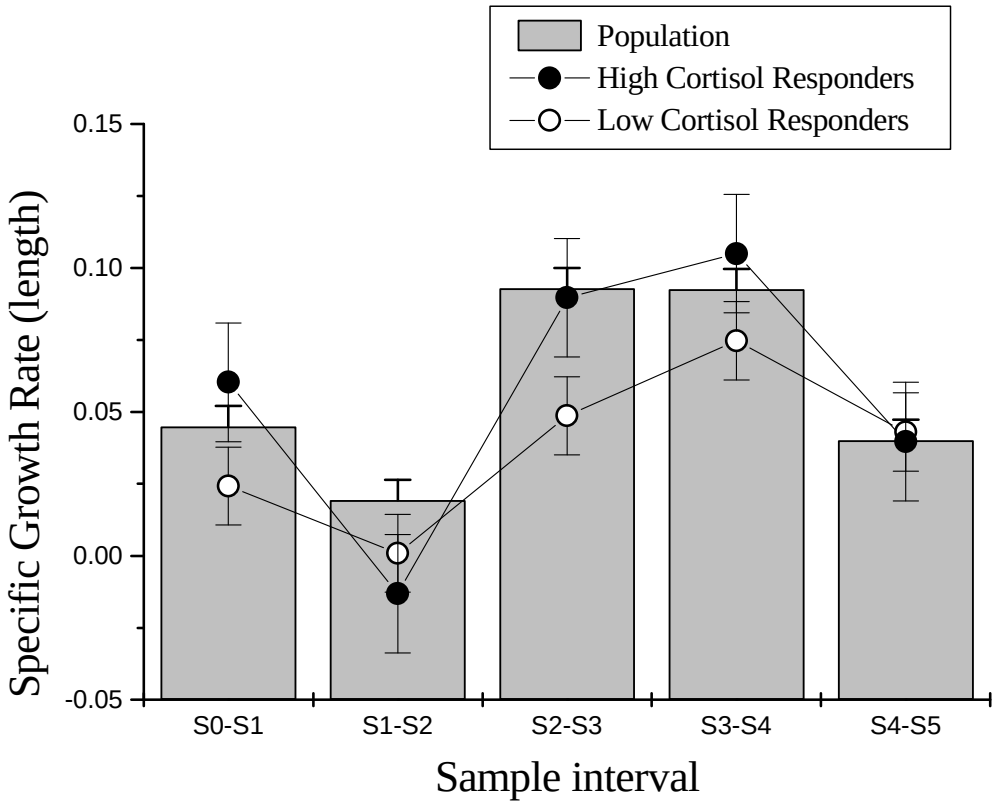

Figure 6. Mean specific growth rates (length) for striped bass males selected for high cortisol responsiveness (HCR) (n=10), low cortisol responsiveness (LCR) (n=10) and the remaining unselected fish (population n=47) for samples taken from July to November, 2002. HCR were not significantly different from LCR on any of the sample intervals, but when averaged across sample dates, the difference between HCR and LCR was suggestive of a possible trend (p=0.10). Vertical brackets represent the SEM.

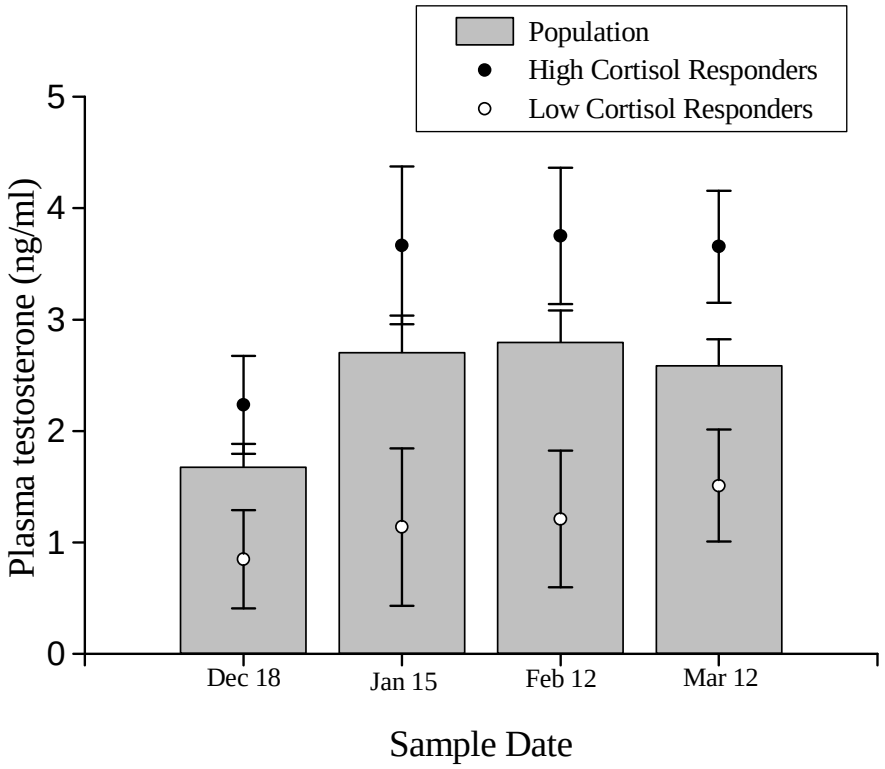

Figure 7. Mean plasma testosterone levels one hour after a two-minute net challenge for striped bass males selected for high cortisol responsiveness (HCR) (n=10), low cortisol responsiveness (LCR) (n=10) and the remaining unselected fish (population, n=47) for samples taken four months prior to the spawning season. HCR levels were significantly greater than those of LCR on all sample dates. Vertical brackets represent the SEM.

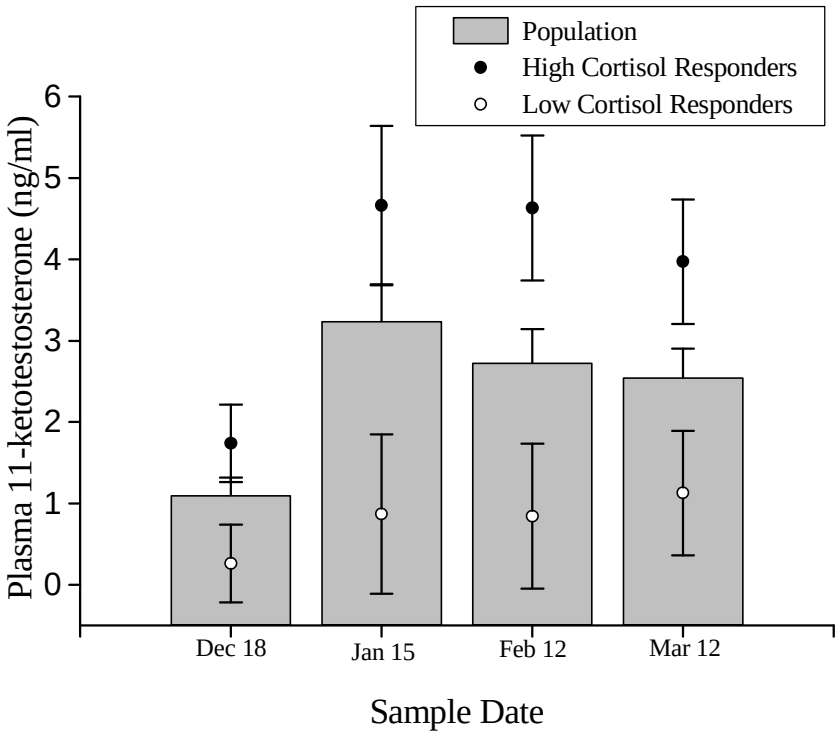

Figure 8. Mean plasma 11-ketotestosterone levels one hour after a two-minute net challenge for striped bass males selected for high cortisol responsiveness (HCR) (n=10), low cortisol responsiveness (LCR) (n=10) and the remaining unselected fish (population, n=47) for samples taken four months prior to the spawning season. HCR levels were significantly greater than those of LCR on all sample dates. Vertical brackets represent the SEM.

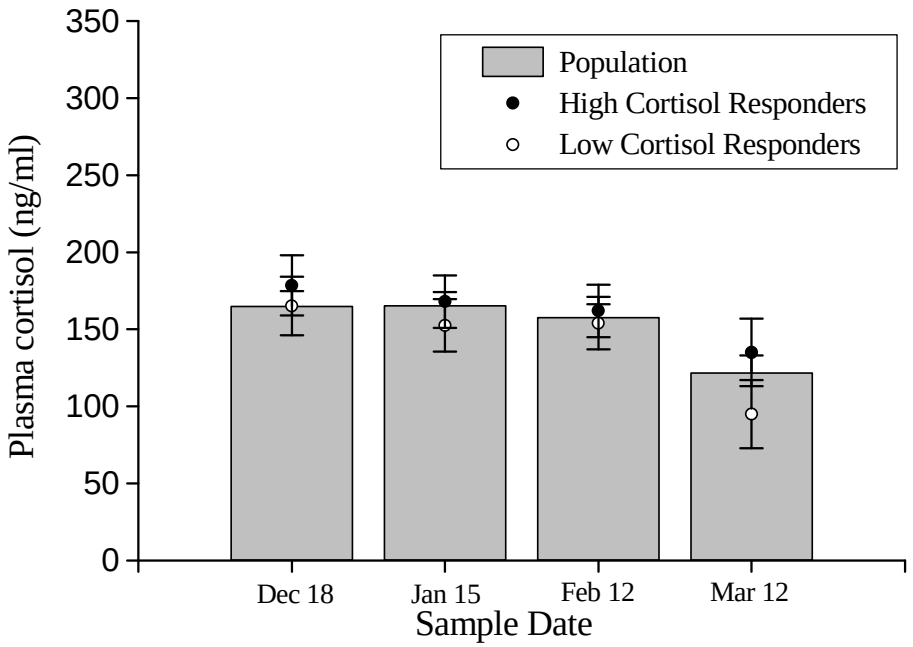

Figure 9. Mean plasma cortisol levels one hour after a two-minute net challenge for striped bass males selected for high cortisol responsiveness (HCR) (n=10), low cortisol responsiveness (LCR) (n=10) and the remaining unselected fish (population, n=47) for samples taken four months prior to the spawning season. No significant differences between responder groups were detected on any of the sample dates. Vertical brackets represent the SEM.

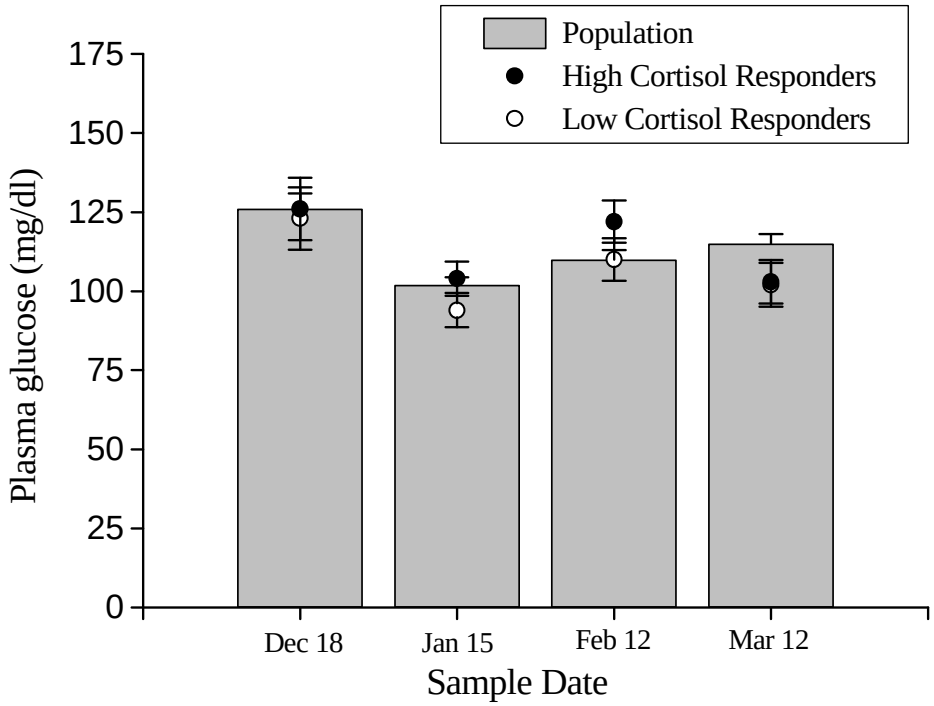

Figure 10. Mean plasma glucose levels one hour after a two-minute net challenge for striped bass males selected for high cortisol responsiveness (HCR) (n=10), low cortisol responsiveness (LCR) (n=10) and the remaining unselected fish (population, n=47) for samples taken four months prior to the spawning season. No significant differences between responder groups were detected on any of the sample dates. Vertical brackets represent the SEM.

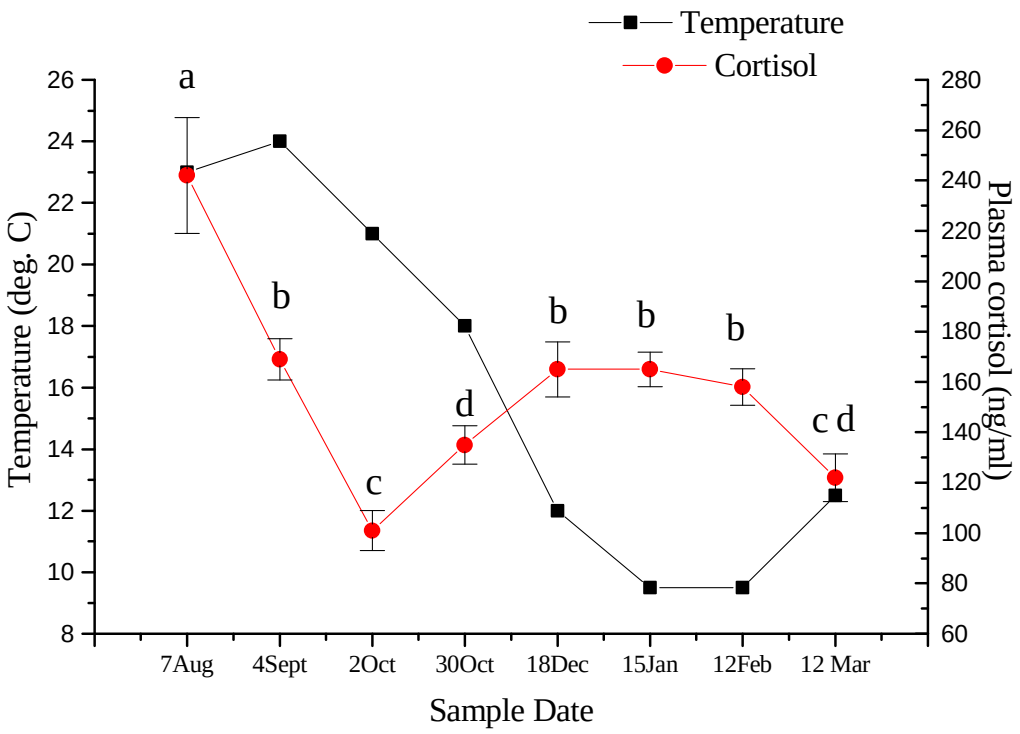

Figure 11. Mean plasma cortisol levels for the entire population (n=67) of striped bass males on all stress sample dates prior to the spawning season, plotted along with corresponding water temperatures. Identical letters signify no significant difference at $p < 0.05$. Vertical brackets represent the SEM.

and fourth to fifth stress samples. Mean plasma cortisol levels were not significantly different between stress samples five, six and seven. A significant decrease in the mean plasma cortisol level was seen from stress sample six compared to stress sample seven.

Mean plasma T and 11-KT values for the entire population of striped bass males (n = 67) followed the same trend of significance between sample dates (Fig. 11). Mean plasma androgen levels increased significantly between the first four sample dates that they were measured on. Mean plasma androgen values were not significantly different on the last three sample dates (Fig. 11).

Plasma hormone, and glucose levels of HCR and LCR during the spawning season

Mean plasma cortisol levels of HCR (122 ± 13.4 ng/ml) (n=10) were not significantly different ($p>0.05$) from those of LCR (97 +10.4 ng/ml) (n=10) when measured immediately following the thirty-second net challenge during the spawning season and averaged across all sample dates ($p = 0.16$) (Fig. 12). Although mean plasma glucose levels were significantly higher in HCR than in LCR on two sample dates (Fig. 13), the mean glucose levels of HCR (90 ± 2.6 mg/dl) averaged across all sample dates was not significantly different from LCR (85 ± 2.6 mg/dl) ($p = 0.76$). Mean plasma T and 11-KT levels were significantly greater ($p<0.05$) in HCR (T = 2.7 ± 0.46ng/ml, 11-KT = 2.2 ± 0.28 ng/ml) than in LCR (T =1.0 ± 1.7ng/ml, 11-KT = 0.75 ± 0.28 ng/ml) when averaged across all samples (Figs. 12 & 13). Mean plasma T and 11-KT levels were also significantly greater ($p < 0.05$) in HCR compared to LCR on all sample dates except the last sample date, May 5, 2002 (Figs. 14 & 15). The

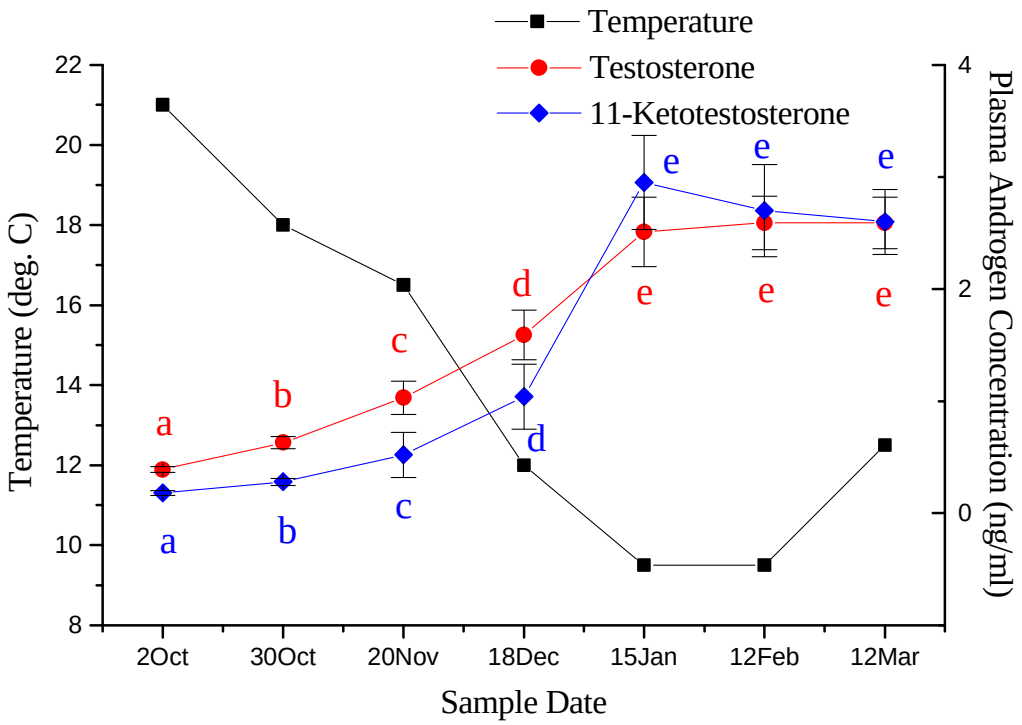

Figure 12. Mean plasma testosterone and 11-ketotestosterone levels on sample dates from October 2002 through March 2003 for the entire population (n=67) of striped bass males, plotted with corresponding water temperatures. Identical letters represent no significant difference at $p < 0.05$ comparing the same androgen on different dates. Red letters correspond to testosterone, and blue letters correspond to 11-ketotestosterone. Vertical brackets represent the SEM.

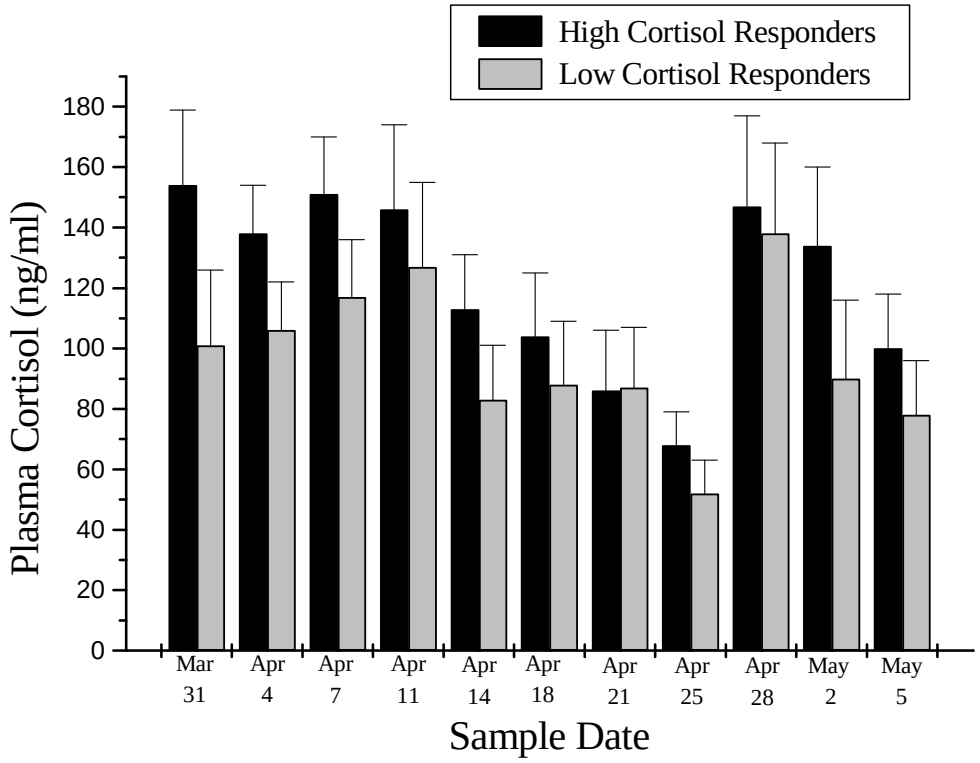

Figure 13. Mean plasma cortisol levels of samples taken immediately following a thirty second net challenge for striped bass males selected for high cortisol responsiveness (HCR) (n=10) and low cortisol responsiveness (LCR) (n=10). Samples were taken during the spawning season. HCR plasma levels of cortisol were not significantly different from those of LCRs ($p>0.05$). Vertical brackets represent the SEM.

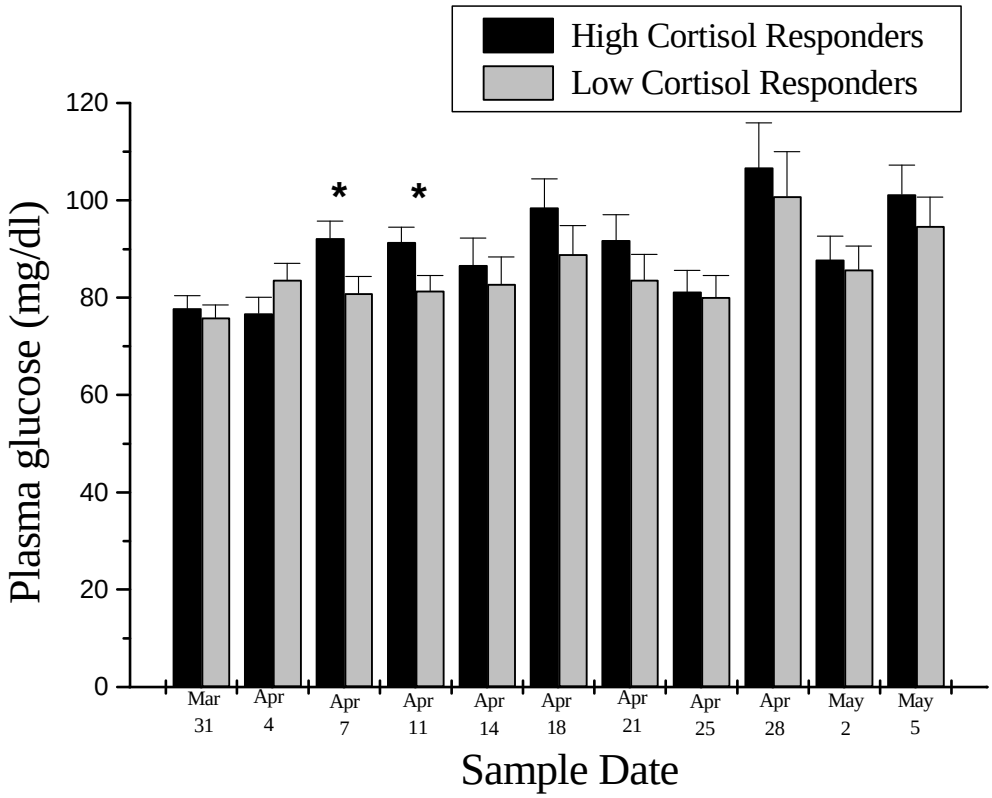

Figure 14. Mean plasma glucose levels of samples taken immediately following a thirty second net challenge for striped bass males selected for high cortisol responsiveness (HCR) (n=10) and low cortisol responsiveness (LCR) (n=10). Samples were taken during the spawning season. Although HCRs had significantly greater glucose levels on two sample dates (*), the overall difference across sample dates was not significantly different. Vertical brackets represent the SEM.

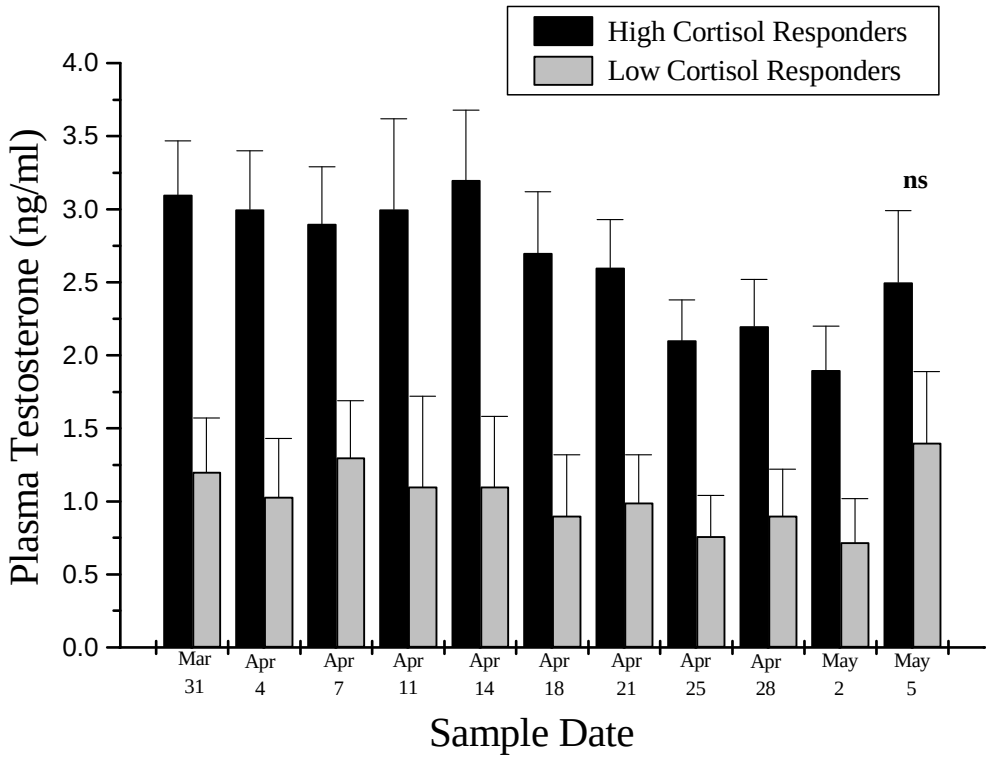

Figure 15. Mean plasma testosterone (T) levels of samples taken immediately following a thirty second net challenge from striped bass males selected for high cortisol responsiveness (HCR) (n=10) low cortisol responsiveness (LCR) (n=10). Samples were taken during the spawning season. HCR plasma levels of T were significantly greater than those of HCR on all sample dates except May 5, 2003. Averaged across all sample dates, HCR mean levels of T were greater than those of LCR ($p<0.05$). Vertical brackets represent the SEM.

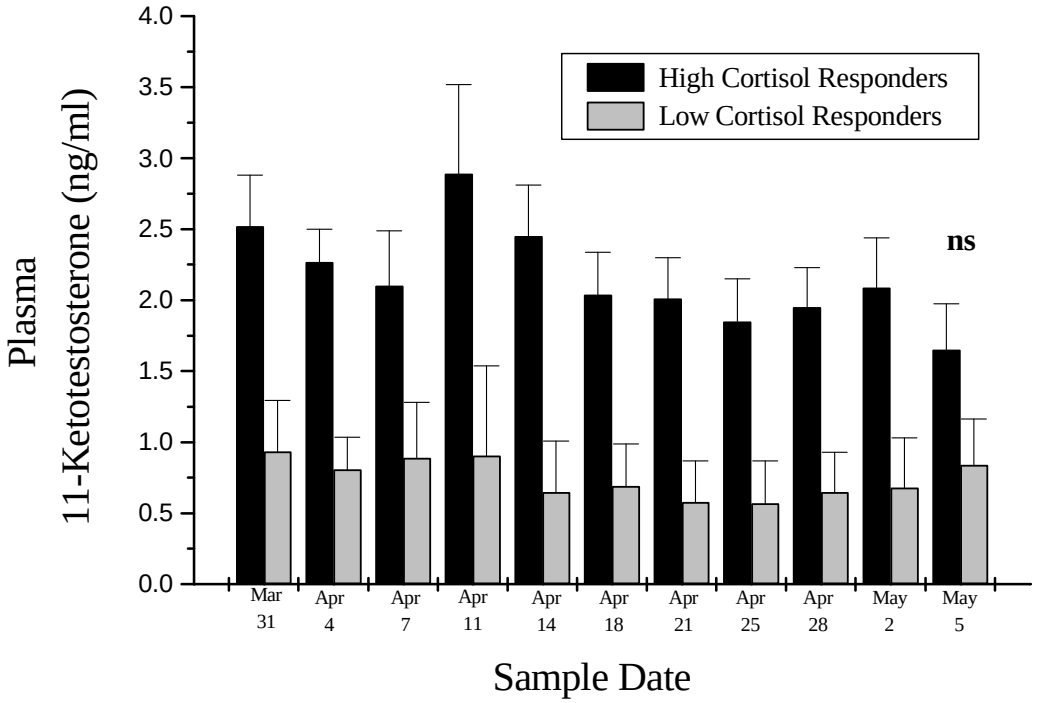

Figure 16. Mean plasma 11-ketotestosterone (11-KT) and levels of samples taken immediately following a thirty second net challenge from striped bass males selected for high cortisol responsiveness (HCR) (n = 10) low cortisol responsiveness (LCR) (n=10). Samples were taken during the spawning season. HCR plasma levels of 11-KT were significantly greater than those of HCR on all sample dates except May 5. 2003. Averaged across all sample dates, HCR mean 11-KT levels were greater than those of LCR ($p<0.05$). Vertical brackets represent the SEM.

difference in mean hematocrits of HCR and LCR during the spawning season was not significant ($p = 0.93$) (Fig 16). The effect of sample day on hematocrit was significant ($p < 0.0001$). A trend of decreasing hematocrits through the spawning season sampling was seen.

HCR and LCR sperm quality analysis

No significant differences were detected ($p > 0.05$) between HCR and LCR for mean percent motile, mean duration, or spermatocrit (Table 2). HCR fish did begin to spermiate earlier, and continued to spermiate longer than LCR fish (Fig. 17). The overall differences in spermiation between HCR and LCR were found to be significant ($p < 0.0001$).

HCR and LCR time course

Mean plasma cortisol levels of HCR were significantly different from the mean plasma cortisol levels of LCR at 0, 3, 6, 12, and 24 hours post stress ($p < 0.05$) (Fig. 18 A). The difference between mean plasma cortisol levels of HCR and LCR was not significant at 48 hours post stress. The effect of sample time on mean plasma cortisol levels was significant ($p = 0.0005$). The interaction of sample time and cortisol responsiveness was not significant ($p = 0.44$). The effect of cortisol responsiveness on plasma glucose levels was significant ($p = 0.015$). The effect of sample time on plasma glucose levels was also significant ($p < 0.0001$). The interaction between sample time and cortisol stress responsiveness was significant ($p = 0.001$) (Fig 18 B).

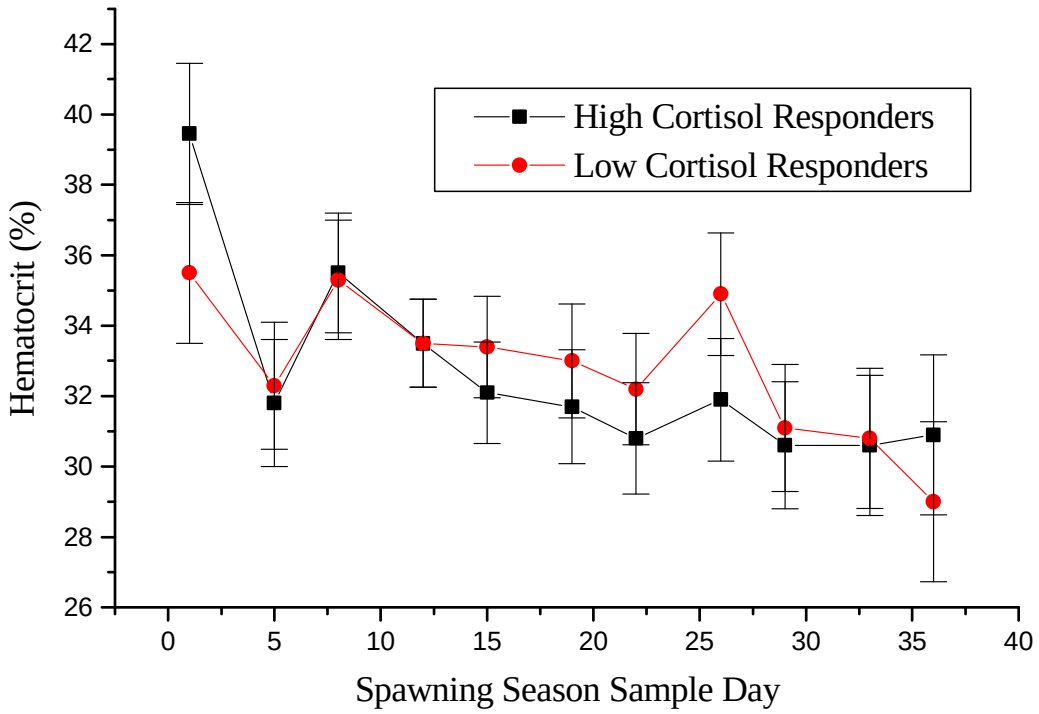

Figure 17. Mean hematocrits of samples taken immediately following a thirty second net challenge from striped bass males selected for high cortisol responsiveness (HCR) (n=10) and low cortisol responsiveness (LCR) (n=10). Samples were taken during the spawning season. HCR and LCR mean hematocrits were not significantly different during the spawning season ($p = 0.93$). The effect of sample day on mean hematocrits was significant ($p < 0.0001$). Vertical brackets represent the SEM.

Table 2. Mean ± SEM percent motile, duration of motility, and spermatocrit for high (HCR) and low cortisol responding (LCR) populations for each sample week during the spawning season. No significant differences between HCR and LCR were found for any parameter on any sample date, but the sample date effect was significant for all parameters.

	Percent Motile		Duration of Motility (s)		Spermatocrit (%)	
Sample Date	HCR	LCR	HCR	LCR	HCR	LCR
April 2	75±3.9	73±3.9	20.4±0.9	20.3±0.9	90±1.7	91±1.7
April 9	75±4.5	72±4.5	19.1±0.9	19.6±0.9	90±1.4	87±1.4
April 16	67±3.2	65±3.5	17.6±0.9	19.0±0.7	93±1.1	94±1.3
April 23	61±3.7	66±4.7	16.6±0.4	17.2±0.6	93±0.8	95±1.4
April 30	50±5.6	65±7.9	16.0±1.1	18.1±1.6	96±0.8	97±1.1

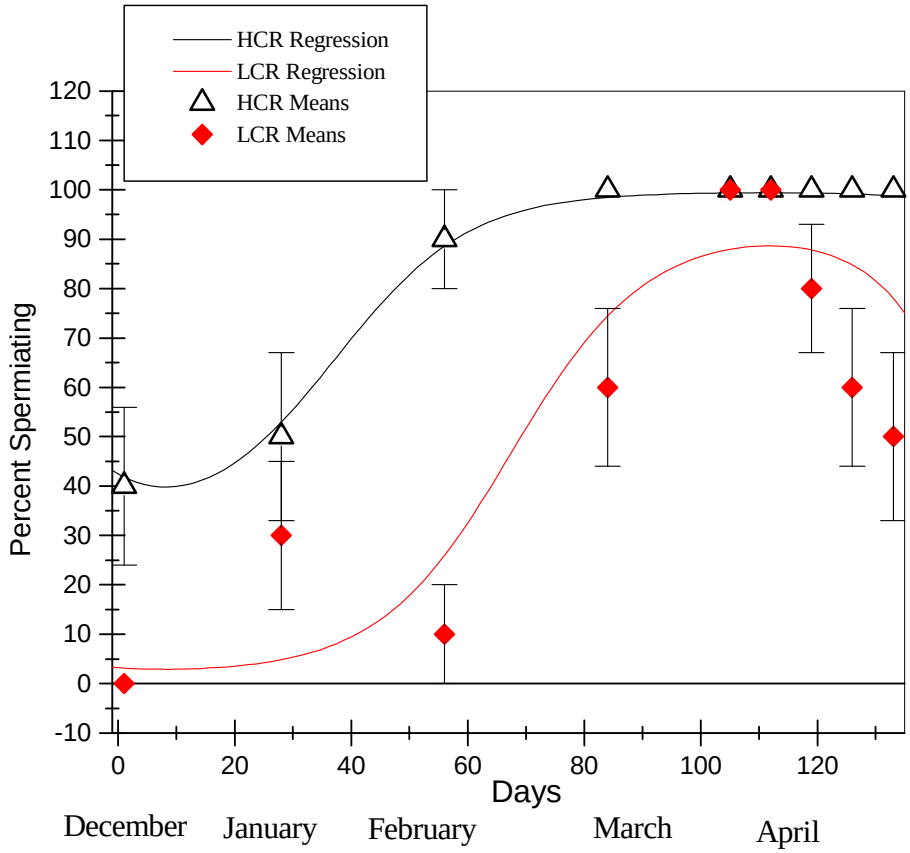

Figure 18. Mean percent of striped bass males selected for high cortisol responsiveness (HCR) (n=10) and low cortisol responsiveness (LCR) (n=10) spermiating, shown with vertical bars representing the SEM. Samples were taken monthly before the spawning season and weekly during the spawning season. Shown with regression lines fitting the data HCR (logit) $y = -0.303 - 0.0275day + 0.00180day^2 - 0.0000183*day^3$ LCR (logit) $y = -3.40 - .0275day + 0.00180day^2 - 0.0000183day^3$. The difference between HCR and LCR number spermiating across all sample dates was significant ($p < 0.0001$).

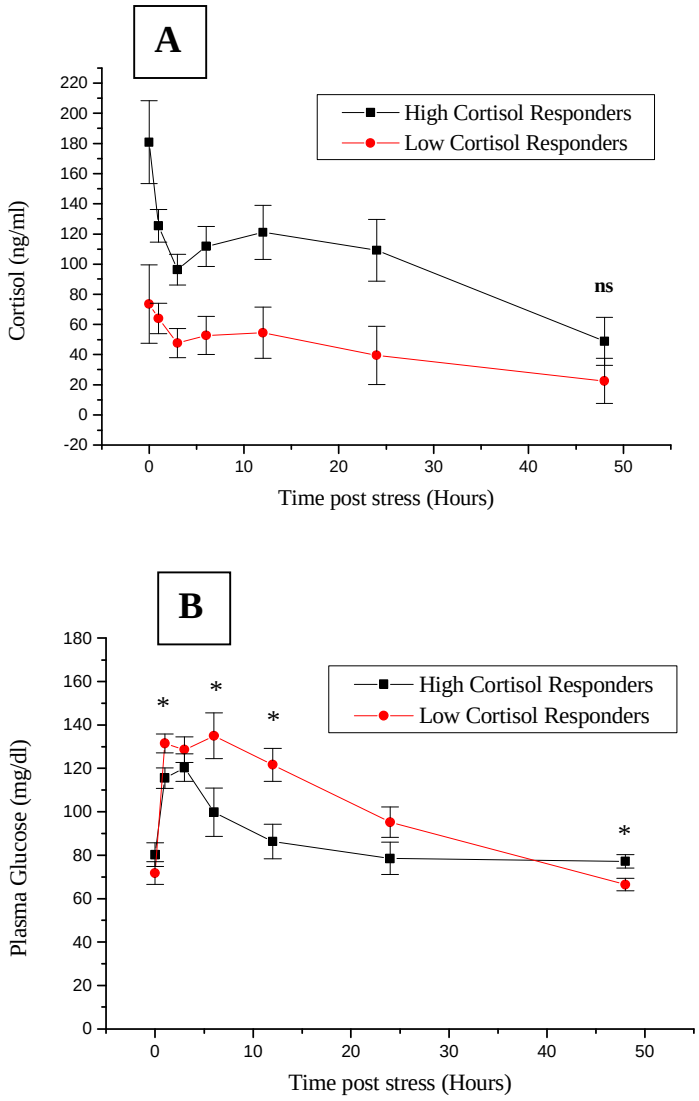

Figure 19. Mean plasma cortisol (A) and glucose (B) levels of samples taken at varying times after a two-minute net challenge for striped bass males selected for high cortisol responsiveness (HCR) (n=8) and low cortisol responsiveness (LCR) (n=9). HCR plasma levels of cortisol were significantly different from those of LCR ($p < 0.0001$). The effect of responsiveness on plasma glucose levels was also significant ($p = 0.015$). Mean plasma glucose levels of HCR were significantly different from those of LCR at some intervals (*) ($p < 0.05$). Vertical brackets represent the SEM.

The effect of selecting for high and low cortisol responsiveness did not have a significant effect on mean hematocrit values during the 48 hour time course study (Fig. 19). The differences in mean hematocrits were not significant at any interval post stress that was measured, but at 12 hours post stress the p-value was suggestive of a difference ($p = 0.059$). The effect of sample time on mean hematocrit values was significant ($p < 0.0001$).

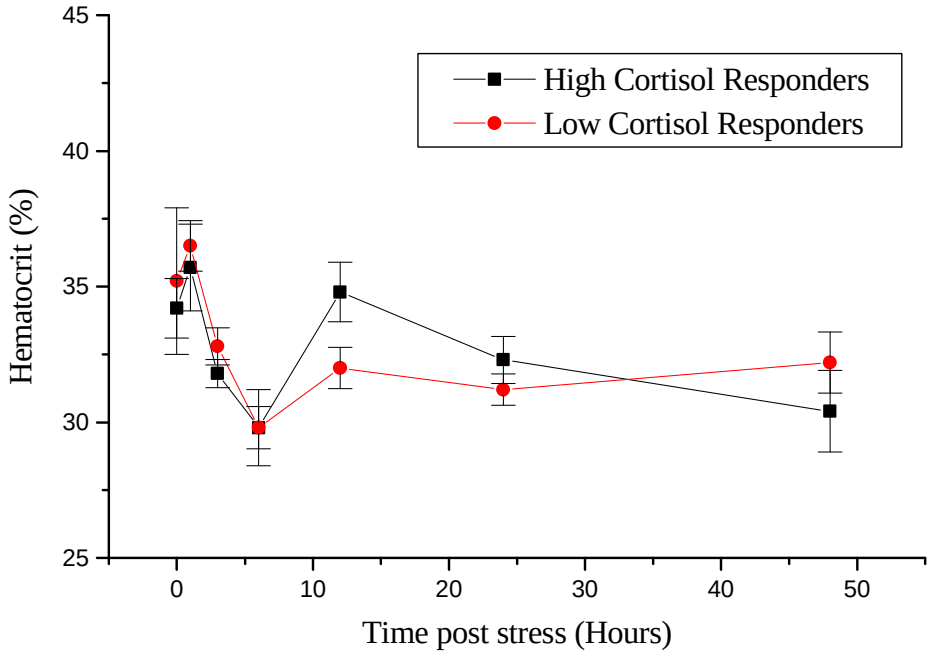

Figure 20. Mean hematocrit values of samples taken at varying times after a two-minute net challenge for striped bass males selected for high cortisol responsiveness (HCR) (n=8) and low cortisol responsiveness (LCR) (n=9). Means of HCR and LCR were not significant at any of the sample times, but the p-value at 12 hours post stress was suggestive of a difference ($p = 0.059$). Across all sample times HCR hematocrits were not significantly different from LCR. The effect of sample time on hematocrit values was significant ($p < 0.0001$). Vertical brackets represent the SEM.

DISCUSSION

This study demonstrates that male striped bass broodstock selected for high cortisol stress responsiveness show increased androgen production and length of spermiation when compared to low responding fish when stressed during the spawning season. This information, coupled with the overall higher specific growth rates in HCR compared to LCR, shows that if the stress response is proven to be a heritable trait in striped bass, selecting for higher cortisol responding male striped bass may impart desirable characteristics to their progeny. It is still unclear whether selecting for stress responsiveness will co-select for some as yet undiscovered undesirable trait (Pottinger & Carrick, 2000).

Through the first four stress sample dates mean cortisol values of HCR and LCR were significantly different, which is not surprising since these were the samples used to determine which fish would be categorized as HCR and LCR. Mean plasma glucose levels for HCR and LCR during this time period were not significantly different when compared across all sample dates, which is similar to what was found by Wang et al., (2003). On the first stress sample date (August), HCR fish had significantly greater mean plasma cortisol levels compared to LCR fish. It is unclear if the hyperglycemia seen in HCR was due to a catecholaminergic response, or if it was related to the elevated cortisol levels. If the difference was due to an elevation in cortisol, the difference would be expected across sample dates, since cortisol was significantly higher across all four sample dates. However, the glucose response may

be related to the magnitude of the cortisol response, because the largest difference between HCR and LCR cortisol levels was seen on the first stress sample date. ACTH has been shown to have a direct effect on catecholamine release in some fish species (Reid et al., 1998), so drastic differences in ACTH levels may have caused a differing catecholamine response, hence the differing glucose response. This explanation would contradict what has been found in rainbow trout. Pottinger and Carrick (2001) found that HCR rainbow trout had a significantly higher cortisol response to a weight-dependent dose of ACTH than LCR rainbow trout when DEX was used to block the secretion of ACTH by the fish, indicating that the divergence between HCR and LCR lies, at least partly, in the interrenal tissue. It is unknown if the divergence between HCR and LCR male striped bass are also due to a divergence in the sensitivity of the interrenal tissue to ACTH.

In an attempt to clarify if resting levels of cortisol were different between HCR and LCR, sample fish were anesthetized and bled without the standardized stress challenge on two sample dates, one in July 2002 and one in November 2002. Cortisol levels appeared to be elevated when compared to resting levels that have been measured by Noga et al. (1994), of less than 10 ng/ml, or by Davis and Parker (1990) of between 5.5 and 69.1 ng/ml at temperatures ranging from 5ºC and 25ºC. Husbandry conditions can vary greatly from one laboratory to another and must be taken into account when trying to compare values across labs. The striped bass males in this study were kept at 5 ppt salinity. Rainbow trout, adapted to freshwater, showed significant increases in plasma cortisol levels in response to an increase in salinity to 5 ppt salinity, which was believed to be associated with the change in

osmoregulatory demands (Orozco et al., 2002). In gilthead seabream (*Sparus aurata*), cortisol is associated with osmoregulatory function in both hyperosmotic and hyposmotic environments (Mancera et al., 2002). *In vitro* experiments on tilapia (*Oreochromis mossambicus*) have shown that cortisol plays an important role in the release of prolactin and growth hormone, both of which play important roles in osmoregulation (Uchida et al., 2004). Relatively high circulating levels of cortisol seen in these striped bass males may be associated with osmoregulatory regulation at 5 ppt salinity. Further research is needed to determine if relatively high baseline cortisol levels are associated with salinity levels in striped bass.

Another possible reason for the relatively high baseline values is due to the stress that the fish experienced from the placement of the fish concentrator into the tank. It is interesting to note, however, that HCR mean cortisol values were not different from that of LCR on the July 2002 sample date, though they were different on the November 2002 sample date. It should be noted that the HCR mean plasma cortisol levels were not different from the means of the unselected fish on either of those sample dates. If the plasma cortisol values attained on these two sample dates are representative of baseline cortisol values, then it appears that HCR and unselected fish have identical circulating cortisol levels, and LCR fish have significantly lower circulating levels of cortisol. If the plasma cortisol values from these two dates represent a cortisol response to a mild stressor (insertion of the fish concentrator), then HCR fish have a similar plasma cortisol response to a mild stressor as the unselected fish. LCR fish on the other hand, have a significantly lower cortisol stress response when compared to HCR and unselected fish.

Greater specific growth rates (weight) in HCR compared to LCR followed a similar pattern to work that has been done in salmonids. Rainbow trout selected for high cortisol responsiveness have been shown to have significantly greater weights, lengths (Pottinger and Carrick, 1999a), and coefficient of condition (Pottinger and Carrick, 1999, Pottinger et al., 1994) when compared to fish selected for low cortisol responsiveness. A higher mean specific growth rate (weight) in HCR compared to LCR differed from what has been found in striped bass in a previous study. Wang et al., (2003) found that HCR had significantly lower coefficient of condition compared to LCR, and no difference was found in specific growth rate (weight). That experiment used a one-minute net challenge, as apposed to the two-minute net challenge that was used in this study, so it is plausible, though unlikely, that different fish were selected because the stressor was not identical. The two-minute net challenge was used to avoid the sample-to-sample decrease in plasma cortisol in response to the acute stressor that was seen in the study by Wang et al., (2003)

The physiological cause for increased growth in HCR fish is unknown. Because high cortisol levels are associated with a change in osmotic function and the fish were in an environment that was hypertonic to their tissue, it is possible that the increased cortisol levels caused hemodilution, and a subsequent increase in tissue water content. This difference in tissue water content could be the cause of the difference seen in mean specific growth rate calculated using weight between HCR and LCR. In an attempt to substantiate or discount this explanation, specific growth rates were also calculated using length. The specific growth rate of HCR was not found to be significant compared to LCR when length was used, though the p-value

was suggestive of a difference. This data does not clarify whether the specific growth rates calculated using weights were significantly different because of tissue growth, or edema. It is unknown if increased water retention in HCR is an adaptive or maladaptive response to stress, if that is what is occurring. It should be noted that some fish had decreases in length from one sample date to another caused by tail erosion. In future experiments, fork length or caudal length may be better growth measures because they would not be affected by tail erosion.

Weil et al., (2001) suggests that the rate at which cortisol is cleared from the system may be more important than peak cortisol levels in rainbow trout. Previous research on striped bass males selected for high and low cortisol stress responsiveness showed that HCR did not have faster post-stress reductions in plasma cortisol when compared to LCR striped bass males (Wang et al., 2003), so it is unlikely that this is the mechanism causing differences in growth rates in this species.

Plasma androgen levels were higher, and spermiation started earlier and lasted longer in HCR compared to LCR. Low plasma levels of GTH-II are believed to play a role in decreased milt volumes (Mylonas and Zohar 2001). Implantation of GnRHa elicited an increase in plasma 11-KT levels in captive striped bass males (Mylonas and Zohar, 2001). The differences in plasma androgen levels and duration of spermiation seen between HCR and LCR may be due to differences in the levels of GnRH or GTH-II being released, but we do not have any data to support this. In fish, there is no data on the stress related regulation of GnRH (Pottinger, 1999). No significant difference in cortisol levels was found leading up to the spawning season (December 2002 through March 2003) one hour post stress, or immediately after

stress during the spawning season. Due to this lack of difference in cortisol levels, it is highly unlikely that the differences in androgen production and spermiation were a direct effect of cortisol. A more plausible explanation is that selecting for cortisol responsiveness inadvertently co-selects for differences in reproductive endocrine functions, though this is hard to prove without further study.

No differences were detected in any of the sperm quality measures used. Duration and percent motility of sperm were not found to be significantly different between HCR and LCR. In addition to providing a qualitative measure of the ability of the fish's gonad to produce high quality gametes, it has been shown that percent motile is correlated with fertilization in the common carp (Linhart et al., 2000). Without doing fertilization trials on HCR and LCR broodstock, assumptions cannot be made about fertilization rates, progeny survival rates, or the heritability of the stress response.

Post-stress cortisol levels for the entire population were high on the first stress sample date and declined to their lowest point on the October 2nd 2002 sampling. Cortisol values then rose slightly and appeared to plateau. When cortisol values are looked at in conjunction with temperature, it looks like there may be a temperature effect on cortisol values, which has been documented for yearling striped bass not undergoing reproductive development (Davis and Parker, 1990). Adaptation could also be a possibility, because similar declines in plasma cortisol levels have been seen in striped bass exposed to a monthly acute stress with temperature being held constant (Wang et al., 2003). Gonadal development and subsequent increases in plasma androgens may have played a role in the attenuation of the stress response. In

rainbow and brown trout, implants containing T and 11-KT caused a significant attenuation of the stress response in male fish when compared to fish with sham implants (Pottinger et al., 1996). Any combination of these factors, including temperature, probably played a role in the fluctuations in the cortisol response seen in the whole population.

In an attempt to determine if differences in recovery from an acute stressor and accompanying decreases in plasma cortisol could explain some of the differences between HCR and LCR, as has been suggested by Weil et al. (2001), a time course experiment was conducted. The rate at which plasma cortisol levels declined was not different between HCR and LCR. HCR had significantly greater mean plasma cortisol values at 0, 1, 3, 6, 12, and 24 hours post stress. Unexpectedly, the peak response time was not at one hour, but instead, was at the 0 hour. It is unclear if this initial peak response was caused by the two-minute net challenge, or if it was caused by the placement of the fish concentrator into the tank before sampling. The glucose responses of HCR were significantly less than those of LCR at 1, 6, and 12 hours post stress. These increased glucose values seen in LCR are most likely due to a catecholaminergic response due to the low levels of cortisol seen in LCR throughout the time course. LCR striped bass showing significantly elevated plasma cortisol levels when compared to HCR striped bass is similar to what has been seen in HCR and LCR rainbow trout (Trenzado et al., 2003). In response to a tank transfer and confinement stress, LCR rainbow trout lines had significantly lower plasma cortisol and liver glycogen levels and significantly higher plasma glucose and lactate levels, when compared to HCR rainbow trout lines, indicating a counterintuitive metabolic

divergence between HCR rainbow trout and LCR rainbow trout (Trenzado et al., 2003). Significantly greater plasma glucose levels of LCR compared to HCR striped bass males is suggestive of a similar metabolic divergence, but further research is needed to substantiate this claim.

It is unknown if fish with higher cortisol stress responses are actually more stressed than individuals with low cortisol stress responses (Barton, 2002), but this study shows that selecting for cortisol responsiveness does have an impact on other measurable factors. Increased growth and better reproductive function between HCR compared to LCR may represent an increase in adaptive ability by HCR fish. The action of glucocorticoids on physiological functions is complex and varied. Some scientists hypothesize that not all of these actions are deleterious, but that glucocorticoids aid in the down regulation of other stress responses, preventing harm to the system (Sapolski et al., 2000). For example, stress causes rapid activation of the immune response, which is then down regulated by glucocorticoids which can have beneficial anti-inflammatory effect, but can also increase the risk of subsequent infection (Sapolski et al., 2000). In rainbow trout, cortisol injections caused an inhibition of the increase in circulating leukocytes that was seen in fish receiving saline injections (Narnaware and Baker, 1996). Glucocorticoids have been shown to have an inhibitory effect on many different phases of the immune response, but the mechanisms through which glucocorticoids inhibit immune function is not clear (Maule and VanderKooi, 1999).

In most species in which the relationship has been investigated, glucocorticoids have been shown to have a negative impact on reproductive function

through *in vitro* and *in vivo* studies (Sapolski et al., 2000). In the European eel (*Anguilla anguilla*), however, cortisol was shown to stimulate a ten-fold increase in GTH-II from pituitary tissue *in vitro* (Huang et al., 1999). Cortisol injections also caused a significant increase in pituitary GTH-II content when compared to saline injections *in vivo* in the European eel (Huang et al., 1999). It is possible that the high pre-spawning levels of cortisol seen in HCR striped bass may have been partially responsible for the increase in androgen production if cortisol causes a similar increase in pituitary levels of GTH-II as seen in European eels. Although both striped bass and European eels are classified as teleosts, European eels are considered a more primitive species compared to the striped bass (Bond, 1996), making any assumptions of similarity in endocrine function pure speculation.

Another plausible explanation for the increase in androgen production, increased number of fish spermiating, and greater mean specific growth rate of male striped bass selected for high cortisol responsiveness compared to those selected for low responsiveness are existing factors that accompany the selection for cortisol responsiveness. This hypothesis is reinforced by the lack of difference in mean plasma cortisol concentration in the time leading up to, and during, the spawning season when the divergence in other factors was found.

Because of the many negative implications associated with the stress response, the assumption is made that if animals could be bred to have an attenuated stress response, they would perform better under stressful conditions when compared to animals with a normal stress response (Pottinger and Pickering, 1997). The difficulty becomes deciding how the stress response is measured. A myriad of

physiological changes occur as a result of stress. In this experiment plasma levels of cortisol were used to measure the stress response in male striped bass, but selecting fish for low cortisol responsiveness seemed to have the opposite effect of what is described above by Pottinger and Pickering (1997). It is unclear whether selecting striped bass for cortisol stress responsiveness could increase productivity. This study suggests that fish that have higher cortisol stress responsiveness may be found to be more desirable than fish with low cortisol responsiveness.

CONCLUDING REMARKS

Increased androgen production, specific growth rate, and number spermiating seen in male striped bass HCR compared to LCR are quite interesting results, but without further research, it may become anecdotal. Further investigations into the heritability of the stress response in striped bass are needed, as well as studies on fertilization rates using sperm from HCR compared to LCR. The existence of negative cofactors associated with selecting fish for high cortisol responsiveness need to be explored. Determining exactly where the divergence between HCR and LCR lies may help explain some of the results found in this study.

A crucial piece of information left unknown by this study is if similar results would be found if female striped bass were selected for their responsiveness to stress. Determining if circulating levels of E_2 and vitellogenin were different between female HCR and LCR would help determine if selecting for cortisol responsiveness co-selected for similar divergences as seen in males. Crosses could then be made between HCR males and HCR females, and LCR males and LCR females, and heritability could be determined. The viability of using cortisol stress responsiveness as a selective marker could be determined through progeny studies. Such work could aid in the domestication of striped bass.

Another possible avenue for this research is to select white bass females for stress responsiveness and determine if such selection results in improved reproductive or growth performance. Crossing HCR striped bass males with HCR white bass

females, and crossing LCR striped bass males with LCR white bass females, and following the progeny, could help determine if selecting for stress responsiveness could produce a superior performing sunshine bass hybrid. Since the majority of commercial *Morone* culture is of the sunshine bass hybrid, such results could be beneficial to the aquaculture industry.

REFERENCES

Barton B.A. 2002. Stress in Fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology. 42, 517-525.

Brown K.I., Nestor K.E. 1973. Some physiological responses of turkeys selected for high and low adrenal response to cold stress. Poultry Science 52, 1948-1954.

Bond C.E. 1996. Biology of Fishes Second Edition. Saunders College Publishers, Fort Worth.

Carlsfeld J., Powell J.F.F., Park M., Fischer W.H., Craig A.G., Chang J.P., Rivier J.E., Sherwood N.M. 2000. Primary structure and function of three gonadotropin-releasing hormones, including a novel form, from an ancient teleost, herring. Endocrinology. 141, 505-512.

Carragher J.F., Sumpter J.P. 1990. The effect of cortisol on the secretion of sex steroids from cultured ovarian follicles of rainbow trout. General and Comparative Endocrinology. 77, 403-407.

Carragher, J.F., Sumpter, J.P., Pottinger T.G., Pickering A.D. 1989. The deleterious effects of cortisol implantation on reproductive function in two species of trout, *Salmo trutta* L. and *Salmo gairdneri* Richardson. General and Comparative Endocrinology. 76, 310-321.

Carsia R.V., Weber H., Satterlee D.G. 1988. Steroidogenic properties of isolated adrenocortical cells from Japanese quail selected for high serum corticosterone response to immobilization. Domestic Animal Endocrinology. 5 (3) 231-240.

Carsia R.V., Weber H. 1986. Genetic-dependent alterations in adrenal stress response and adrenocortical cell function of the domestic fowl (*Gallus domesticus*). Proceedings of the Society for Experimental biology and Medicine. 183, 99-105.

Chow M.M., Kight K.E., Gothilf Y., Alok D., Stubblefield J., Zohar Y. 1998. Multiple GnRHs present in a teleost species are encoded by separate genes: analysis of the sbGnRH and cGnRH-II genes from striped bass, Morone saxatilis. Journal of Molecular Endocrinology. 21, 277-289.

Cleary J.J., Pankhurst N.W., and Battaglene S.C. 2000. The effect of capture and handling stress on plasma steroid levels and gonadal condition in wild and farmed snapper *Pagrus auratus* (Sparidae). Journal of the World Aquaculture Society. 31 558-569.

Constien, D., Lambert J.G.D., Komen H., Goos H.J.Th. 2002. Corticosteriods affect the testicular androgen production in male common carp (*Cyprinus carpio* L.). Biology of Reproduction. 66, 106-111.

Constien, D., Lambert J.G.D., Goos H.J.Th. 2001. Cortisol affects testicular development in male common carp, Cyprinus carpio L., but not via an effect on LH secretion. Comparative Biochemistry and Physiology Part B. 129, 671-677.

Contreras-Sanchez W.M., Schreck C.B., Fitzpatrick M.S., Pereira C.B. 1998. Effects of stress on the reproductive performance of rainbow trout (*Oncorhynchus mykiss*). Biology of Reproduction. 58, 439-447.

Cooper E.L. 1983. Fishes of Pennsylvania and the North Eastern United States. The Pennsylvania State University Press. University Park.

Damron S. W. 2003. Introduction to Animal Science, Global, Biological, Social, and Industry Perspectives second edition. Prentice hall. Upper Saddle River.

Davies B., Bromage N. 2002. The effects of fluctuating seasonal and constant water temperatures on the photoperiodic advancement of reproduction in female rainbow trout, Oncorhynchus mykiss. Aquaculture 205, 183-200.

Davis K.B., Parker N.C. 1990. Physiological stress in striped bass: effect of acclimation temperature. Aquaculture. 91, 349-358.

FAO (Food and Agriculture Organization). (2002) The state of world fisheries and aquaculture. Part 1. World review of fisheries and aquaculture. FAO, Rome.

Fevolden S.E., Roed K.H., Fjalestad K. 2003. A combined salt and confinement stress enhances mortality in rainbow trout (*Oncorhynchus mykiss*) selected for high stress responsiveness. Aquaculture. 216, 67-76.

Fevolden S.E., Roed K.H., Fjalestad K.T. 2002. Selection of cortisol and lysozyme in rainbow trout and correlation to growth. Aquaculture. 205, 61-75.

Fevolden S.E., Refstie T., Roed. 1991. Selection for high and low cortisol stress response in Atlantic salmon (*Salmo Salar*) and rainbow trout (*Oncorhnchus mykiss*). Aquaculture. 95: 53-65.

Haddy J.A., Pankhurst N.W. 1999. Stress-induced changes in concentrations of plasma sex steroids in black bream. Journal of Fish Biology. 55, 1304-1316.

Hazon N., Balment R.J. 1998. Endocrinology. *In* "The Physiology of Fishes Second Edition". D.H. Evans (ed.) CRC Press, Boca Raton. 448-463.

Harrell R. M. 1997. Hybridization and Genetics. *In* "Striped Bass and Other Morone Culture". R.M Harrel (ed.). Elsevier, Amsterdam.

Harrell R. M., Webster D.W. 1997. An overview of Morone culture. *In* "Striped Bass and Other Morone Culture". R.M Harrel (ed.) Elsevier, Amsterdam.

He S., Woods III, L.C. 2003a. The effects of osmolality, cryoprotectant and equilibration time on striped bass *Morone saxatilis* sperm motility. Journal of the World Aquaculture Society. 34, 255-265.

He S., Woods III, L.C. 2003b. Effects of glycine and alanine on short term storage and cryopreservation of striped bass (*Morone saxatilis*) spermatozoa. Cryobiology. 46, 17-25.

Hassin S., Claire M., Holland H., Zohar Y. 2000. Early maturity in the male striped bass, *Morone saxatilis*: follicle-stimulating hormone gene expression and their regulation by gonodotropin-releasing hormone analogue and testosterone. Biology of Reproduction. 63, 1691-1697.

Hodson R.G., Clark R.W., Hopper M.S., McGinty A.S., Weber G.M., Sullivan C.V. 1999. Reproduction of domesticated striped bass: commercial mass production of fingerlings. UJNR Technical Report No. 28. 23-32.

Huang Y.S., Rousseau K., Sbaihi M., Le Belle N., Schmitz M., Dufour S. 1999. Cortisol selectively stimulates pituitary gonadotropin β-Subunit in a primitive teleosts, Anguilla anguilla. Endocrinology. 140, 1228-1235.

Jennings S., Kaiser M. J., Reynolds J.D. 2001. Marine Fisheries Ecology. Blackwell Science. Malden MA.

Jeong K.H., Jacobson L., Widmaier E.P., Majzoub J.A. 1999. Normal suppression of the reproductive axis following stress in corticotropin –releasing hormone-deficient mice. Endocrinology. 140 (4) 1702-1710.

Linhart O., Rodina M., Cosson J. 2000. Cryopreservation of sperm in common carp (*Cyprinus carpio*): Sperm motility and hatching success of embryos. Cryobiology. 41, 241-250.

McDonald G. and Milligan L. 1997. Ionic, osmotic and acid-base regulation in stress:*In* : "Fish Stress and Health in Aquaculture". Iwana G.W., Pickering A.D., Sumpter J.P. and Schreck C.B. (eds) Cambridge University Press, Cambridge. 119-144.

Mancera J.M., Carrion R.L., del Pilar Martin del Rio M. 2002. Osmoregulatory action of PRL, GH, and cortisol in the gilthead seabream (*Sparus aurata* L.). General and Comparative Endocrinology. 129, 95-103.

Maule A.G., VanderKooi S.P. 1999. Stress-induced immune-endocrine interaction. *In:* " Stress Physiology in Animals". P.H.M. Balm (ed.). Sheffield Academic Press. Sheffield.

Mazeaud M.M., Mazeaud F., and Donaldson E.M. 1977. Primary and secondary effects of stress in fish: some new data with a general review. Transactions of the American Fisheries Society. 106, 201-211.

Mylonas C.C., Zohar Y. 2001. Endocrine regulation and artificial induction of oocyte maturation and spermiation in basses of the genus *Morone.* Aquaculture. 202, 205-220.

Mylonas C.C., Zohar Y., Woods L.C.III., Thomas P., Schulz R.W. 1998. Hormone profiles of captive striped bass Morone saxatilis during spermiation and long-term enhancement of milt production. Journal of the World Aquaculture society. 290 (4) 379-392.

Mylonas C.C., Scott A.P., Zohar Y. 1997. Plasma gonadotropin II, sex steroids, and thyroid hormones in wild striped bass (*Morone saxatilis*) during spermiation and final oocyte maturation. General and Comparative Endocrinology. 108, 223-236.

Narnaware Y.K., Baker B.I. 1996. Evidence that cortisol may protect against the immediate effects of stress on circulating leukocytes in the trout. General and Comparative Endocrinology. 103, 359-366.

Noga E.J., Kerby J.H., King W., Aucoin D.P., Giesbrecht F. 1994. Quantitative comparison of the stress response of striped bass (Morone saxatilis) and hybrid striped bass (*Morone saxatilis* X *Morone chrysops* and *Morone saxatilis* X *Morone americana*). American Journal of Veterinary Research. 55 (3) 405-409.

Orozco A., Villalobos P., Valverde C. 2002. Environmental salinity selectively modifies the outer-ring deiodinating activity of liver, kidney, and gill in the rainbow trout. Comparative Biochemistry and Physiology Part A. 131, 387-395.

Pall M.K., Mayer I., Borg B. 2002. Androgen and behavior in the male three-spined stickleback, Gastereus aculeatus I. – changes in 11-ketotestosterone levels during the nesting cycle. Hormones and Behavior. 41, 377-383.

Pankhurst N.W., Van Der Kraak G., Peter R.E. 1995. Evidence that the inhibitory effects of stress on reproduction in teleost fish are not mediated by the action of cortisol on ovarian steroidogenesis. General and Comparative Endocrinology. 99, 249-257.

Pankhurst N.W., Van Der Kraak G. 1997. Effects of stress on reproduction and growth of fish. *In.* Fish Stress and Health in Aquaculture. G.K. Iwana, A.D. Pickering, J.P. Sumpter and C.B. Schreck (eds). Cambridge University Press, Cambridge.

Pankhurst N.W., Van Der Kraak G. 2000. Evidence that acute stress inhibits ovarian steroidogenesis in rainbow trout *in vivo* through the action of cortisol. General and Comparative Endocrinology. 117, 225-237.

Pickering A.D., Pottinger T.G., Carragher J., Sumpter J.P. 1987. The effects of acute and chronic stress on the levels of reproductive hormones in plasma of mature male brown trout, *Salmo trutta* L. General and Comparative Endocrinology. 68, 249-259.

Pottinger T.G. 1999. The impact of stress on animal reproductive activities. *In*: "Stress Physiology in Animals". P.H.M. Balm (ed.). Sheffield Academic Press. Sheffield.

Pottinger T.G., Carrick T.R. 2001. ACTH does not mediate divergent stress responsiveness in rainbow trout. Comparative Biochemistry and Physiology Part A. 129, 399-404.

Pottinger T.G., Carrick T.R. 2000. Indicators of reproductive performance in rainbow trout *Oncorhynchus mykiss* (Walbaum) selected for high and low responsiveness to stress. Aquaculture Research. 31, 367-375

Pottinger T.G., Carrick T.R. 1999a. A comparison of plasma glucose and plasma cortisol as selection markers for high and low stress-responsiveness in female rainbow trout. Aquaculture. 175, 351-363.

Pottinger T.G., Carrick T.R. 1999b. Modification of the plasma cortisol response to stress in rainbow trout by selective breeding. General and Comparative Endocrinology. 116, 122-132.

Pottinger T.G., Carrick T.R., Hughes S.E., Balm P.H.M. 1996. Testosterone, 11-ketotestosterone, and estradiol-17β modify baseline and stress induced Interrenal and corticotropic activity in trout. General and Comparative Endocrinology. 104, 284-295.

Pottinger T.G., Moran T.A., and Morgan J.A.W. 1994. Primary and secondary indices of stress in the progeny of rainbow trout (*Oncorhynchus mykiss*) selected for high and low responsiveness to stress. Journal of Fish Biology. 44, 149-163.

Pottinger T.G., Pickering A.D. 1997. Genetic basis to the stress response: selective breeding for stress-tolerant fish. *In* "Fish Stress and Health in Aquaculture". G. K. Iwana, A.D. Pickering, J.P. Sumpter, C.B. Schreck (eds.). Cambridge University Press, Cambridge.

Pottinger T.G., Pickering A.D., Hurley M.A. 1992. Consistency in the stress response of individuals of two strains of rainbow trout, *Oncorhynchus mykiss*. Aquaculture. 103, 275-289.

Querat B. 1995. Structural relationships between fish and tetrapod gonadotropins. Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish. F.W. Goetz and P. Thomas (eds). Fish Symposium 95, Austin.

Rakitin A., Ferguson M.M., Trippel E. A. 1999. Spermatocrit and spermatozoa density in Atlantic cod (*Gadus morhua*): correlation and variation during the spawning season. Aquaculture. 170, 349-358.

Reid S.G., Bernier N.J., Perry S.F. 1998 The adrenergic stress response in fish: Control of catecholamine storage and release. Comparative Biochemistry and Physiology. 120C, 1-27.

Schreck C.B., Contreras-Sanchez W., Fitzpatrick M.S. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture. 197, 3-24.

Schulz R.W., Miura T. 2002. Spermatogenesis and its endocrine regulation. Fish Physiology and Biochemistry. 26, 43-56.

Schulz R.W., Vischer H.F., Cavaco J.E.B., Santos E.M., Tyler C.R., Goos H.J.Th., Bogerd J. 2001. Gonadotropins, their receptors, and the regulation of testicular functions in fish. Comparative Biochemistry and Physiology Part B. 129, 407-417.

Sapolski R.M., Romeo L.M., Munck A.U. 2000. How do glucocorticoids influence stress response? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews. 21 (1) 55-89.

Sullivan C.V., Berlinsky D.L., Hodson R.G. 1997. Reproduction. *In* Striped Bass and Other Morone Culture. R.M. Harrell (ed). Elsevier, Amsterdam.

Sumpter J.P. 1997. The endocrinology of stress. *In* "Fish Stress and Health in Aquaculture". G. K. Iwana, A.D. Pickering, J.P. Sumpter, C.B. Schreck (eds.). Cambridge University Press, Cambridge.

Tate A.E., Helfrich L. A. 1998. Off-season spawning of sunshine bass (*Morone chrysops* X *M. saxatilis*) exposed to 6- or 9-month phase shifted photothermal cycles. Aquaculture. 167, 67-83.

Tanck M.W.T., Vermeulen K., Bovenhuis H., Komen H. 2001. Heredity of stress related cortisol response in androgenetic common carp (*Cyprinus carpio* L.). Aquaculture. 199, 283-294.

Teitsma C., Lethimonier C., Tujague M., Anglade I., Saligaut D., Bailhache T., Pakdel F., Kah O., Ducouret B. 1998. Identification of potential sites of cortisol actions on the reproductive axis in rainbow trout. Comparative Biochemistry and Physyiology Part C. 119, 243-249.

Trenzado C.E., Carrick T.R., Pottinger T.G. 2003. Divergence of endocrine and metabolic responses to stress in two rainbow trout lines selected for differing cortisol responsiveness to stress. General and Comparative Endocrinology. 133, 332-340.

Uchida K., Yoshikawa-Ebesu J.S.M., Kajimura S., Yada T., Hirano T., Grau E.G. 2004. In vitro effects of cortisol on the release and gene expression of prolactin and growth hormone in the tilapia, *Oreochromis mossambicus*. General and Comparative Endocrinology. 135, 116-125.

Van Der Kraak G., Chang P., Janz D.M. 1998. Reproduction. *In* "The Physiology of Fishes Second Edition. D.H. Evans (ed.) CRC Press, Boca Raton. 465-488.

Wang, C., King W. V., Woods L. C. III. 2003. Physiological indicators of divergent responsiveness in male striped bass broodstock. Aquaculture. *In press*.

Williams J.E. 2000. The Coeficient of Condition of Fish. Chapter 13 *In* "Manual of fisheries survey methods II: with periodic updates." Schneider, James C. (ed.) 2000. Michigan Department of Natural Resources, Fisheries Special Report 25, Ann Arbor.

Wedemyer G.A., Barton B.A., McLeay D.J. 1990 Stress and Acclimation. *In* Methods for fish biology. C.B Schreck and P.B. Moyle (eds). American Fisheries Society, Bethesda.

Wedemeyer G.A. 1997. Physiology of Fish in Intensive Culture Systems. Chapman and Hall, New York.

Weil L.S., Barry T.P., Malison J.A. 2001. Fast growth in rainbow trout is correlated with a rapid decrease in post-stress cortisol concentrations. Aquaculture. 193 373-380.

Wendelaar Bonga S.A. 1997. The stress response in fish. Physiological Reviews. 77, 591-625.

Woods L.C. III., Sullivan C.V. 1993. Reproduction of striped bass, *Morone saxatilis* (Walbaum), broodstock; monitoring maturation and hormonal induction of spawning. Aquaculture and Fisheries Management. 24, 211-222.