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Continuous exercise enhances swim performance and alters growth rate, IGF-I, and cortisol in juvenile marine finfish in aquaculture

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Continuous Exercise Enhances Swim Performance and Alters Growth Rate, IGF-I, and Cortisol in Juvenile Marine Finfish in Aquaculture

A thesis submitted in partial satisfaction of requirements for the degree of:

Masters of Science in Marine Science

By

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Chapter One: General Introduction

With the decline in wild fish stock due to over fishing, habitat loss and the increased demand for fish as a food source, the aquaculture industry has become an important component in the global economy not only in a consumable goods sense but also as a means of stock replenishment through release programs. Capture fisheries reached their maximum sustainable yield of 100 million metric tons (MMT) more than ten years ago, and the demand for seafood has shown no signs of abating (FAO 2004). The Food and Agriculture Organization (FAO) of the United Nations' annual report states that in 2002, aquaculture supplied 29.9% of the global supply (FAO 2004). In aquaculture, fish are commonly reared in net cages, ponds, tanks, and raceways. Aquaculture systems are typically designed to optimize water quality, which results in flow parameters that may not be optimal for growth. Fish with increased growth rates reach marketable size faster and with a higher proportion of muscle mass. A faster growth rate also allows individuals to more rapidly achieve a size suitable for over-wintering in tanks, possibly decreasing the high mortality seen in fingerlings in aquaculture. This would lead to decreasing the release time, enhancing the stock replenishment, and decreasing the holding costs to the hatchery. The early rearing conditions have a substantial influence on muscle growth and proliferation of muscle fibers (Johnston 2001a) making exploration of growth conditions for early juveniles an attractive area of focus for aquaculture.

Continuous swimming during rearing improves growth rates (Davison 1997; Yogata and Oku 2000; Martin and Johnston 2005), enhances aerobic

potential (Farrell, et al. 1991; Martinez, et al. 2003), and in some cases reduces stress (Young and Cech Jr. 1994b; Davison 1997; Milligan 1997) in a variety of fish species. The rigor of the exercise regime in terms of speed and duration is an important factor in these studies, because optimal conditions vary greatly by species and because excessive exercise can result in lower growth rates or increased stress (Kieffer 2000). The activities of glycolytic and oxidative enzymes are often used as indicators of anaerobic and aerobic metabolism in fish. Studies have shown that these enzymes positively correlate with growth rates, locomotory habits and food availability and as more recent studies have shown sustained exercise (Somero and Childress 1980; 1990; Pelletier, et al. 1995; Buhr 2002). Such metabolic enhancement may translate into improved performance or survival in fish released or transferred into net pens, where current flows may occasionally exceed velocities encountered during aquaculture.

Growth in fish is largely controlled by hormones. Both environmental and nutritional factors indirectly influence growth by affecting the release of these growth hormones. This is especially true for ectotherms like teleost fish, which rely on temperature, photoperiods, and food availability to trigger developmental processes. Insulin-like growth factor (IGF) and growth hormone (GH) are two fundamentally important anabolic factors in fish. Pituitary GH stimulates liver production of IGF-I. IGF-I then directly stimulates tissue growth and mediates much of the growth promoting effects of GH. As exercise produces the same somatic growth enhancement as optimal feeding conditions (Pierce, et al. 2005), it is reasonable to suspect that sustained swimming will exert its effect through the

IGF-GH axis. Reducing stress is critically important for health management in aquaculture. Although acute stressors can elicit a coordinated set of behavioral and physiological responses enabling the animal to overcome the strain (fuel mobilization, fight or flight response), chronic stress leads to negative effects on important physiological systems, including immunosuppression (Wendelaar Bonga 1997; Barton 2002). Since excessive exercise can result in increased stress (Kieffer 2000), it is important to establish optimal swimming conditions specific for each fish species.

Purpose of this project

The purpose of this study was to investigate the effects of continuous exercise training on three finfish with distinct swim styles; California yellowtail, white seabass, and California sheephead. Four flow regimes were used in order to determine the optimal flow speed that enhances growth, reduces stress, and therefore may lead to increased survival and overall health in an aquaculture setting. Somatic measurements along with muscle fiber cross sectional area were collected to determine the effects of exercise on growth. Metabolic activity of aerobic (citrate synthase) and anaerobic (lactate dehydrogenase) metabolism were measured as possible indicators of enhanced aerobic conditioning and amplified capacity for burst swimming due to the training. Levels of plasma IGF-I and cortisol were also observed to determine the effects of exercise on the endocrine system and the stress response of each species due to the endurance training. The findings of this investigation not only add three relatively unknown fish species to the field of exercise physiology but also provide evidence that proper endurance

training could increase the growth rates, aerobic potential, and overall health of fish in an aquaculture setting.

Chapter Two: Literature Review

I. Metabolic Enzymes

Sustained-aerobic and burst-anaerobic swimming rely on different muscle fibers (red vs. white) and different metabolic pathways to provide the ATP used for muscle contractions. Burst swimming relies on anaerobic metabolism and glycolytic enzymes in white muscle for ATP production. The specific activity of key enzymes along with the levels of anaerobic fuel stores in the white muscle have been suggested as indicators of the fish's anaerobic performance. Glycogen stores, activity of enzymes along the glycolytic pathway (PK, PFK, LDH), and buffering capacity have all been used to assess fish anaerobic capacity (Somero and Childress 1980; 1990; Gibb and Dickson 2002). Sustained swimming relies on the aerobic metabolism and mitochondrial enzymes in red muscle to produce ATP. The ability of the circulatory system to deliver oxygen and fuel to the muscle cells, the activity of enzymes involved in the intra-mitochondrial aerobic pathways like CS, and the number of mitochondria in the cell are all used to assess the aerobic capacity of fish (Gibb and Dickson 2002).

Early studies by Somero and Childress (1980) have demonstrated that glycolytic enzymes tend to scale with body size for a variety of fish species. The activities of aerobically adapted enzymes (i.e. mitochondrial) tend to decrease with size (Somero and Childress 1980; Tripathi and Verma 2004). The scaling coefficients for glycolytic enzymes appear to be dependent on the lifestyle of the species, and benthic species exhibit much lower scaling coefficients then pelagic species (Somero and Childress 1980; 1990). The size-dependent scaling may not occur in all species and in all size ranges. In white muscle of striped bass, LDH

and PK were positively scaled to size only in small fish and did not scale in fish > 1000g; the aerobic enzymes were mass-independent in both the red and white muscle (Norton, et al. 2000). In a study with cod, white muscle glycolytic enzymes (PK and LDH) were directly correlated with growth rate; the authors suggest this was not solely caused by an increase in size as suggested by Somero and Childress, because enzyme activity in white muscle was shown to be size-independent over a larger size range than the cod study provided for (Pelletier, et al. 1995). Muscle metabolic capacities have also been shown to vary with the position along the fish. In Atlantic cod, glycolytic enzyme (LDH, PFK, PK) levels increased from head to tail and red muscle mitochondrial enzyme (CCO, CS) levels were highest in the caudal sample (Martinez, et al. 2003).

Close links between the activity of some metabolic enzymes to food availability and growth rate have been shown for several fish species (Sullivan and Somero 1983; Yang and Somero 1993; Pelletier, et al. 1995). Starvation significantly affected the metabolic capacities of white and red muscle in Atlantic cod with levels of glycolytic enzymes (LDH) changing more in white than red muscle. Swimming endurance was linked with the metabolic capacities of the red and white muscle and starvation in the cod (Martinez, et al. 2003).

Muscle mitochondrial density and mitochondrial metabolic enzyme activity have been shown to consistently increase when teleost fishes are cold-acclimated; the acclimation allows the fish to elevate metabolic rate (Moerland and Egginton 1998; Sidell 1998; McClelland, et al. 2006). In late spring, CS activity in the axial muscle of three spine stickleback increased as a direct result

of cold acclimation and was positively correlated with burst swimming speed—while growth rate was correlated with LDH in pectoral and axial muscles (Guderley, et al. 2001). When the same experiment was conducted in the fall, cold- and warm-acclimated fish showed similar growth rates but showed no differences in burst swimming capacity, suggesting that photoperiod is an influencing factor in the stickleback's response to thermal acclimation. CS activity in white muscle was shown to correlate with oxygen consumption rate. It was suggested that fishes that produce more lactate in white muscle require a higher aerobic capacity to restore glycogen stores within the white muscle– indicating that CS in white muscle and oxygen consumption should correlate (Dickson, et al. 1993). In adult zebra fish exercise significantly increased CS and LDH levels relative to controls (McClelland, et al. 2006).

In order to accurately predicate changes in swim performance using biochemical indices, it is imperative to separate out the effects of body size, temperature and ontogenetic stage (Gibb and Dickson 2002). In halibut, white muscle LDH activity and U_{max} increased with size. However, when size effects were removed, there was no correlation between LDH and swim speed. The same results were observed with red muscle enzyme indicators in juvenile scombrids (Gibb and Dickson 2002). Following a 28 day training bout at up to 60% U_{crit}, CS increased in both red and white muscle of rainbow trout along with no significant increase in U_{crit} and decrease in condition factor (Farrell, et al. 1991).

II. Exercise in Fish

About 50 years ago, the study of exercise physiology in fish began with the pioneering works of Brett (1964) and Black (1966). Their work demonstrated that fish have the capacity for both aerobic sustained swimming and anaerobic burst type swimming. Fish are mobile, and can generally be easily manipulated to swim against a current, which makes them ideal subjects for exercise training. However comparisons between studies are challenging due to differences in swimming chambers and training protocols. Additionally, the results are species-specific; relating to the life styles and swim styles of each species (Hammer 1995; Davison 1997; Johnston 2001a). Regardless, since the first experiments of Brett and Black, teleosts have become one of the most studied vertebrates with respect to exercise (Kieffer 2000). Swimming in fish is generally characterized as either aerobic or anaerobic (Beamish 1978). During sustained exercise, muscle metabolism is generally aerobic and is supported by the red musculature. During events such as predator-prey interactions and spawning migrations the capacity of the red muscle is exceeded and burst-type exercise occurs. This burst-type action is largely supported by anaerobic glycolysis within the white muscle (Brett 1964; Black, et al. 1966; Beamish 1978; Kieffer 2000).

Sustained Exercise

Numerous studies have shown that endurance training modifies muscle morphology, enzyme activity, and aerobic capacities in fish (Davison 1997; Kieffer 2000). It is generally assumed that at favorable swimming speeds (≤ 1.5

bl sec^{-1}), exercise training leads to increased growth, whereas training at substantially higher speeds has distinct negative effects on growth in any fish (Davison 1997). Although the speeds vary among species and reflect differences in training methods, exercise has been shown to improve growth rate of Atlantic salmon, brown *Salmo trutta* and rainbow *Oncorhynchus mykiss* trout, striped bass *Morone saxatilis*, yellowtail *Seriola quinqueradiata* (Yogata and Oku 2000), whiting *Merlangius merlangus,* Danube bleak *Chalcalnurnus chalcoides mento,* and Arctic *Salvelinus alpines* and brook *S. fontinalis* charrs, as reviewed by Davison (1997). In the few studies where growth seemed unaffected or retarded (Kiessling, et al. 1994; Davison 1997; Bjornevik, et al. 2003), the confined conditions of the experimental raceway may have stressed the fish therefore inhibiting growth, particularly in the Chinook salmon (*Oncorhynchus tshawytscha*). In a review by Davison (1997), numerous studies in non-salmonids species are given showing the effects of exercise on muscle morphology. In general, an increase in cell diameter and number of aerobic fibers is common as a result of endurance exercise. The increase in fiber size and number leads to a greater proportion of total red muscle along with increased capillarization (Young and Cech Jr. 1993a; Sanger and Stoiber 2001). The oxidation potential of white muscle may also be affected in some species, even at low speeds (Mommsen and Moon 2001). Interestingly, in the salmonids such changes are not reported (Davison 1997; Sanger and Stoiber 2001). Particularly important for aquaculture fish destined for human consumption, exercise was found to increase flesh

firmness in Atlantic salmon and sea bream and long term exercise (9 months) did not affect the flesh quality in farmed cod (Bjornevik et al. 2003).

Changes in non-growth parameters such as aerobic capacity, behavior, and internal body chemistry also occur as a result of exercise training. Conversion efficiency, defined as mass gained relative to the amount of food consumed, is an important measure in aquaculture, as fish food is a large operational expense. In many salmonids and a few non-salmonid species, exercise has been shown to increase feed conversion efficiency. During the exercise training, appetite is stimulated and the fish eat more. Although the exercise may have a higher energy demand, a unit mass of growth is generally achieved at a faster rate while requiring less food as compared to unexercised fish (Davison 1997). This phenomenon was manifested in Arctic charr, where exercised fish experienced a decreased food ration with no negative effects on growth (Hammer 1995).

Mommsen and Moon (2001) suggested that at least a part of the increased growth could be due to decreased aggressiveness, that diluted the dominance structure and with it feeding dominance, consequently increasing appetite. Particularly in subordinate individuals, swimming may cause a reduction of energy expenditure due to the relief from chronic stress and a subsequent increase in growth rate (Yogata and Oku 2000). Reduced aggression during exercise has been observed in several studies including salmonids (reviewed in Davison 1997), Arctic charr (Adams, et al. 1995) and yellowtail(Yogata and Oku 2000).

Much of the biochemical work done on swimming muscles in fish is somewhat contradictory but general trends are noted (Davison 1997; Kieffer 2000; Sanger and Stoiber 2001). Exercise training increases protein turnover in most tissues in rainbow trout (Houlihan and Laurent 1987). Exercise also raises hematocrit, and consequently raises hemoglobin concentration, increasing the blood oxygen carrying capacity. When coupled with increased myoglobin in the muscles, this allows for better transport of oxygen in muscle tissue (Davison 1997; Sanger and Stoiber 2001). There is strong evidence supporting that endurance exercise training increases the aerobic potential in red--and in some cases white--muscle due to better tissue capillary supply, increased mitochondrial and lipid content, and increases in enzyme activity (Sanger and Stoiber 2001). The enzymes predominantly affected are those involved in lipid metabolism, suggesting that lipids provide the primary fuel for swimming (Davison 1997; Sanger and Stoiber 2001). An increase in the glycolytic potential of white muscle suggests increased lactate production; along with the corresponding capillary supply, there is an enhanced ability to clear the lactate (Sanger and Stoiber 2001). After exercise, rainbow trout had higher levels of enzymes associated with aerobic metabolism in red, white, and cardiac muscle, with high levels of 3-hydroxyacyl-coenzyme A dehydrogenase (HOAD) in white muscle, indicating a shift toward lipid metabolism (Sanger and Stoiber 2001). In striped bass, trained fish reached peak lactate levels later and lactate returned to rest values faster, with the improvement of the muscle buffering capacity resulting in a smaller decrease in pH (Davison 1997).

Numerous endocrine and paracrine factors affect muscle growth in fish. During sustained exercise, these factors exhibit changes in plasma levels of cortisol and the growth hormone axis. Although not observed in every species, lower cortisol levels were reported for steelhead trout, Atlantic salmon, Arctic charr, and striped bass as a result of sustained exercise training (Young and Cech Jr. 1994b; Davison 1997). In rainbow trout, sustained exercise correlates with increased growth and protein turnover, and with higher plasma levels of cortisol and growth regulating hormones. Mommsen and Moon (2001) suggest that the concomitant increase in cortisol may increase protein degradation, offsetting some of the growth-promoting effects of growth hormones, resulting in an observed increase in protein turnover. Fish placed in a current following an acute stressor showed reduced plasma cortisol levels more rapidly compared to control fish, indicating swimming may enhance (or decrease the time of recovery) the recovery process (Young and Cech Jr. 1994b; Davison 1997; Milligan 1997). GH levels have been positively correlated with exercise in steelhead trout (Barret and McKeown 1988) and rainbow trout (Nielsen, et al. 1994), but in each instance GH was not a good marker for growth performance. Other hormones such as IGF may be a better marker for the exercise effects on growth in fish. Specifics on the effects of the GH/IGF axis on growth in fish are discussed in section [IGF Axis in Fish].

Exhaustive Exercise

The pioneering work of Black and his colleagues (1966) established the beginnings of what is now a comprehensive model used to explain the large

anaerobic capacity of fish. Black's study created the benchmarkin exercise physiology using fatigue or exhaustive exercise experiments. Exhaustive exercise studies have been used to quantify the aerobic swimming performance of fish, to understand fish muscle recovery after exhaustion, and more recently to understand the processes of protein synthesis, lactate removal and glycogen resynthesis associated with high energy, fatigue studies (Hammer 1995; Kieffer 2000; Milligan 2003).

Many studies in fish exercise physiology have focused on determining the maximum sustained swim speed or U_{crit} of different fish and under different conditions. This "critical speed" is generally classified as the measure of highest sustainable swim speed achieved and is associated with the aerobic maximum of the fish (Brett 1964; Black et al. 1966; Beamish 1978; Hammer 1995). Although called "aerobic maximum", as speeds approach U_{crit}, aerobic swimming alone cannot sustain swimming at high speeds. Studies with Chinook salmon (Geist, et al. 2003) and rainbow trout (Burgetz, et al. 1998) reported that anaerobic metabolism was required to support swimming at speeds equal to or greater than 80% U_{crit}. For determination of the aerobic maximum (U_{crit}), fish are usually placed in a flow tunnel and required to match the speed of the water for a predetermined amount of time. After the fish has swum for the predetermined time, the velocity is increased by some increment. This is repeated until exhaustion occurs. The U_{crit} is then calculated by a formula originally developed by Brett (1964), taking into account the fraction of time the fish was able to swim at the final incremental speed (Beamish 1978; Hammer 1995). The size of the

water velocity increments and the time between increments is highly variable among studies making comparisons between species and experiments difficult. A very extensive review by Hammer (1995) in an attempt to standardize these parameters has led to a more consistent incremental test. The review suggests that velocity increments should be 0.25 – 0.5 body lengths (BL) per second with an interval in the range of 30 minutes (Hammer 1995). As a fish approaches this U_{crit}, muscle metabolism switches from aerobic to anaerobic as the capacity of the red muscle is reached and fast twitch muscle mass is heavily recruited. Anaerobic swimming can only be maintained for a short time, so white muscle use rapidly leads to exhaustion. Because of this terminal change in muscle utilization during the Ucrit determination, a new measurement termed U_{sust} has been recently developed. The procedure is similar to U_{crit} determination with the exception that the U_{sust} speed is the speed that the fish was able to maintain for a complete 30 minute period while swimming using a gait characterized by steady, continuous tail beats (Sepulveda and Dickson 2000; Gibb and Dickson 2002). This speed is assumed to be powered solely by the slow twitch aerobic muscles and a better measure of aerobic swimming performance than U_{crit}.

U_{crit} is dependent on many external and internal factors further complicating comparative studies. The absolute swim speed of fish increases with size, although the relative speed decreases as size increases (Brett 1964; Hammer 1995). U_{crit} increases to an optimum as temperature rises and then decreases again (Beamish 1978; Claireaux, et al. 2006). The optimal temperature not only depends on the species but on acclimation temperatures, season, and in

some cases light conditions (Hammer 1995). Other environmental factors shown to affect critical swim speeds include pollutants, ambient gas concentrations, life styles and food availability (Randall and Brauner 1991). Life style and size may not affect swim performance in young fish. In a comparative study of maximum sustainable speeds in juvenile kawakawa tuna and chub mackerel (Sepulveda and Dickson 2000), a significant difference in U_{crit} was not seen. This is surprising as tuna are known to obtain such high aerobic speeds. A positive effect of sustained exercise training on U_{crit} has been demonstrated in a variety of species, (Houlihan and Laurent 1987; Young and Cech Jr. 1994a; Hammer 1995; Davison 1997), but the results with the salmonids are inconsistent. The inconsistencies in results among studies are largely due to differences in experimental design, which emphasizes the importance of a standardized training regime (Hammer 1995). Physiologically the amount of stored red blood cells and the proportion of red to white muscle mass have also been shown to affect the critical swim speed in fish (Hammer 1995).

In the early stages of exhaustive exercise, energy is largely derived from the hydrolysis of phosphocreatine (PCr) and ATP. Muscle PCr and ATP levels tend to decline as exercise continues, though the extent of depletion is species-specific and variable among studies, possibly reflecting differences in exercise protocol and evolutionary and physiological differences between species (Milligan 1996; Kieffer 2000; Kieffer, et al. 2001). As PCr and ATP levels decline, glycogenolysis is activated providing the majority of ATP required to sustain the exercise. As a result, glycogen levels may be reduced by 90%

(Milligan 1996). Associated with the reduction in muscle glycogen is an accumulation of lactate and H^+, reducing the muscle pH. Some of the acid and lactate are leaked in the blood, increasing blood lactate levels and reducing the pH of the blood (Milligan 1996; Kieffer 2000). Following the exercise bout, the recovery of ATP and PCr is rapid, usually returning to pre-exercise levels in 2 hours. In contrast, the removal of muscle lactate is rather slow, as is glycogen replenishment. In rainbow trout, peak levels of lactate in the blood are usually not reached until 2 hours into recovery, and basal levels are generally not seen for 8 hours (Milligan 1996). Regeneration of muscle ATP, PCr, and glycogen and clearance of the lactate load are necessary if fish are to regain their ability to sprint- or burst-perform (Milligan 2004).

A large research focus in exercise physiology has been to determine the metabolic fate of lactate and to find out how and where glycogen is resynthesized, particularly in vertebrates, since vertebrate skeletal muscle is not capable of gluconeogenesis. In mammals, lactate produced in the muscle is released into the blood and converted into glucose in the liver via the Cori cycle, which is then released to the blood and utilized by muscle to support glycogen synthesis (Alberts, et al. 2002). In fish the Cori cycle has little if any physiological role in glycogen resynthesis in skeletal muscle (West, et al. 1994). Instead, muscle glycogen synthesis occurs via lactate-based *in situ* glycogenesis (Milligan 1996). By retaining the lactate in the muscle, fish are able to recycle the lactate carbon *in situ*, which would not be possible via the Cori cycle (Milligan 1996; Milligan 2003). This understanding explains how lactate is cleared in fish muscle but does

not give insight into what fuels the regeneration of ATP, PCr, and glycogen during the recovery process. In a recent review by Milligan (2004), lipids were suggested as the primary substrate to fuel the regeneration of ATP, PCr, and glycogen in rainbow trout via novel fatty acid transport proteins in the red and white muscle. Future research is needed to confirm this in other species and to better understand fat transport and metabolism in fish.

During or soon after exhaustion, a rise in plasma cortisol and catecholamines is observed in fish (Milligan 1996; Kieffer 2000; Milligan 2003). In general, cortisol levels peak 1-2 hrs post exercise and remain elevated for up to 6 hrs, while plasma levels of noradrenaline and adrenaline are highest immediately following exercise and quickly return to basal levels by 2 hrs (Milligan 1996; Kieffer 2000). The release of catecholamines is believed to function in the mobilization of energy stores and oxygen delivery to the tissues. Interestingly, allowing the trout to swim against a slow current after exercise prevented the rise in plasma cortisol compared to fish held in still water (Milligan, et al. 2000). Studies with rainbow trout suggest the increase in cortisol after exercise exerts a negative influence on lactate and glycogen recovery metabolism (Milligan 1996). The continued elevation of cortisol to over 4 hrs post-exercise appears to exert an inhibitory effect on muscle glycogen resynthesis, which prevents restoration of glycogen stores (Milligan 1996; Milligan 1997). When cortisol synthesis was blocked in rainbow trout, there was an associated rapid decrease in glycogen phosphorylase *a* (Phos *a*) activity and a rapid increase in glycogen synthase activity as compared to controls, therefore hastening the

restoration of muscle glycogen stores (Milligan 2003). Complete restoration was accomplished at 2 hrs post-exercise in treated fish, compared to well over 4 hrs in control fish. Since muscle glycogenolysis results in lactate production and cortisol stimulates hepatic lactate gluconeogenesis, stimulation of glycogenolysis in muscle may provide lactate for hepatic gluconeogenesis (Milligan 1997). The associated decrease in Phos *a* following the elevated plasma cortisol may reflect its general role as an energy mobilizing hormone, keeping the muscle primed for rapid energy mobilization via glycogenolysis (Milligan 2003).

Several biotic and abiotic factors limit both aerobic and burst exercise performance and the recovery process from exhaustion in fish. The large intra-specific variability in the physiological response to exercise in fish may be the result of these factors (Randall and Brauner 1991; Kieffer 2000). Body size limits the amount of white muscle and therefore the amount of energy stores available in a fish. All the energy stores available for burst activity scales with body size in rainbow trout: smaller trout tend to have greater amounts of PCr and lower amounts of ATP and glycogen. Associated with the available energy stores, anaerobic capacity also appears to scale with body size, although there appears to be a cap on this source of energy production (Kieffer 2000). The relationship between anaerobic metabolism and body size is inconsistent among fish species; however, when comparing fish with similar locomotive habits (e.g. frequent burst activity), a scaling relationship for glycolytic enzyme activity (LDH) does occur (Somero and Childress 1990).

Temperature is one of the most important environmental factors to an ectothermic organism, such as a fish. Acute temperature change has been shown to influence muscle fiber recruitment, muscle contractile properties, PCr levels, the clearance rate of lactate, and the recovery rates for ATP and glycogen (Randall and Brauner 1991; Kieffer 2000). Long fasting periods form part of the natural life cycle in most species. This is due to the spatial and temporal patchiness of food associated with the aquatic environment. Fasting, whether occurring naturally or induced experimentally, depletes glycogen stores in fish (Kieffer 2000). The reduction in glycogen levels has been shown to set limits to burst performance and lower the anaerobic capacity in fish (Kieffer 2000). And finally, endurance training has been shown to enhance the buffering capacity of pH and blood (Young and Cech Jr. 1994a), enzyme concentrations (Davison 1997), and the aerobic and anaerobic capacity (Davison 1997; Kieffer 2000) in a variety of fish species.

As an important note, the preceding review of exercise in fish presents the general consensus for most species. Contradictory results do exist, particularly in regard to muscle morphology and biochemistry. This may be a result of the differences in training procedures (condition and duration of exercise), the use of species unaccustomed to aerobic swimming in the wild (benthic fish), and as discussed in the previous paragraph, changes in a variety of environmental factors (Davison 1997; Kieffer 2000; Sanger and Stoiber 2001).

III. Muscle Growth in Fish

Fish growth in many species is indeterminate, although relative growth rate decreases with age as more energy is invested in reproduction. The same does not apply to absolute growth rates that have been shown to stay constant in certain species such as carp (Johnston 2001a). The majority of growth is invested in accretion of muscle tissue, particularly the fast twitch, anaerobic white muscle fibers. Muscle may account for more than half of the fish's body mass and due to its low fractional rate of protein synthesis and highest growth rate efficiency, compared to other tissues, an increase in muscle mass is an excellent overall measure of fish growth (Johnston 2001a; Mommsen 2001). Muscle organization, fiber type segregation, and muscle growth patterns in fish show unique differences from other vertebrates. For example, fish skeletal muscle fibres are multinucleate syncitia. Also, fish muscles grow by hyperplasia, an increase in cell number, and hypertrophy, an increase in cell size. With only a few exceptions, post-hatch hyperplasia is not important in any other vertebrate groups where muscle fiber number is fixed at birth (Johnston 2001b).

Non-hormonal Growth

Post larval growth of skeletal muscle is attributed to proliferation and differentiation of muscle satellite cells. These small cells are located between the sarcolemma and the basal lamina of differentiated muscle fibers. During growth, satellite cells either fuse with pre-existing muscle fibers resulting in hypertrophic growth or fuse together to form myotubes that differentiate into new fibers in

hyperplastic growth (Fauconneau and Paboeuf 2001; Johnston 2001a; Levesque, et al. 2008). The massive increase in girth between hatching and maturity takes place by the hypertrophy of muscle fibers but since the white muscle fibers rarely exceed 200 μm in diameter, growth to a large body size must require the recruitment of new muscle fibers (Johnston 1999). Although the contribution of hyperplasia declines with size, hyperplasia has been shown to persist in white seabass with standard lengths of greater than 91 cm (Zimmerman and Lowery 1999). The proliferation and differentiation of satellite cells are regulated by transcription factors called myogenic regulatory factors. Hormones along with extrinsic factors such as temperature and pollutants have also been shown to affect proliferation and differentiation of satellite cells in fish (Fauconneau and Paboeuf 2001).

The number and size distribution of the fibers is an important parameter in aquaculture, as it relates to the quality of the flesh: in general more fibers equal greater firmness in the flesh and therefore better quality. Extrinsic factors such as feeding regime and diet composition are known to influence fiber growth and therefore flesh quality (Johnston 1999). Other non hormonal factors that impact growth parameters include temperature, pressure, and osmotic conditions, and intrinsic components such as tension, innervation or activity patterns (Mommsen 2001). Exercise, osmotic shock, and feeding alter myofibrillar swelling in mammals and recently found to occur in teleosts. The cell swelling re-orients cell metabolism toward anabolic pathways, encouraging sustained protein and lipid synthesis with a parallel decrease in the rate of degradation. Also factors

controlling other energy demanding processes will have a bearing on energy potentially allocated to growth, for example growth is slowed during breeding. In addition, decreasing osmoregulatory demands has been shown to increase growth (Mommsen and Moon 2001).

Endurance exercise training is a powerful stimulus for muscle fiber hypertrophy in a variety of teleosts. The optimal velocity for growth varies among species and training regimes. In general long term swimming at low speeds ($\leq$ 1.5 bl sec^{-1}) will lead to increased growth in most species. Training at substantially higher speeds has distinct negative effects on growth in any fish (Davison 1997).

Hormonal Growth

Growth in fish and most vertebrates is largely controlled by growth hormones. Both environmental and nutritional factors are able to indirectly influence growth by affecting the release of these growth hormones. This is especially true for ectotherms like teleost fish, which rely on temperature, photoperiods, and food availability to trigger developmental processes. Information received from both external stimuli and the internal state of the body is processed, integrated, and responded to by the brain for the appropriate modifications of growth through hormonally mediated pathways (Duan 1998). A central step in this endocrine pathway is the growth hormone (GH) and the insulin-like growth factor (IGF) axis.

The somatotropic cells of the anterior pituitary gland produce and release GH under the regulation of hypothalamic hormones. Once released, the GH has a variety of targets throughout the body. The GH binds to its receptors in target organs, mainly the liver and muscle, stimulating synthesis and release of IGF-I and IGF-II. Many local physiological functions of GH in muscle are thought to be mediated by the IGFs since the evidence for direct effects of GH on muscle is not strong although it appears that muscle contains 2.5 times the total GH binding capacity as the liver (Mommsen and Moon 2001). Although aptly named, growth hormone has important functions beyond the regulation of somatic growth in fish. The hormone has an important role in smoltification and seawater adaptation in salmonids and in the hepatic biosynthesis of antifreeze proteins in marine teleost (Mommsen 2001; Mommsen and Moon 2001).

Exogenous GH will increase the rate of body mass accretion in feeding fish, and numerous reports on the effectiveness of GH to accelerate the rate of fish muscle growth are available (Mommsen, et al. 1999; Mommsen and Moon 2001; Levesque, et al. 2008) albeit under the tight control of IGF-I. It is generally believed that small fibers are earlier stages of larger ones. Injection of GH in rainbow trout (Fauconneau et al. 1997) and Atlantic salmon (Levesque et al. 2008) increased the proportion of small diameter fibers, indicating a key function of GH in the regulation of hyperplastic muscle growth. In several studies with salmonids, Fauconneau reported length increases or no effect of growth hormone in trout and salmon, although specific growth rate and muscle fractional protein synthesis rates were increased. Also, the immunoneutralization of GH was found

to decrease muscle protein synthesis (Fauconneau et al. 1997). Undoubtedly, GH plays an essential role in the maintenance of protein synthesis with the muscle identified as the most important target. GH enhances the rate of amino acid uptake from the intestine and at the same time, GH inhibits lipogenesis and activates lipolysis in adult fish. The free fatty acids create an oxidative substrate diverting amino acids away from oxidation and into growth (Mommsen 2001). Although muscle contains a much smaller amount of mass specific GH binding sites compared to the, the bulk of the muscle tissue more than compensates for the deficiency in receptor activity per gram of tissue (Mommsen and Moon 2001).

In addition to its direct effect, growth hormone can also alter the rate of muscle protein synthesis by a number of indirect routes. GH has been shown to increase appetite, dominance (Jonsson, et al. 1998), nitrogen retention, and amino acid incorporation (Cheema, et al. 2005). GH has been shown to accelerate the rate of amino acid uptake from the gut and from the plasma into muscle. Since muscle growth may be limited by the availability of building blocks, effects of GH on amino acid uptake could be particularly important in stimulating muscle protein synthesis (Cheema et al. 2005). It has been suggested that growth hormone also controls muscle lipid storage, but data on this in teleosts are rare (Mommsen and Moon 2001). These effects show the positive influence GH has on muscle growth in fish and many of these effects are mediated by local or hepatic production of IGF-I.

IV. IGF Axis in Fish

Overview

The IGF axis consists of IGF-I and -II, the IGF receptors, and the IGF binding proteins (IGFBPs). IGFs are polypeptides with high sequence similarity to insulin. The key importance of IGF for normal development and growth has been illustrated by a growth-retardation phenotype exhibited by IGF-I, IGF-II or IGF-I receptor "knockout" mouse (Duan 1998). In mammals, IGF-II is thought to be the primary growth factor for early development while IGF-I becomes prominent later in life. In fish the function of IGF-II is not restricted to the early developmental process because systemic differences between adults and juveniles do not occur (Duguay, et al. 1996; Evans 1998). IGF-I and –II have been detected in all developmental stages in fish, including unfertilized eggs (Moriyama, et al. 2000).

Although the highest concentrations of IGF-I and -II transcripts are found in the liver, they are abundant in every tissue of fish surveyed (Evans 1998). In general, circulating IGF-I arise from the IGF-I produced and secreted from the liver, while local IGF-I is produced by each tissue. As fish grow older the liver experiences the greatest decrease in expression of IGF-I transcripts compared to other tissues (Evans 1998). Locally produced IGF-I has been found in all tissues tested to date, which include the brain, gills, heart, gut, kidney, spleen, testis, and muscle (Duan 1998; Caelers, et al. 2004). The function of the locally produced peptide may act in a paracrine and autocrine fashion, and may compensate for the

decrease in the hepatic IGF production as a fish ages (Moriyama, et al. 2000; Chauvigne, et al. 2003; Wood, et al. 2005). Unlike liver IGFs, the non-hepatic transcripts are unresponsive to GH.

IGF-I is involved in the regulation of protein, lipid, carbohydrate, and mineral metabolism in the cell, differentiation and proliferation of the cells, and ultimately body growth (Moriyama et al. 2000). Actions of IGF-I are mediated by the IGF-I receptor and IGFBPs. In the extracellular environment, IGFs are bound to IGFBPs. The IGFBPs mediate the actions of IGF by transporting it to cell surface proteins or by sequestering it in the blood stream. There are two classes of high-affinity specific IGF receptors: the IGF-I receptor and the IGF-II receptor. The biological actions of IGFs are primarily mediated by the IGF-I receptor

Structure

There is high degree of structural similarity between IGF-I, IGF-II, and proinsulin genes. These genes are translated into prehormones. PreproIGF consists of a signal peptide, B-, C-, A-, D-, and E- domains in turn from the N-terminal. Mature IGFs are produced when the signal peptide and the E-domain are proteolytically removed from the preprohormones (Moriyama et al. 2000). The IGF-I gene has become more complex in higher vertebrates. In salmon, the IGF-I gene spans about 20 kb while in humans the gene is over 100 kb (Johnston 2001a). At least 4 different forms of proIGF-I have been found in salmon, and since then at least 1 form has been found in other fish species (Chen, et al. 1998;

Moriyama, et al. 2000; Hill and Goldspink 2003; Cheema, et al. 2005; Goldspink 2006). These variants are a result of alternate gene splicing and differ only in the E-domain (Hill and Goldspink 2003).

Evolutionarily, IGFs are ancient growth factors present in all vertebrates. A duplication of an ancestral "insulin-like" gene gave rise to the predecessors of insulin and IGF around the time of the emergence of vertebrates (Adams, et al. 1995; Kelley, et al. 1996; Duan 1998; Moriyama, et al. 2000; Dyer, et al. 2004b; Pierce, et al. 2005). Since their divergence, insulin and IGF evolved distinct anabolic roles with the insulin gene expressed exclusively in pancreatic β cells while IGFs are expressed in a variety of cell types where they serve as ubiquitous cellular growth promoters (Kelley et al. 1996). IGFs are highly conserved among vertebrates. Human and fish IGF-I are equally potent in mammalian and fish bioassay systems and nutritional, developmental, and seasonal patterns of IGF-I levels in fish are quite similar to those in mammals (Moriyama et al. 2000). Homology at the amino acid level is very well conserved with mammalian IGF-I and -II, so much so that human IGF-I is biologically active in fish (Duan 1998; Johnston 2001a). A divergence does exist in the lower fish families. Agnatha has only one form of IGF cDNA while all teleost and elasmobranches tested thus far have both IGF-I and IGF-II. This suggests IGF-I and II may have had an early divergence in vertebrate evolution between Gnathostmata and Agnatha (Shamblott and Chen 1992; Moriyama, et al. 2000). Another divergence may have occurred between fish and higher vertebrates. Depending on the teleost species, adult fish muscle has a two – six fold greater ability to bind IGF-I then

insulin. This situation is diametrically opposite that in mammals (Duan 1998; Johnston 2001a).

V. Regulation of IGF in Fish

Hormonal

There is good evidence that GH regulates IGF-I expression similarly in teleosts and in mammals; it is believed like that in mammals, GH is the primary positive regulator of IGF-I production in the teleost fishes (Moriyama et al. 2000). In a number of teleost species, IGF-I bioactivity in serum and tissue responsiveness to IGF-I are found to be GH-dependent in the hepatic tissues. Additionally, IGF-I exerts significant negative feedback on pituitary GH secretion inhibiting GH release (Moriyama, et al. 2000; Mommsen and Moon 2001; Dyer, et al. 2004b; Pierce, et al. 2005). Injection of GH significantly increased the IGF-I mRNA levels in the liver of coho salmon, rainbow trout, ell, and seabream (Duan 1998, Moriyama et al 2000). The increase in the IGF-I mRNA expression, induced by GH, was also accompanied by an increase in circulating IGF-I peptide levels (Moriyama, et al. 2000; Wilkinson, et al. 2006). Unlike in mammals, relative abundance of IGF-I mRNA in non hepatic tissues of fish seems not to be regulated by GH because injection of GH in non-hepatic tissues had no effect (Duan 1998; Perez-Sanchez and Le Bail 1999). In the case of salmon, there is differential expression and hormonal regulation of alternately spliced IGF-I mRNA transcripts in which Ea-1 and Ea-3 transcripts are restricted to the liver while Ea-4 is expressed universally. Duan et al. (1994) showed that while GH dramatically increased Ea-1 and Ea-3 transcripts, Ea-4 transcripts remained unaltered. Insulin-like growth factor-II shows little dependence on GH-- although

one study showed that GH can regulate the IGF-II gene in rainbow trout (*Oncorhynchus mykiss)* (Greene, et al. 1999).

Insulin may also play a role in regulating hepatic IGF-I expression in fish. Insulin alone has no effect on IGF-I mRNA levels but co-incubation of insulin with GH significantly enhanced the stimulatory effect of GH in salmon (Duan, et al. 1994). The insulin may act synergistically with GH to stimulate hepatic IGF-I expression in salmon; this effect is restricted to the liver. Growth-promoting effects of IGF-I are usually assessed by its capacity to stimulate sulfate incorporation into branchial cartilage. In this ability both IGF-I and insulin act indistinguishably. Effects from both of these hormones are not additive indicating a common intracellular pathway with a possible dimeric insulin receptor (Evans 1998). IGF expression in muscle is controlled by a number of hormonal factors, not least insulin and IGF-I, leading to autocrine feed-forward activation of IGF-I gene expression and implying independence of the growth hormone system (Mommsen and Moon 2001).

Environmental / Nutritional

The principal environmental regulator of the GH-IGF axis is nutritional status (Duan 1998; Perez-Sanchez and Le Bail 1999; Moriyama, et al. 2000). During fasting, energy is no longer used for growth and instead utilized to support metabolism. These effects are in part mediated by the GH-IGF axis where it is now widely accepted that food restriction causes plasma IGF-I decreases and elevation of plasma GH levels in a range of different fish species (Shamblott and

Chen 1992; Duan, et al. 1994; Duan 1997; 1998; Perez-Sanchez and Le Bail 1999; Beckman, et al. 2004a; Wilkinson, et al. 2006). The paradoxical increase in plasma GH with decreased plasma IGF-I is explained by the development of resistance to GH at the tissue level and regulation of the hepatic GH receptors (Duan 1997, 1998; Perez-Sanchez and Le Bail 1999). Presumably as a result of this GH resistance, hepatic IGF mRNA and in turn circulating IGF-I concentrations are shown to be significantly reduced in a variety of nutritionally limited fish species (Duguay, et al. 1996; Meton, et al. 2000; Pierce, et al. 2001; Beckman, et al. 2004a; Peterson and Small 2004; Pierce, et al. 2005; Wilkinson, et al. 2006). In salmonids, food deprivation resulted in a cessation of growth and decrease in serum IGF-I concentrations but a significant increase in serum GH and IGFBP-1 concentrations within 4 days (Moriyama, et al. 1994; Duan 1998). The starvation induced rise in plasma GH concentrations is associated with a significant decrease in hepatic GH binding sites, again suggesting a possible resistance to GH at the tissue level therefore causing a decline in the production of IGF- I at the tissue (Duan 1997; 1998; Pierce, et al. 2005). Dietary restrictions produce similar results in fish, as it does in mammals. Refeeding was shown to recover GH and IGF-I concentrations to control levels although the response time is longer in fish relative to mammals (Chauvigne, et al. 2003), regardless of composition or ration size (Metone et al. 2000). If GH is principally regulated by negative feedback from total plasma IGF-I then you would expect total plasma IGF-I to decrease prior to an increase in GH during a fasting response (Pierce et al. 2005; Wilkinson et al. 2006). When fed a variety of diet formulas a positive

correlation between growth rate and IGF-I concentrations was observed in barramundi and Atlantic salmon. Plasma IGF-I also significantly increased with increasing ration size in barramundi suggesting that IGF-I concentration may provide a tool for assessing the potential for new diets (Dyer, et al. 2004a; Dyer, et al. 2004b). It appears both protein and energy intake are critical in the regulation of IGF-I concentrations in fish.

Findings by Duan (1998) and Plisetskaya (1998) suggests that nutritional status regulates IGF-I production at the mRNA level. Starvation of coho salmon decreased hepatic IGF-I mRNA levels, whereas refeeding led to a rise of IGF-I mRNA. In fish a much longer starvation (3-4 weeks) and refeeding (1 week) is needed in order to produce a change in hepatic IGF-I mRNA concentrations compared with mammals (Duan, et al. 1993). This may be due to the generally slower metabolism of the ectothermal animals. In addition diurnal modulation of hepatic IGF-I mRNA levels occurred following food intake in the Gilthead sea bream, suggesting food intake may play a role in regulating the anabolic effects of IGF-I (Meton, et al. 2000). Also the nutritional regulation of IGF-I is restricted to the liver in salmon with no significant changes detected in non hepatic tissues. This may be explained by the differential expression and hormonal regulation of alternately spliced IGF-I mRNA transcripts in salmonids (Duan, et al. 1994). In contrast refeeding of rainbow trout after a fasting period resulted in a dramatic increase in muscle IGF-I mRNA while the negative growth regulator myostatin (MSTN) mRNA levels remained unchanged during the fasting and refeeding (Chauvigne et al. 2003). These results indicate that muscle IGF-I may contribute

to muscle compensatory growth in rainbow trout and that the autocrine / paracrine action of IGF-I may be able to support normal post natal growth and development emphasizing the importance of locally produced IGF-I (Sjogren, et al. 1999; Yakar, et al. 1999). Refeeding following a 1, 2, and 4 week fast in rainbow trout resulted in compensatory growth and associated recovery of IGF-I mRNA expression in liver and muscle A similar result was presented in the gilthead sea bream. The muscle IGF-I mRNA expression had a faster response capacity to refeeding than the liver indicating that the two IGF isoforms are regulated in distinct manners by nutritional status (Montserrat, et al. 2007a; Montserrat, et al. 2007b).

Temperature profoundly influences growth of heterothermic vertebrates. As in other heterotherms, exposure to cool temperatures generally slows metabolism and growth while high temperatures along with sufficient food will generally increase metabolism and growth in fish. Although only a few studies have investigated the effects of temperature on growth and the IGF axis in fishes, recent research has suggested IGF-I may mediate growth at different temperatures (Wood, et al. 2005; Luckenbach, et al. 2007). Channel catfish reared at two temperatures exhibited expected faster growth and higher plasma IGF-I levels at higher temperatures (Silverstein, et al. 2000). Similar studies with Atlantic salmon (McCormick, et al. 2000), post-smolt (Beckman, et al. 2004b) and adult (Larsen, et al. 2001) coho salmon and Rainbow trout (Gabillard, et al. 2003) have produced similar results. In contrast juvenile flounder reared at 23 ^{o}C exhibited significantly higher growth, plasma IGF-I levels, and muscle IGF-I mRNA

compared to 28 ^{o}C reared flounder (Luckenbach et al. 2007). The 28^{o}C temperature may have exceeded the thermal optimum for this species. Further research is needed to confirm this. In one study with IGF-II, IGF-II mRNA in muscle was not correlated to temperature-associated differences in rainbow trout growth (Gabillard et al. 2003).

Developmental

IGF-I and IGF-II are detected in all developmental stages in fish although IGF-II is expressed predominately during the larval and post-larval stage. Soon after hatching the liver becomes the principle site for endocrine IGF-I production under the regulation of GH. IGF-II mRNA levels in the gilthead seabream were highest one day after hatching and decreased thereafter. In contrast IGF-I mRNA increased in 12 and 16 day old larvae (Duguay, et al. 1996). The steady increase of IGF-I mRNA has also been recorded in salmon larvae (Duguay, et al. 1992) and 11 day old turbot fish (Berwert, et al. 1995).

During the juvenile stage of the anadromous salmon, the fish undergo a parr-smolt transformation. During this smoltification stream salmon (par) transform to seawater adaptable forms (smolt) and plasma GH, insulin, and hepatic IGF-I mRNA levels are elevated (Duan 1997). Similar results have been observed during smoltification in rainbow trout and coho salmon (Duguay, et al. 1994). These changes in GH and IGF are closely correlated with the growth rates of the fish and environmental cues. In salmonids and many other species, mature males grow faster than non-mature ones. Immature coho salmon plasma IGF-I

and 41 kDa IGFBP levels increased in response to androgen in a significant dose dependent manner, while GH was unaltered. This suggests that during reproductive maturation the gonadal steroids (testosterone and 11-ketotestosterone) may play a significant role in regulating IGF-I and its binding proteins in fish. This may provide a growth regulatory mechanism for bringing about dimorphic growth patterns observed between sexually maturing and non-maturing cohorts of a given species (Larsen, et al. 2004).

Biological Action of IGF Factors in Fish

The biological activity of IGF is highly conserved among vertebrates and its biological actions in fish are diverse. IGF-I has been shown to stimulate DNA synthesis, cartilage sulfation and protein synthesis, nutrient uptake, enhance sea water adaptability, stimulate spermatogenesis and induce final oocyte maturation (Duan 1994, 1997, 1998; Moriyama et al. 2000). It enhances proliferation and differentiation of myoblasts while also regulating apoptosis, mitosis, chemotaxis, and renal Na^+ transport. There is evidence to say it also suppresses the rate of protein degradation, accelerates the rate of amino acid and glucose uptake by tissue, and increases whole body parameters as diverse as aggressiveness, food consumption, and growth (Mommsen and Moon 2001). Mammalian IGF-I has been shown to stimulate protein synthesis in the muscles of the gulf killifish (Negatu and Meier 1995) and appeared to accelerate salmon growth when infused through implanted mini osmotic pumps (McCormick 1996). Others have shown that injection of IGF-I improved the ability of salmonids to maintain plasma osmoregulation and sodium levels (McCormick 1996; Moriyama, et al. 2000).

Further, IGF-I has been shown to act directly in stimulating gill Na^+, K^+ - ATPase in Atlantic salmon (McCormick 1996). But IGF-Is ability to increase salinity tolerance and sea water adaptation is not limited to anadromous fish such as salmonids. Treatment with GH or IGF-I also increases salinity tolerance and/or gill Na^+, K^+ -ATPase activity of killifish, tilapia, and striped bass (Mancera and McCormick 1998).

IGFs play an essential role in somatic growth and in particular muscle growth in fish. IGF-I acts on muscle, cartilage, and most cell types to increase growth by stimulating nutrient uptake, protein synthesis, and eventually cell division (Dyer, et al. 2004a; Dyer, et al. 2004b) IGF-I is a potent stimulator of DNA synthesis and proteoglycan synthesis of skeletal tissues in fish. In addition, the hepatic IGF-I mRNA levels and plasma IGF-I concentration are positively correlated with growth rate in juvenile coho salmon (Duan et al. 1997; Dyer et al. 2004b) and maturing Chinook salmon (Shimizu, et al. 2000). Generally fish species with higher growth rates exhibit higher plasma IGF-I levels compared with slow growing species and, interestingly, warm water fish show higher plasma IGF-I levels than cold water (Pierce, et al. 2001; Beckman, et al. 2004b). In an intra species comparison study, Peterson et al. (2004) found that IGF-II mRNA levels were greater in muscle and surprisingly liver of fast growing strains of channel catfish compared to slow growing channel catfish. IGF-II mRNA levels in the liver and muscle were also higher than IGF-I mRNA levels, a first for any species, indicating that IGF-II may play a more important growth promoting role then IGF-I in certain species and instances.

IGF Binding Proteins

IGFBPs prolong the half life of IGF, coordinate, and transport IGF in blood and across capillaries, regulating the bioavailability of IGF (Peterson and Small 2004). Unlike insulin, IGFs tend to circulate through the blood bound tightly to specific IGF binding proteins (IGFBPs). For example in mammals IGFBP-3, which carries greater than 80% of circulating IGFs in serum, exists primarily as a 150-kDa ternary complex made up of IGF, IGFBP-3, and a bound acid-labile subunit (ALS) (Shimizu, et al. 1999; Kelley, et al. 2002a; Peterson and Small 2004). In contrast IGFBP-1 is distributed in a 40 kDa binary complex made up of IGF and IGFBP-1, carrying about 20% of IGF-I in circulation (Shimizu, et al. 1999). At any one time less than 1% of IGF circulates in the free form. These large ternary and binary complexes function to maintain a sustainable and functional IGF reservoir within circulating blood. For example the IGFBPs have been shown to increase the half life of IGF-I from less than 10 minutes to up to 12 hours (Kelley, et al. 2002). The IGFBPs have a very high affinity for IGFs rivaling the IGF receptors and not only block the potential of IGFs to have insulin-like side effect, but they are also important modulators of IGF ligand-receptor interaction. In contrast, insulin has no affinity for any of the IGFBPs (Kelley, et al. 1996).

At least six IGFBPs (IGFBP 1-6) have been cloned and sequenced in mammals thus far and appear to be highly conserved among vertebrate, with up to an 80% nucleotide sequence homology between species (e.g. rat and human). This is evident by the ability of IGFBPs from fish, amphibians, reptiles, birds and

metatherian mammals to specifically bind mammalian IGF-I (Kelley, et al. 1996; Siharath, et al. 1996). The six IGFBPs differ in size, site of synthesis, and physiological roles with some being inhibitory (high affinity biding of IGF blocking cell receptor binding) and others promoting ligand/receptor interaction (Moriyama, et al. 2000; Kelley, et al. 2002a). The IGFBPs are derived from a super family of cystine-rich growth factors and are considered growth integrators having important influences on distribution and bioavailability of IGF in the cell and physiological environment (Kelley, et al. 2002). IGFBPs exhibit an array of specialized properties including interactions with proteins that post-translationally modify them, direct IGF-dependent cellular actions, and intracellular nuclear actions. They function to prolong the half life of IGFs, regulate their metabolic clearance, and modulate the interaction of the IGFs with their receptors thereby indirectly controlling the IGFs biological action (Duan 1997, 1998; Moriyama, et al. 2000; Kelley, et al. 2002; Peterson and Small 2004).

Unlike in mammals where IGFBPs are primarily hepatic in origin, they are produced by variety of tissues and cells in some fish. In striped bass the kidney, spleen, brain, gonad, pituitary, gill filament, bone, cartilage, heart, gut, muscle were found to produce single 30 kDa IGFBP *in vitro* while liver produced both 30 and 24 kDa IGFBPs. This non hepatic "local" tissue IGFBP expression appears to be regulated by a variety of physiologically relevant factors (Kelley, et al. 2002). At basal levels IGFBPs and IGFs are far less prominent features of fish plasma compared to mammals and a large IGFBP ternary complex has yet to be found in fish. In coho salmon most IGF-I was eluted around 40 kDa suggesting

the major form of bound IGF-I in circulation may be in a 40 kDa binary complex rather than in a 150 kDa ternary complex, as in mammals (Shimizu, et al. 1999). The total amount of IGF in fish plasma is normally around 25 ng mL^{-1} with little more than 0.1% of this total IGF present in the biologically active form compared to a circulating mammalian concentration between 500-800 ng mL^{-1} (Kelley, et al. 1992; Plisetskaya 1998). However when provoked physiologically, levels of one or more IGFBPs can be substantially altered (Kelley, et al. 2002b). Based on these findings Kelley, et al. (2002) propose that in fish, enhancement or inhibition of growth requires an increase IGFBP concentrations from low basal levels, in contrast to mammals that maintain a standing reservoir of these growth factors.

In vertebrates, the mature IGFBP-1 protein is ~30 kDa in size. IGFBP-1 is strongly expressed in the liver and kidney and its regulation is primarily associated with metabolism and reproduction. The IGFBP-1 gene is strongly expressed under catabolic conditions including fasting and is the primary IGFBP found in amniotic fluid and fetal plasma (Kelley, et al. 1996). In general, IGFBP-1 exhibits inhibitory growth effects in vertebrates. The IGFBP-2 protein is around 31 kDa in rats and 36 kDa in humans. IGFBP-2 is expressed in a number of reproductive tissues and is the major IGFBP in cerebrospinal fluid. Metabolic factors regulate IGFBP-2 in a similar but lesser extent to IGFBP-1 (Kelley, et al. 1996). The IGFBP that carries the most of the circulating IGF is IGFBP-3. IGFBP-3 is positively regulated by GH and IGFBP-3 levels in the blood stream are positively correlated with somatic growth. But IGFBP-3 is also a well documented inhibitor of cellular proliferations. Although generally up-regulated

during growth, IGFBP-3 can play both a stimulatory and inhibitory role in vertebrate growth. IGFBP-4 is the smallest of the six IGF binding proteins at 24 – 28 kDa. In general, IGFBP-4 inhibits the cell function of IGF by sequestering the local IGF and therefore preventing the IGF –cell receptor interaction. IGFBP-5 is produced at ~29 kDa size. It is the predominant IGFBP is bone and is believed to function to promote IGF ligand-receptor interaction in the bone matrix. In contrast to the bone, IGFBP-5 is associated with growth inhibition in the kidney. Little is known of IGFBP-6. In humans it is ~30 kDa and unique to the IGFBPs has a higher affinity for IGF-II over IGF-I. IGFBPs have become complex players in the growth regulatory axis, with some IGF binding proteins exhibiting both stimulatory and inhibitory roles. At this time the potential identity comparisons between mammals and fish are based on similar molecular size and similar actions and inducers. Currently research is being conducted to identify similarities in the IGFBP gene and protein sequence of fish and mammals.

Of the six binding proteins found in mammals at least three are also found in fish, a higher molecular weight 35-50 kDa-IGFBP and two IGFBPs in the ≤ 31 kDa size range (Duan, et al. 1999; Moriyama, et al. 2000; Kelley, et al. 2001; Kelley, et al. 2002b; Maures, et al. 2002; Maures and Duan 2002). Based on similar molecular weights and endocrine regulation to that of mammals, numerous experiments have proposed that the 35-50 kDa fish IGFBP may be analogous to mammalian IGFBP-3, while the ≤ 31 kDa fish IGFBPs may be counterparts of mammalian IGFBPs -1 and/or -2. The 35 – 50 kDa fish IGFBP shows positive regulation by pituitary GH and is directly correlated with somatic

growth rate very similar to the physiological roles of mammalian IGFBP-3 (Siharath, et al. 1996; Duan 1998; Kelley, et al. 2001; Kelley, et al. 2002a; Kajimura, et al. 2003; Peterson and Small 2004; Duan and Xu 2005). In the Chinook salmon Pierce et al. (2005) observed that during fasting, plasma IGFBP of 41 kDa declined, following IGF-I levels closely and both were stimulated by exogenous GH. An up regulation of the 35 – 50 kDa IGFBPs by GH stimulation and a lack of effect or decrease in concentration by stress (starvation, cortisol) was seen in goby (Kelley et al. 2002), channel catfish (Peterson and Small 2004), tilapia (Park, et al. 2000; Kajimura, et al. 2003), rainbow trout (Kelley, et al. 1992; Fukazawa, et al. 1995), striped bass (Siharath et al. 1996), and Coho salmon (Kelley, et al. 1992; Shimizu, et al. 1999; Shimizu, et al. 2005). In mammals IGFBP-3 declines during fasting and is stimulated by GH (Pierce, et al. 2005), indicating that the 35- 50 kDa IGFBP in fish may be analogous to IGFBP-3.

The role of IGFBP-3 is not exclusively stimulatory. In solution IGFBP-3 competes with the IGF receptors for IGF. IGFBP-3 has a stronger affinity for IGF then the IGF receptor and it also as the ability to bind directly to the receptors, thereby preventing the IGF-membrane receptor binding (Kelley, et al. 1996). Channel catfish (*Ictalurus punctatus)* produce a negative atypical physiological response of the 35-47 kDa IGFBPs to GH treatment (Johnson, et al. 2003). Compared with other teleosts, plasma IGF-I levels are 3 – 10 fold lower after GH treatment and plasma IGFBP-3 (35-47 kDa) levels are reduced, indicating a potential inhibitory role of the IGFBPs in catfish (Johnson, et al.2003).

The ≤ 31 kDa fish IGFBPs are up regulated in catabolic states, negatively regulated by insulin, and inversely correlated with somatic growth, indicating a possible relationship to mammalian IGFBP-1 and -2 (Siharath, et al. 1996; Duan 1998; Kelley, et al. 2000; Kelley, et al. 2001; Kelley, et al. 2002b; Peterson and Small 2004; Peterson, et al. 2004). Kelley, et al. (2002) found that in fish experiencing catabolic conditions, 30 and 24 kDa IGFBPs increased in serum in association with increased cortisol levels and suppression of growth. After 60 min handling stress the Kelley lab found serum IGFBP (30 kDa) in jack mackerel (*Trachurus symmetricus)* increased substantially along with an 8 fold increase in serum cortisol. These findings indicate that fish 30 and 24 kDa IGFBPs display physiological response comparable to that of mammalian IGFBP-1. Stress induction brought similar results in a 25 kDa IGFBP of striped bass (Siharath, et al. 1996), 24 and 30 kDa IGFBP of goby (Kelley et al. 2001), ≤ 31 kDa in tilapia (Park, et al. 2000; Kajimura, et al. 2003), channel catfish (Peterson and Small 2004, 2005), coho salmon (Shimizu, et al. 1999) and zebra fish (Duan, et al. 1999; Maures, et al. 2002; Maures and Duan 2002). Exogenous cortisol increased 20 kDa IGFBP and decreased plasma IGF-I levels without observed differences in liver IGF-I mRNA expression in channel catfish (*Ictalurus punctatus*) (Peterson and Small 2005). Exogenous cortisol in tilapia administered rapidly, increased 24,28,30, and 35 kDa IGFBPs within 2 hours without altering plasma levels of GH or IGF-I, resulting in an inhibitory effect on IGF-I action. Plasma IGF-I decreased after 24 hours indicating cortisol induces a rapid increase in plasma IGFBP and more delayed decrease in IGF-I production (Kajimura, et al. 2003). In

postnatal and fetal stages IGFBP-1 and -2 may be a conserved physiological mechanism to restrict IGF stimulated growth and development process under stressful conditions (Kajimura, et al. 2005; 2006; Kajimura and Duan 2007). During early development in zebra fish, hypoxic conditions elevated IGFBP-1 expression therefore delaying growth and development of the embryos by inhibiting IGFs growth promoting actions (Kajimura, et al. 2005, 2006).

IGFBP-1 and -2 are not solely inhibitory and have been reported to potentiate IGF action in some cell systems. Recent *in vivo* studies have indicated that IGFBP-1 serves as a molecular switch by restricting IGF signaling and diverts the limited energy resources away from growth and development and towards those metabolic processes essential for survival (Kajimura and Duan 2007). IGFBP-1 can simultaneously inhibit binding of IGF-I to cells and stimulate their mitogenesis suggesting it may be potentiative by providing a sustained release of IGF-I to the cells. IGBP-1 is also involved in variety of reproductive functions including endometrial cycling, oocyte maturation, and gestational development. Its reproductive function is strengthened as IGFBP-1 is predominant IGFBP in amniotic fluid and fetal plasma (Kelley et al 1996). In early embryonic stages of zebra fish IGFBP-I was initially expressed in multiple embryonic tissues. In the embryos, hypoxia induced IGFBP-I mRNA expression in the pharyngeal arches, ventricle atrium, and brain. Interestingly after hatching hypoxia-induced IGFBP-I expression became liver specific in the zebra fish alluding to the developmental role IGFBP-I may have in fish (Maures, et al. 2002).

IGF Receptors

The biological actions of IGFs are mediated by receptors, which are found in a variety of cell types. In mammals there are two known receptors that specifically recognize IGFs, the IGF-I receptor and the IGF-II receptor. They are able to discriminate among the various members of the insulin / IGF family and show specific binding properties. However a certain cross-interaction between ligands and receptors occurs. For example under experimental conditions, IGF-II exerts its mitogenic actions through the insulin and IGF-I receptors and not through its own. It appears that the IGF-II receptor may only bind IGF-II to clear it from circulation and thus regulate the levels of IGF-II reaching the target tissues (Planas, et al. 2000). In fish and mammals alike the IGF-II receptor is a molecule structurally unrelated to the insulin or IGF-I receptors and does not share a common ancestry with the receptors of the insulin/IGF-I family (Planas, et al. 2000). When bound with IGF-II, the IGF-II receptor shows no known growth signaling function (Kelley, et al. 2002a) although the binding capacity of cells for IGF-II is more prominent than IGF-I binding in trout yolk sac larvae suggesting a possible role in development (Planas, et al. 2000). In all fish tissues examined IGF-I receptors appear to be more abundant than insulin receptors with the fish heart and brain containing the greatest number of IGF-I receptors. This appears to be the opposite in higher vertebrates where all endothermic species have higher numbers of insulin receptors then IGF-I receptors. The low number of insulin receptors in fish tissues may partially explain the known poor utilization of carbohydrates by fish tissue (Mommsen and Moon 2001; Planas, et al. 2000).

In fish the insulin and IGF-I receptors will mediate the actions of their prospective ligands. Fish insulin and IGF-I receptors are heterotetrameric proteins consisting of two extracellular α subunits containing the ligand binding site, which are linked by disulfide bridges to two membrane anchored β subunits containing the tyrosine kinase domain. Ligand binding studies using human and salmon IGF-I found high affinity binding sites on membranes from fish tissues indicating that the IGF receptor in fish seems to be highly conserved in structure (Parrizas, et al. 1995), with a higher than 80% sequence similarity to human IGF-I receptors (Planas, et al. 2000). In addition to the structural similarities the receptors show functional similarities with other vertebrate insulin and IGF-I receptors in that they are able to undergo autophosphorylation and to phosphorylate exogenous substrates when binding with IGF-I (Kelley, et al. 2002). Ligand binding studies using human and salmon IGF-I found high affinity biding sites on membranes prepared from several fish tissues. This confirms that IGF receptor in fish is conserved both structurally and functionally (Moriyama, et al. 2000). IGF-I receptor regulates cell proliferation by a number of routes controlled by different regions of the receptor. One route is transformation, a signal most likely transmitted by the C terminal sections of the receptor. Two other control routes are inhibition of apoptosis and activation of mitogenesis. The activation of mitogenesis is likely the most relevant function to hyperplastic muscle growth in fish (Mommsen 2001). In white skeletal muscle the number of IGF-I receptors is up regulated in situations when circulating levels of IGF-I are elevated, such as feeding (Planas, et al. 2000).

VI. Cortisol and the Stress Response in Fish

The integrated stress response in teleosts is characterized by primary, secondary, and tertiary responses. Physiological responses are grouped as primary and include activations of brain centers resulting in large releases of catecholamines (CAs) mainly from the chromaffin cells (homologous to the adrenal medulla) and corticosteroids from inter-renal cells. Secondary responses represent the immediate actions and effects of these hormones at blood and tissue levels including changes in metabolism and hydromineral balance while tertiary responses focus on the organism and population level and include growth inhibition, reproduction, behavior and overall resistance to disease (Wendelaar Bonga 1997; Mommsen, et al. 1999; Barton 2002). Stressors produce effects that threaten or disturb the homeostatic equilibrium and they elicit a coordinated set of behavioral and physiological responses enabling the animal to overcome the strain. During chronic stress the adaptive value normally used to alleviate the stressor may be lost resulting in inhibition of growth, reproductive behavior, and a suppressed immunity to pathogens. The brain coordinates the stress response in vertebrates through two major routes: the hypothalamic-autonomic nervous-adrenal medulla axis and the hypothalamic-pituitary-adrenal axis via CAs and glucocorticoids as primary messengers (Wendelaar Bonga 1997). Although both pathways are extremely important in the integrated stress response in fish, this review will focus on the hypothalamic-pituitary-adrenal axis and its adrenocortical end products, specifically cortisol.

Stress has intense effects on vertebrate systems both physiologically and behaviorally to overcome or adapt to the stressor. The endocrine control mechanisms in response to stress seen in mammals also occur in fish. These adaptive changes often lead to detrimental effects in livestock production such as inhibition of growth, reduced immunity response, and reproductive failure (Wendelaar Bonga 1997). Aquaculture systems are particularly susceptible to stress-related effects due to the ease in which chemicals and pollutants can enter the body of the fish via the gills and digestive tract in an aquatic environment. Fish often possess extremely sensitive sensory systems and therefore respond to external stressors at (concentration) levels well below that of terrestrial animals (Wendelaar Bonga 1997).

Catecholamines

In fish as in all vertebrates the catecholamines epinephrine and norepinephrine are released into the blood during periods of enhanced blood oxygen transport and mobilization of energy substrates such as the "fight or flight" response. After any acute severe stressor a quick and immediate increase in CA levels is typically followed by an equally rapid drop (< 10 min). Chronic stress allows elevated CA levels to persist for hours or even days. Typically a massive CA release is followed by a rapid rise in plasma glucose concentrations, gill blood flow, cardiac output, and oxygen uptake and transfer. Inhibition of hydromineral balance and liver glycogen levels is also common (Wendelaar Bonga 1997). Chronic stressors not only stimulate the release of CAs but may also indirectly modify the actions of these hormones. After a prolonged physical

stress a decreased responsiveness of chromaffin cells to the stressor was observed in rainbow trout (Reid and Perry 1994). This may reflect a conservation mechanism of liver glycogen stores in stressed fish (Wendelaar Bonga 1997).

Cortisol

The endocrine control of cortisol secretion is complex in teleosts with multiple hormones including GH having corticotropic actions as well as cortisol exhibiting self expression by negative feedback directly on the interrenal gland. Hypophysectomized fish exhibit significant reductions in plasma cortisol levels indicating the pituitary gland dominates the endocrine control of cortisol secretion (Young 1993). α-Melanophore-stimulating hormone (α -MSH) and adrenocorticotropic hormone (ACTH) are the main candidates for this type of control. Circulating ACTH then stimulates the interregnal cells embedded in the kidney to synthesize and release corticosteroids into circulation. Unlike catecholamine release from chromaffin cells, cortisol synthesis and release has a lag time of several minutes (Barton 2002). Therefore proper sampling can allow measurements of resting levels for this hormone in fish. A rise in plasma ACTH in conjunction with a rise in cortisol has been observed in a variety of salmonid species under acute stressors as well as in mammals. In contrast, in tilapia, handling stress elevated cortisol levels without the accompanied rise in plasma ACTH suggesting the relationship between ACTH and cortisol may be less restricted in this species (Wendelaar Bonga 1997). The role of α-MSH in hypothalamic control is suggested to be more in stress adaptation than direct cortisol secretion. Brown trout subject to handling and confinement stress

induced a rise in cortisol without raising α-MSH levels. α-MSH levels elevated only after thermal shock was combined with the other stressors suggesting that α-MSH may modulate the pituitary interrenal axis only in constantly stressed fish with chronically elevated ACTH levels (Wendelaar Bonga 1997).

In teleosts the function of cortisol combines actions comparable to the mineralocorticoid aldosterone and the glucocorticoids in terrestrial vertebrates. The gills, intestine, and liver are important cortisol targets as these organs function as major sites for hydromineral balance and energy metabolism, the two major actions of cortisol in fish. Cortisol also functions in reduction of growth rate and suppression of reproductive and immune functions (Wendelaar Bonga 1997). Characteristic cortisol elevations of fishes in response to acute stressors range within 30 and 300 ng ml^{-1} with most fish species exhibiting their peak levels within an hour of the stressor (Barton 2002). Notable exceptions due occur. Mazik et al. documented plasma cortisol levels to nearly 2,000 ng ml^{-1} after a handling and transport stress in striped bass (*Morone saxatilis)* (Mazik, et al. 1991). The developmental stage of the fish may also affect its response to a stressor. A fish's stress response generally develops within 2-4 weeks post hatch in most species (Barry, et al. 1995; Barton 2002). The first 1-2 weeks after hatching refer to a stress hyporesponsive period which may function to keep cortisol levels low and constant during a critical developmental period when these steroids could have a permanent detrimental effect (Barry, et al. 1995). In juvenile yellowtail (*Seriola quinqueradiata)* baseline whole-body immunoreactive cortisol concentrations increased concomitantly with the onset and development of

aggressive behavior, after the transition from larval to the juvenile stage. In the larva, stress tolerance was 100% and decreased during and after the transition to the juvenile stage from day 23. This may suggest the hypothalamic-pituitary-adrenal axis and stress response are not functional during the larval stages and become activated during the metamorphosis from larvae to juvenile in yellowtail (Sakakura, et al. 1998). Fish with a more rapid rate of development may elicit a stress response much earlier.

In euryhaline fish the effect of cortisol is stimulatory on branchial Na^+ and Cl^- extrusion, essential for hydromineral control in a marine environment. Generally interrenal cells proliferate, and cortisol secretion and clearance are stimulated after transfer to seawater, and will continue long after acclimation. However in freshwater fish the hydromineral control of cortisol has a stimulatory effect on the uptake of ions such as Na^+ and Cl^- rather than the extrusion of these ions in seawater. In andronomous fish such as the striped bass (*Morone saxatilis)* the hydromineral control maybe more complex particularly for fresh water acclimation. Striped bass stocked at a fresh water site had significantly elevated cortisol levels and lower survival compared to a brackish site. Adding salts to transport tanks has also been shown to reduce cortisol levels induced by transport stressors (Wallin and Van Den Ayle 1995). Regardless of the salinity, cortisol promotes the differentiation of chloride cells and increases the specific activity of ion-transporting enzymes, in particular Na^+ -K^+ -ATPase in gills, intestine, and kidneys (Wendelaar Bonga 1997).

In fish the effects of cortisol on carbohydrate, lipid, and protein metabolism are similar but possibly less prominent than the glucocorticoid actions in terrestrial vertebrates. In fresh water catfish (*Clarias batrachus)*, cortisol injection significantly increased citrate synthase (CS) activity and decreased lactate dehydrogenase (LDH). This suggests that cortisol induces the aerobic and inhibits the anaerobic processes in catfish (Tripathi and Verma 2003). Cortisol may stimulate lipolysis and a decrease in lipogensis was seen in fish with cortisol implants (Vijayan, et al. 1991; Mommsen, et al. 1999). Administration of cortisol is also frequently followed by hyperglycemia in fish but the mechanisms involved have only recently been studied. During stress, cortisol acts in the glucose-regulation and glycogen-repletion processes, both of which are important pathways for the recovery from stress. This action of cortisol is probably in conjunction with other glucoregulatory hormones such as epinephrine, glucagon and insulin. Cortisol may also be playing a role in the peripheral mobilization of substrates, thereby providing precursors for hepatic gluconeogenesis (Mommsen et al. 1999) and lactate-based *in situ* glycogenesis (Milligan 1996; Milligan 1997) in fish. The hepatic gluconeogenesis may be limiting the stimulatory action of cortisol and this may contribute to the loss of mass seen during chronic stress although the effects are substantially different among species suggesting the glucocorticoid actions of cortisol may vary depending on habitat and life style (Vijayan, et al. 1991). Cortisol can act rapidly to alter amino acid mobilization and metabolism following exhaustive exercise in rainbow trout. Cortisol may be inhibiting protein synthesis and stimulating protein catabolism therefore

increasing tissue free amino acids (Milligan 1997; Mommsen, et al. 1999). The liver is the most likely site of cortisol-stimulated proteolysis because it has the highest affinity cortisol receptors of all tissues examined, liver protein and amino acid turnover is very rapid, and branched-chain amino acids are seen to increase after exercise and cortisol elevation in mammals (Milligan 1997).

Because of its abundance and consistent elevation during stress, plasma cortisol is the most widely used stress indicator in fish. Cortisol plasma elevation is an easy and reliable technique because basal levels are very low in most species (< 5 ng/ml) and peak values of 10 to over 100 fold have been reported (Barton 2002). After a few minutes of exposure to an acute stressor, plasma cortisol levels rise and may remain elevated for one or more hours. During chronic stress, levels will remain elevated but typically below peak values. But natural variations in plasma cortisol levels have been demonstrated including elevated levels during the final stages of maturation, spawning, and also diurnal and seasonal cycles (Wendelaar Bonga 1997). It is important to note that the absence of a cortisol response does not guarantee the absence of a stressor. In salmonids, chronic plasma cortisol levels as low as 10 ng ml^{-1}, levels below those often associated as "unstressed" have been implicated in depression of immune function and disease resistance (Pickering and Pottinger 1989). Poor water quality, including pollutants and toxins, have also been shown to impair the cortisol response in several fish species (Mommsen, et al. 1999; Barton 2002). It is also well established that plasma cortisol levels are related to social rank in schooling

fish with subordinate fish having elevated levels relative to dominant individuals (Sakakura, et al. 1998; Gregory and Wood 1999).

Stressors have a profound effect on the hydromineral control in fish, making a disturbance of water and ion homeostasis one of the most characteristic aspects of stress in fishes (Wendelaar Bonga 1997). This is primarily due to the intimate relationship between the extracellular fluid in the gills and the ambient water. A common effect of stressors on fish gills is the high incidence of apoptotic cell death along with necrosis reflecting an increased turnover or accelerated aging of the branchial epithelial cells during stress (Wendelaar Bonga 1997). Similar effects have been observed in epithelial cells, where rates of necrosis, apoptosis, and mitosis occurred in the upper layer of the skin along with hypersecretion of mucus and dilation of intercellular spaces (Wendelaar Bonga 1997). Aside from the structural deterioration of the gills, stressors cause passive ion losses and water uptake in fresh water fish, increase passive ion fluxes and water loss in marine species, and inhibit active ion exchange in both as reviewed by Wendelaar Bonga (1997). These effects are related to the disturbance of ion homeostasis and acid-base balance.

While in a stressful state metabolic energy is relocated away from growth and reproduction, and toward activities to restore homeostasis including respiration, locomotion, tissue repair, and hydromineral regulation. During chronic stress this reduces the performance capabilities of the fish and the recovery phase following the stress. Inhibited growth is commonly seen in a variety of species following physical, social, and chemical stressors including but

not limited to changes in temperature, handling, confinement, and many kinds of pollutants (Wendelaar Bonga 1997). Food intake is also often reduced in stressed fish (Pickering and Pottinger 1989). In aquaculture, stocking densities influence food intake in a species-specific way. High densities have been shown to reduce intake and growth rate in coho salmon, brook char, and rainbow trout as reviewed by Wendelaar Bonga (1997). An increased metabolic rate is typically associated with acute and chronic stress. Barton (2002) reported a 25% increase in metabolism after brief disturbances along with a reduction in growth rate. Cortisol treatment had no sig effect on swimming performance (U_{crit}) in juvenile rainbow trout. Given that swimming is the predominant behavioral reaction to stressors in fish, it seems appropriate not to impair this strategy (Gregory and Wood 1999).

Reproductive success depends on complicated endocrine control and particularly in fish, many environmental variables including temperature, food availability, daily photoperiod, and water quality. These many factors make successful reproduction very dependent on environmental conditions. Toxins and pollutants have been reported to reduce fecundity, larval survival, and gonadal size and development (Wendelaar Bonga 1997; Mommsen, et al. 1999; Goos and Consten 2002). Stress is commonly associated with a reduction of reproductive performance in vertebrates including fish. Cortisol has often been indicated as the major factor suppressing reproduction in many vertebrates. In fish the evidence is not fully consistent. In reviews by Barton (2002) and Wendelaar Bonga (1997), exogenous cortisol was reported to reduce circulating estradiol, vitellogenin, and

androgens levels in some species with little to no change of the hormones in others. They concluded that cortisol might exert its effects at a level higher than gonadal steroidogenesis. Another study found elevated cortisol in female damselfish could transfer to the egg yolk resulting in smaller larvae at hatching (McCormick 1998). Goos and Consten (2002) reported that adaption to temperature stress caused retardation of testicular development, mediated by cortisol.

Stressors have an intense and diverse effect on the defense system of fish. Many stressors both natural and artificial in origin suppress components of both the innate and adaptive arms of the immune system predisposing fish to infectious diseases. In general stress causes a rapid increase in circulating neutrophils and a reduction in lymphocyte levels. Both B- and T- cell functions become inhibited and this is associated with a decreased resistance to opportunistic pathogens resulting in disease and mortality (Wendelaar Bonga 1997). In most reports high cortisol levels and reduced immune responses were positively correlated.

Growth is under multiple endocrine controls with GH and the IGF axis as the pivotal factors and since catecholamines and cortisol are the major hormones with growth-inhibiting effects, one would expect to see a stress induced response on the endocrine system. When studies exclusively focus on circulating GH levels in stressed fish the reports are inconsistent and difficult to interpret. GH levels increase, decrease, or remain the same depending on the species (Wendelaar Bonga 1997). Results become more consistent once GH is observed in correlation with the IGF axis. Chronic stress and increased cortisol have been

reported to increase low weight (< 32 kDa) IGFBPs relative to controls in a variety of fish species: 25 kDa IGFBP in striped bass (Siharath, et al. 1995), the 24 kDa and 30 kDa in the mud sucker goby (Kelley, et al. 2001), the 20 kDa IGFBP in channel catfish (Peterson and Small 2004), the 31 kDa in the zebra fish (Duan et al. 1999), the 24, 28, 30, and 32 kDa in tilapia and in jack mackerel caused by handling stress for 60 min (Kelley, et al. 2001). Associated with the IGFBP increase was decreased plasma IGF-I levels without observed differences in liver IGF-I mRNA expression. This suggests increased cortisol may play a role in the production of glucose by increased glycogenolysis in (Mommsen, et al. 1999). Thus one of the metabolic roles of cortisol during stress in fish may be glucose regulation processes. In the above studies cortisol levels returned to normal after 45 days suggesting glucose serves as a signal of nutritional status controlling cortisol response (Peterson and Small 2004). In experiments involving high level exogenous injections (400 mg/kg for 4 weeks) of cortisol, plasma IGF-I levels were significantly reduced. When low levels of cortisol were injected IGF-I decreased despite unchanged GH mRNA levels which suggests the sensitivity of IGF-I production to GH is reduced by cortisol (Kajimura, et al. 2003; Peterson and Small 2005). This indicates cortisol induces a rapid increase in low weight plasma IGFBP with a more delayed decrease in IGF-I production. The cortisol decreases plasma IGF-I levels through the attenuation of IGF-I gene expression in liver. This suggests longer exposure is required for the inhibitory effects of cortisol on IGF-I production to contribute to growth retardation (Kajimura, et al. 2003). As in mammals this may be one of the mechanism in

which cortisol reduces growth. Other mechanisms include variable feeding patterns, lower conversion efficiencies and appetite suppression reported when plasma cortisol levels are elevated directly reducing the growth rate of the fish species (Gregory and Wood 1999; Barton 2002).

The effects of cortisol are not limited to just the IGFBPs. Coupled with myostatin and GH, cortisol can act in concert to control muscle growth. Myostatin (MSTN) functions to negatively regulate muscle growth in vertebrates and it has been suggested to have an inhibitory role in hyperplastic muscle growth of fish (Xu, et al. 2003). In studies where rbGH was injected into rainbow trout, MSTN mRNA levels were reduced relative to controls while liver IGF mRNA and circulating IGF increased (Biga, et al. 2004). Roberts and Goetz observed GH responsive elements upstream of the MSTN promoter region in two different MSTN salmonid forms suggesting GH might down regulate MSTN gene transcription (2003). Trout treated with rbGH also had elevated cortisol levels positively correlated with MSTN-1 mRNA and negatively correlated with MSTN-2 (Biga, et al. 2004). Similar results were found in muscle wasting rainbow trout (Rescan, et al. 2001).

VII. Life History of Yellowtail

California yellowtail (*Seriola lalandi*), also known as yellowtail kingfish and goldstriped amberjack, are the largest members of the Carangidae or "jack" family. *Seriola* are considered a circumglobal species that supports commercial and recreational fisheries worldwide. The major commercial fishery for Yellowtail kingfish is in New South Wales and Japan, but the majority of landings worldwide come from recreational and sport anglers (Nakada 2002). California yellowtail are found in temperate waters of the Pacific and Indian oceans, off South Africa, Japan, and the United States (Gillanders, et al. 2001). Adults are a pelagic, schooling fish usually seen in small to large numbers. They generally inhabit rock shores, reefs, and islands, frequently adjacent to sandy areas in coastal waters in water depths above 50 meters (Gillanders, et al. 2001). Juveniles are usually found far from shore and associated with floating debris or weeds. Yellowtail are ferocious feeders with feeding activity peaking at dusk and dawn. Adults primarily feed on squid and small fish. Juveniles feed on zooplankton and small fish while drifting with the seaweed (Gillanders, et al. 2001). California yellowtail, like all members of the genus *Seriola*, utilize a carangiform swimming mode, are facultative ramventilators, and share many specialized morphological adaptations with the tunas, including a fusiform body shape to reduce drag, fin grooves to increase streamlining, a high aspect-ratio tail with a narrow caudal peduncle, and finlets along the trailing edges of the body (Dewar and Graham 1994; Dewar, et al. 1994).

Because of their relatively fast growth rate and high flesh quality, Japan currently recognizes and cultures 3 species of *Seriola*, including *S. lalandi*. In 1990, total landed quantity of yellowtail in Japan was 213,204 metric tons, of which over 70% was from aquaculture production (Kolkovski and Sakakura 2004). The production of yellowtail reached a maximum of 170,000 metric tones in 1995 (Nakada 2002). Of the 3 species, the market value for *S. lalandi* is highest due to stock size and preference of the Japanese people. In the last decade, Australia and New Zealand have developed an aquaculture industry specifically for *S. lalandi* (Kolkovski and Sakakura 2004).

VIII. Life History California Sheephead

The California sheephead (*Semicossyphus pulcher*) is a carnivorous, temperate, rocky-reef/kelp-bed fish in the Labridae (wrasse) family. *S. pulcher* feeds on a variety of invertebrates including mollusks, echinoids, and polychaetes (Cowen 1990). The California sheephead is considered a crucial species in the kelp forests because they are essential in regulating the urchin populations, which forage on kelp, and therefore have an indirect effect on kelp density and distribution (Dayton, et al. 1998). *S. pulcher* is a protogynous hermaphrodite, meaning all fish are born female and change to male later in life. Both environmental and social factors can affect the size at sex change and population sex ratio (Cowen 1990). California sheephead utilize a labriform swim style where routine movements are powered by the pectoral fins. Adults are diurnally active returning to their home shelter at night. Males are highly territorial of their home range, particularly during mating season (Topping, et al. 2005).

IX. Life History White seabass

White seabass (*Atractoscion nobilis*) are members of the Sciaenidae or "croaker" family. These fish have been found in coastal waters of the Eastern Pacific Ocean from Juneau, Alaska to Magdalena Bay, Baja California and in the northern Gulf of California (Thomas 1968). The white seabass congregate in schools and may also be found to exist solitarily. Throughout their range they are desired for their impressive size, high food quality, and angling challenge. White seabass experience prolonged hyperplastic growth, up to at least 91.8 cm, enabling this species to grow to their impressive ultimate size (Zimmerman and Lowery, 1999). Post larval white seabass have a fusiform body shape and utilize a sub-carangiform swim mode. In sub-carangiform swimming, routine propulsion through the water is powered by undulating the posterior third to half of the body, typical of trout or salmon. During the 1960's the number of seabass significantly declined and in 1984, an experimental restocking program for white seabass was established under the Ocean Resources Enhancement and Hatchery Program (OREHP) to help replenish the stock (Vojkoich and Reed 1983).

Chapter Three: Metabolic Enzyme Study

I. Introduction

Fish are mobile and generally can be manipulated to swim against a current, which makes them ideal subjects for exercise conditioning. However comparisons between studies are challenging due to differences in training protocols. Additionally, the results are species-specific; relating to the life styles and swim modes of each species (Hammer 1995; Davison 1997; Johnston 2001b). In general, continuous swimming during rearing stimulates somatic growth across a wide range of species, albeit the growth enhancement and optimal exercise speed are both species and age specific (Davison 1989; Young and Cech Jr. 1993a; Davison 1997; Yogata and Oku 2000). Swimming at speeds of 1.5 bl s^{-1} or less will enhance growth in most species while training at substantially higher speeds with have negative effects. Since excessive exercise can result in lower growth rates or increased stress (Davison 1997; Ogata and Oku 2000), it is necessary to establish an optimal flow velocity for each species, particularly for fish in aquaculture.

Enhancing growth is not the only important result of exercise in fish. Long term endurance training will induce muscle remodeling in many vertebrates, producing a more aerobic phenotype. In the reviews by Davison (1989; 1997), numerous studies incorporating a variety of species are given showing the effects of exercise on muscle morphology. In general, an increase in cell diameter and number of aerobic fibers is common as a result of endurance exercise. The increase in fiber size and number leads to a greater proportion of total red muscle along with increased capillarization (Young and Cech Jr. 1993a; Sanger and

Stoiber 2001). Interestingly, hypertrophy of the white muscle fibers may also be induced in some species, even at low speeds (Mommsen and Moon 2001; Martin and Johnston 2006).

Changes in non-morphological parameters such as aerobic capacity, behavior, and internal body chemistry also occur as a result of exercise training. Studies have shown that mitochondrial and anaerobic enzymes positively correlate with growth rates, locomotory habits and food availability and as more recent studies have shown sustained exercise (Somero and Childress 1980; 1990; Adams, et al. 1995; Pelletier, et al. 1995; Buhr 2002). The activities of these enzymes are often used as indicators of aerobic and anaerobic metabolism in fish. In the zebra fish, exercise changed the mitochondrial mass and up regulated metabolic pathways involved in aerobic metabolism (McClelland et al. 2006). Such metabolic enhancement may translate into improved performance or survival in fish released or transferred into net pens, where current flows may occasionally exceed velocities encountered during aquaculture.

To address the species specific effects of exercise training, three species of juvenile finfish with distinct swim modes and life styles were used. The California sheephead (*Semicossyphus pulcher*) is a carnivorous, temperate, rocky-reef/kelp bed fish that utilizes a labriform swimming pattern for routine movement. *S. pulcher* is a protogynous hermaphrodite with both environmental and social factors affecting the size at sex change and population sex ratio (Topping et al. 2005). Of the three species in this study, the sheephead have a relatively small territory spending most of their lives in a small home range. The

white seabass (*Atractoscion nobilis*) is the largest of the croaker family (Sciaenidae) on the U.S. Pacific coast. They are found in schools and also occur as solitary individuals generally around coastal kelp beds and rocky offshore islands (Thomas 1968). The white seabass routinely utilize a sub-carangiform swim style for routine movement and range from Baja California to Juneau Alaska. The California yellowtail (*Seriola llandi*) is the most active of the three species in this study. The yellowtail utilize a carangiform swimming mode, are facultative ram-ventilators, and share many specialized morphological adaptations with the tunas, including a fusiform body shape to reduce drag, fin grooves to increase streamlining, a high aspect-ratio tail with a narrow caudal peduncle, and finlets along the trailing edges of the body (Dewar and Graham, 1994).

The goals of this study were to address the species specific effects of exercise in fish. This research represents a beginning to determine an optimal rearing velocity for enhanced growth and aerobic performance for each species in a cultured setting. We hypothesize that the different lifestyles and swimming abilities among species will result in different exercise induced effects observed through: (i) aerobic and anaerobic muscle growth; (ii) changes in key metabolic and mitochondrial enzymes; and (iii) overall aerobic swimming performance.

II. Methods

Subjects

Juvenile white seabass *Atractoscion nobilis* and California sheephead *Semicossyphus pulcher* were obtained from Leon R. Hubbard Hatchery (Carlsbad, CA). Juvenile California yellowtail *Seriola lalandi* were provided by Hubbs-SeaWorld Research Institute marine hatchery (San Diego, CA). The timing of the experiments was based on the availability of the fish species. Juvenile white seabass reached target size (10.8 ± 0.2 cm) in March, yellowtail (8.4 ± 0.3 cm) in August, and the sheephead (8.3 cm ± 0.4 cm) in January.

Experimental raceways

The experimental raceway system was designed to allow for four exercise treatments to run concurrently, utilizing re-circulated water from a common sump to maintain consistency of water chemistry and temperature among tanks. The experimental system was constructed of four fiberglass raceways, each with dimensions of 551.2 x 50.8 x 30.5 cm. The raceways were placed on a rack creating a 2x2 stacked array (Fig. 3.1). Screens were placed at each end of the raceway to shield the water inlet and outlet from the chamber of fish. Water was pumped from a common sump to the inlet chambers at one end of each raceway using two ½ hp Spec pumps. Ball valves were used so the inflow rate of each raceway could be adjusted separately. Matala mat mesh (Matala USA) was used as the screen between the inlet and fish chamber. The inlet chamber was designed to remove energy from the water and create a laminar current pattern into the fish

rearing section. A perforated plastic grid separated the fish chamber form the outflow drain. Standpipes were used to maintain a constant water depth in all four raceways. With this configuration the volume available to the white seabass and California sheephead was 236.2 x50.8x10.2 cm. To obtain higher flow velocities appropriate for the yellowtail, the cross sectional area available to the fish was reduced by inserting cinder blocks in the raceway. A thin sheet of ABS was attached to the sides of the cinder blocks to eliminate any edge effect created from the rough edge of the cinder blocks. This reduced the dimensions available to the fish to 236.2 x 29.2 x 10.2 cm.

Flow velocities maintained in each raceway were determined as percentage of the maximum aerobic potential (U_{crit}) of each species, determined prior to training (see below). The four raceway flow regimes were termed Fast (60% U_{crit}), Moderate (40% U_{crit}), Slow (20% U_{crit}), and a minimal flow Control. Water quality parameters including temperature, dissolved oxygen, and ammonia were measured daily. Flow velocities were maintained using a Flowtracker Handheld ADV (Acoustic Doppler Velocimeter) (SonTek, San Diego, CA) and temperature was monitored using data loggers in each raceway and maintained using a heater placed in the sump. The yellowtail were trained at 21.9 ± 1^{o}C, the white seabass at 18.0 ±1^{o}C, and the sheephead at 17.3 ± 0.7^{o}C. Temperatures were based on the concurrent rearing conditions of the hatchery and fluctuated seasonally. A siphon was used to clean the raceways daily. Minimal cleaning was needed as the flow rate allowed most particulate matter and waste to be easily transported out of the fish chamber. In all trials fish were fed daily to satiation

with a commercial pellet food dispensed with a continuous belt feeder during a 12 hr daylight period.

Ucrit

Aerobic swimming performance tests were performed pre and post endurance training utilizing an incremental velocity test modified from the procedure of the Brett (1964). For the white seabass and California sheephead, a Brett style swimming tunnel was used to measure aerobic capacity (Fig. 3.2B). A variable-speed motor propels the water, so that a fish maintaining its position in the center of the chamber is swimming at the same speed as the flowing water. Fish were placed in the test section (36 cm x 18 cm x 18.1 cm) and allowed to acclimate for a minimum of 4 h during which oxygenated water was circulated through the section at a slow speed. After the acclimation period, the speed was increased to 0.25 BL sec^{-1}. Each experimental run consisted of a 30 min period at each of the designated speeds after which the speed was increased by 0.25 BL sec^{-1} up to the maximum sustainable speed. The experiment ended when the fish could no longer maintain their position in the current using steady continuous tail beats, assumed to be powered by the aerobic slow twitch muscle (Webb 1998). The time and speed of fatigue was recorded and used to determine the U_{crit} based on Brett's equation (1964):

$$U_{\text{crit}} = V_{\text{p}} + \left(\left(\frac{t_{\text{f}}}{t_{\text{i}}}\right) \times V_{\text{i}}\right),$$

Current velocity and laminar flow were monitored using a FlowTracker Handheld ADV® from SonTek. Aerobic capacity for the California yellowtail was

determined using the Brett-type swim tunnel respirometer described in Sepulveda and Dickson (2000) (Fig. 3.2A). At the end of the experiments, the fish were sacrificed using an overdose of MS-222 and frozen at -80^{o}C.

Stocking

On the basis of total length (TL), the fish were sorted and randomly divided into four groups: Fast (60 % U_{crit}), Moderate (40% U_{crit}), Slow (20% U_{crit}), and a minimal flow Control group. Each group contained 75 fish in the white seabass experiment and 53 for the sheephead. For the yellowtail, the Slow group was excluded and the remaining three groups each contained 80 fish. The exercise groups were placed in their corresponding raceways and allowed to acclimate under minimal flow for at least 24 hours. After acclimation, the water flow was increased gradually to appropriate velocities based on the percentage of a predetermined U_{crit}. Fish were exercised continuously under natural photoperiod for 27 days in the sheephead, 34 days for the yellowtail, and 42 days for the white seabass.

Sampling techniques

During the experiments, 10 fish from each group were randomly removed from each raceway prior to daily feeding (i.e., fish had not eaten for 12 hr); Table 3.1 shows the sampling days used for each species. Fish were sampled by netting and sacrificed using an overdose of anesthetic (MS222, tricane methanesulphonate). Tail length and body mass were measured and in all individuals blood samples were collected within 5 min of initial handling. In

smaller individuals, blood was collected using haematocrit tubes following caudal severance; once the fish grew to a larger size, blood was collected from the caudal vein using a syringe and 22-gauge needle. Blood was centrifuged at 3,000 rpm for 3 min and the plasma was removed, aliquoted, and stored at -80 ^{o}C for later analysis.

Red/White muscle ratio (total cross sectional muscle area)

A thin transverse cross sectional cut (1-2 mm) through the body of frozen fish was taken. For California yellowtail, the cut was directly anterior to the first dorsal fin ray of the caudal fin, but posterior to the anal vent representing 55% of the distance from the tip of the nose to the tip of tail. In the white seabass the cut was made directly posterior to the last dorsal fin ray at the beginning of the caudal peduncle or about 70% of the TL of the fish (Fig. 3.3). The location of the cross sections in both species represents an area of high red muscle without interference from the body cavity. The thin section was photographed using a camera attached to a dissecting microscope. The total cross-sectional area of red and white muscle tissues were measured using Image J computer image analysis software. Due to the small amount of visible red muscle in the juvenile California sheephead, a cross sectional red to white muscle ratio could not be obtained.

Enzyme Assays

Enzyme activities were determined in the red muscle and white muscle tissue from fish prior to and after training. White muscle tissue samples were always taken from the same side and from the same location in each frozen fish,

directly below the first dorsal fin (Fig. 3.4). Tissues were homogenized using a 2–mL ground-glass tissue grinder in 80 mM imidazole buffer, pH 8.0 at 20.0^{o}C. Lactate dehydrogenase (LDH) activity was measured using a method modified from Yancey and Somero (1978) and reported as µmol pyruvate converted min^{-1} g muscle^{-1} (units g^{-1}). Citrate synthase (CS) activity was quantified according to a modified method of Somero and Childress (1980) as µmol oxaloacetate converted min^{-1} g muscle^{-1} (unites g^{-1}). Enzyme reactions were conducted at 20^{o}C in a 1.0 mL volume cuvette.

Calculations and Statistical analysis

Length (cm) and mass (g) were used to calculate a condition factor [CF = (mass x total length $^{-3}$) x 100]. Individual specific growth rates (SGR) were calculated using the equation:

$$Specific\ Growth\ Rate = \left(\frac{\ln(W_2\ /W_1)}{(t_2 - t_1)}\right) x100$$

where W_1 represents the initial mass in g or the initial length in cm, W_2 the final mass or length and *t* the number of days exercised. Statistical analyses were performed using SPSS (version 15.0). Statistical comparisons among the four different treatment groups were made using a Kruskall-Wallis ANOVA. If a significant difference was found ($p < 0.05$), then pair wise comparisons between treatments were made using a Mann Whitney U test. Spearman's rank correlation coefficient was used to exam relationships between the biochemical variables and fish size. If a significant correlation ($p < 0.05$) was revealed, the size effect was removed using analysis of residuals from the regression of the biochemical

variable and fish mass. Then Mann Whitney pair wise comparisons between treatments were made to determine if a significant ($p < 0.05$) exercise effect was revealed after the size effect was removed.

Fig. 3.1. Graphical representation of the experimental raceway system. Matala filter mesh (Matala USA) was used as a flow straightener in the front of each raceway (photo).

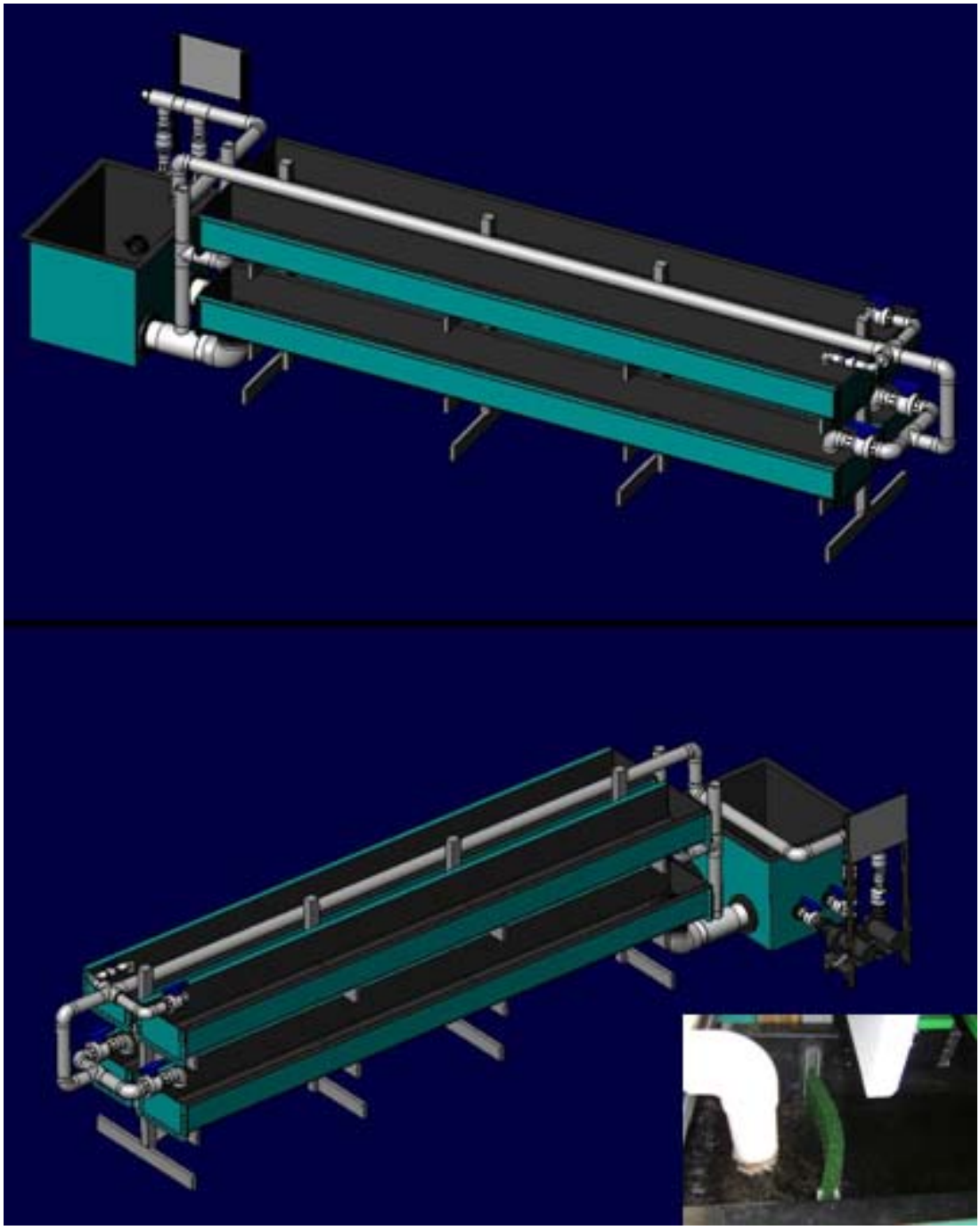
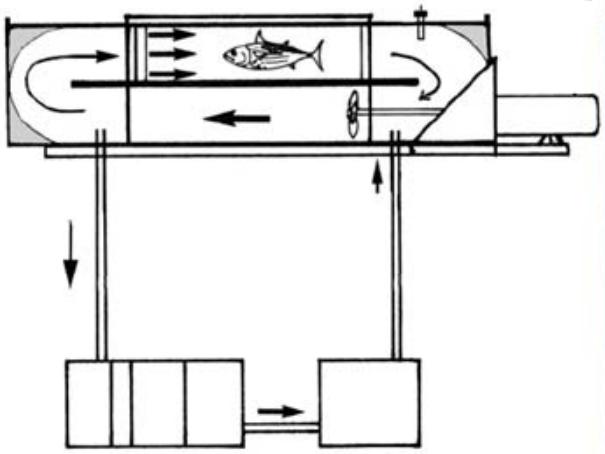


Fig. 3.2. Flumes used to determine U_{crit} in fish. California yellowtail were swum in Flume A. White seabass and California sheephead were swum in Flume B. (Illustration of Flume A from Sepulveda and Dickson 2000).

A



B

Fig. 3.3. Images of a transverse cross section of A*tractoscion nobilis* (A) and *Seriola lalandi* (B) at approximately 70% and 55% of total length. The red muscle (R) is highlighted in yellow. It is positioned along the midline of the fish, the white muscle (W) comprises the majority of the cross section, and the spine (S) is in the middle.

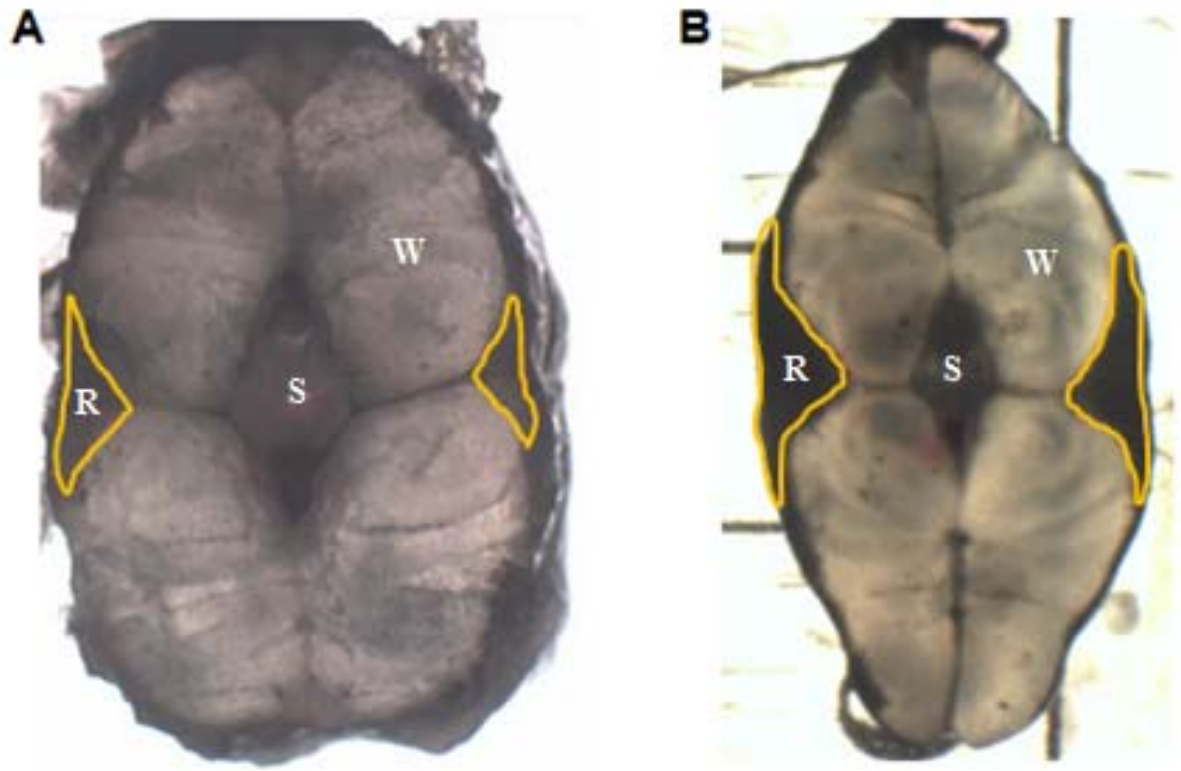

A
W
R
S
B
W
R
S

Fig. 3.4. White muscle was sampled directly below the first dorsal fin in all species (**W**). The red muscle was sampled along the lateral line in the middle of the body (**R**).

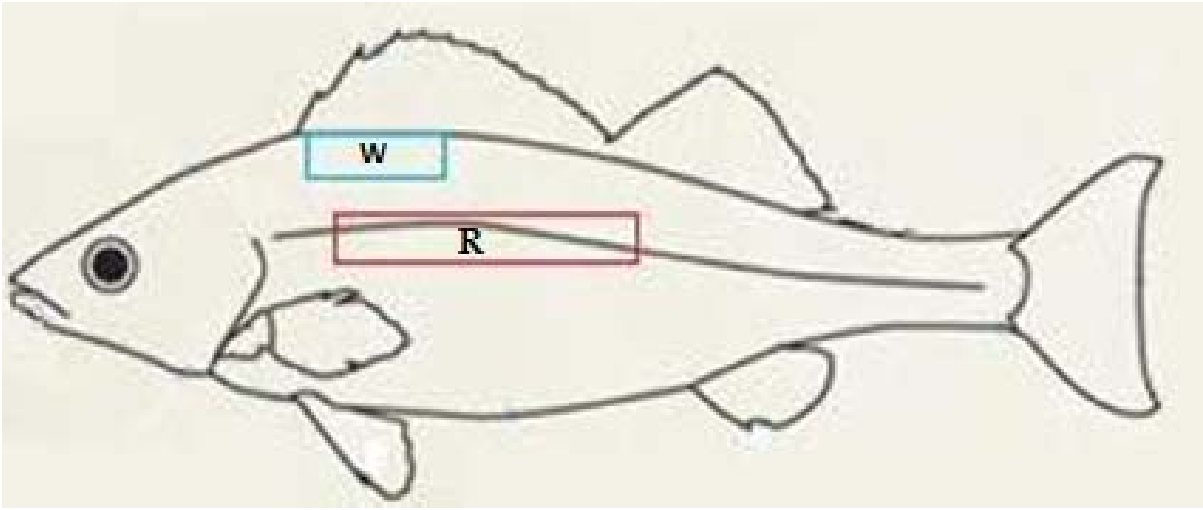

W
R

Table 3.1. Average temperature and list of sample days for each experiment.

Species	Temp (°C)	Raceway	Sample Days
White Seabass			
(Atractoscion nobilis)	18.0 ± 1	Control	
		Slow (20% U_{crit})	pre transfer,
		Moderate (40% U_{crit})	d 1, 3, 7, 14, 28, 42
		Fast (60% U_{crit})	
California Sheephead			
(Semicossyphus pulcher)	17.3 ± 0.7	Control	
		Slow (20% U_{crit})	pre transfer, post transfer,
		Moderate (40% U_{crit})	d 3, 7, 14, 27
		Fast (60% U_{crit})	
California Yellowtail 2007	21.9 ± 1	Control	pre transfer, post transfer,
(Seriola lalandi)		Moderate (40% U_{crit})	d 3, 6, 13, 27, 34
		Fast (60% U_{crit})	

III. Results

Three separate trials were conducted in the experimental raceways, one for each of the three species, California yellowtail, California sheephead, and white seabass. The California yellowtail experimental trial was run for 34 days beginning on August 30 2007. These fish were 55 days old post hatch (DPH) at the start of the experiment with a starting average total length of 8.4 cm with a standard deviation of ± 0.3 cm. Due to a limited number of fish the "slow" raceway was not used in the yellowtail trial. Results of a pilot study indicated that yellowtail swum at the "slow" speed did not exhibit an exercise induced change in growth or aerobic capacity relative to non-exercised control fish and the slow raceway was therefore omitted from the trial. The white seabass trail began on March 26, 2007 and lasted for 42 days. The fish were 148 DPH and total length was 10.8 cm ± 0.2 cm at the beginning of the experiment. The sheephead experiment ran for 27 days beginning on January 12, 2007. The sheephead total length was 8.3 ± 0.4 cm and age was 225 DPH at the start.

Growth and condition parameters

In the California yellowtail (*Seriola lalandi*), the specific growth rates of the fast (60% U_{crit}) group were approximately 24% greater than the unexercised group over the 34 day period. This resulted in a significant increase ($p< 0.05$) in fish mass and condition factor relative to the unexercised control group (Table 3.2). Although not significant at the 0.05 level, length and mass were greater in the moderate (40% U_{crit}) group when compared to the control after 34 days ($p =0.094$).

After 42 days, the condition factor of the fast exercised and control white seabass (*Atractoscion nobilis)* differed significantly ($p < 0.003$). California sheephead (*Semicossyphus pulcher)* did not exhibit an exercised induced enhancement of growth after 27 days of treatment at any exercise level.

Effects of exercise on metabolic enzyme activity

Results from all three studies indicated that the effect of exercise on metabolic enzyme levels was more pronounced in the red than white muscle. Furthermore, of the three species trained, the white seabass exhibited the greatest change in enzyme levels as a result of the exercise (Fig. 3.5). Exercise significantly increased the activity of both citrate synthase ($p < 0.001$) and lactate dehydrogenase ($p < 0.01$) in the red muscle of the white seabass, even after size effects were removed (Fig. 3.5). There was no significant change in enzyme activity in the red muscle of the yellowtail as a result of exercise, while in the red muscle of the California sheephead training at 40% U_{crit} significantly ($P<0.015$) decreased CS activity (Fig. 3.5).

Exercise did not induce a change in either the glycolytic or mitochondrial enzyme in the white muscle of the California sheephead and white seabass. In the yellowtail (Fig. 3.5), training at 40% and 60% of U_{crit} significantly decreased the CS and LDH activity in the white muscle ($p < 0.01$).

It is well known that many muscle enzyme concentrations, including LDH and CS, correlate with body size (Somero and Childress 1990). In the white seabass and yellowtail studies, correlation analysis of unexercised fish revealed

that several muscle biochemical variables were significantly correlated with body size. In the red muscle, both CS and LDH were positively correlated with body mass in the white seabass while only LDH showed a positive correlation to body mass in the white muscle (Fig. 3.6). In the white muscle of California yellowtail, CS exhibited a negative correlation with body size, and LDH was positively correlated (Fig. 3.7). Analysis of residuals was used in order to remove any effect size had on the metabolic enzyme activities associated with different exercise treatments. The asterisks in Fig. 3.5 indicate the significant differences between treatment groups after size effects were removed.

Red: White muscle ratio

Figure 8 indicates the changes in the ratio of total red to total white muscle tissue in cross-sections due to the exercise treatment in California yellowtail and white seabass. After 42 days, there was a significant difference among treatments in the white seabass ($p < .006$), with the unexercised white seabass having the smallest ratio (0.047) and the moderate treatment group possessing the largest ratio (0.100). Analysis of the muscle ratio in the California sheephead was not performed due to the small amount of lateral red muscle visible in cross sections.

In the yellowtail, after 34 days of exercise, the red to white muscle ratio in both the fast (60% U_{crit}) and moderate (40% U_{crit}) treatment groups differed significantly ($p = 0.004$ and $p= 0.037$) from the control group (Fig. 3.8). The fast treatment group had the largest red: white ratio (0.097) followed by the moderate (0.095) treatment group and the control group (0.081).

Aerobic Capacity

The maximum sustainable swimming speeds (U_{crit}) for unexercised California yellowtail ranging in length from 19.6 to 21.2 cm were 2.89 – 4.24 BL s^{-1}. The significantly larger ($p < 0.05$) fast trained yellowtail ranged in length from 18.5 to 21.9 cm and had U_{crit} speeds of 3.04 – 5.53 BL s^{-1} (Table 3.3). When comparing individuals of similar size, the trained fish had a larger U_{crit} than the control fish (Fig. 3.9). The regressions from the two treatment groups were not significantly different from each other. In the white seabass U_{crit} trial, the unexercised controls ranged in length from 14.4 to 16.0 cm with maximum sustained swim speeds of 3.16 to 3.45 BL s^{-1}. The exercised white seabass ranged in length from 12.3 to 15.6 cm and exhibited U_{crit} values that ranged from 3.16 to 4.18 BL s^{-1}. The linear regressions were not significantly different and there was no significant difference between U_{crit} of similar sized individuals, although the average maximum sustained swim speed for each group was significantly different. In all groups and species, U_{crit} (BL s^{-1}) decreased with body size (Fig. 3.9; Table 3.3). Aerobic capacity was not determined in the California sheephead.

Fig. 3.5. Activities of glycolytic (LDH) and mitochondrial (CS) enzymes in the red and white muscle tissue of fish after being exercised at 60% Ucrit (Fast), 40% Ucrit (Moderate), or un exercised (Control). Values are means ± S.E.M. * indicates a significant differences ($p < 0.05$) from the Control treatment among each species.

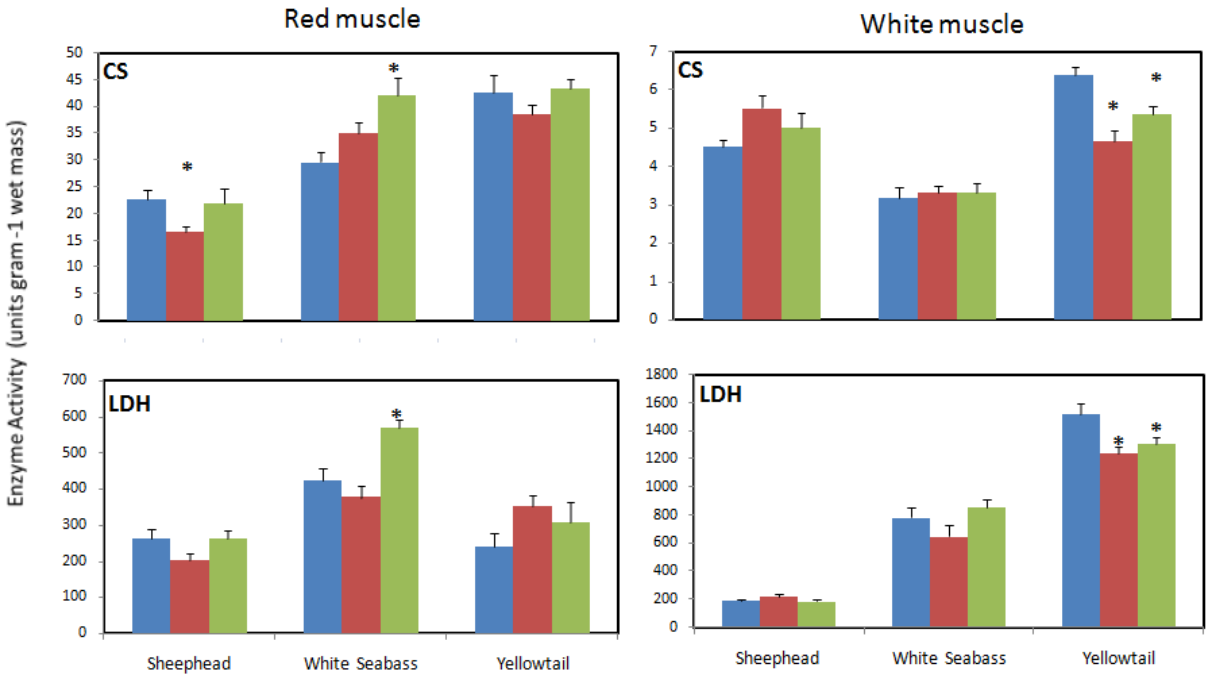

Red muscle
White muscle
CS
LDH
Enzyme Activity (units gram -1 wet mass)
Sheephead
White Seabass
Yellowtail

Fig. 3.6. The correlation of size (g) with lactate dehydrogenase (LDH) and citrate synthase (CS) activity in juvenile white seabass (*Atractoscion nobilis)*. The equations represent the linear relationship. ***** indicate a significant correlation. CS in the red muscle is positively correlated ($P < 0.003$) with mass. LDH in the red muscle is positively correlated ($P < 0.004$) with mass. LDH in the white muscle is positively correlated ($P < 0.001$) with mass.

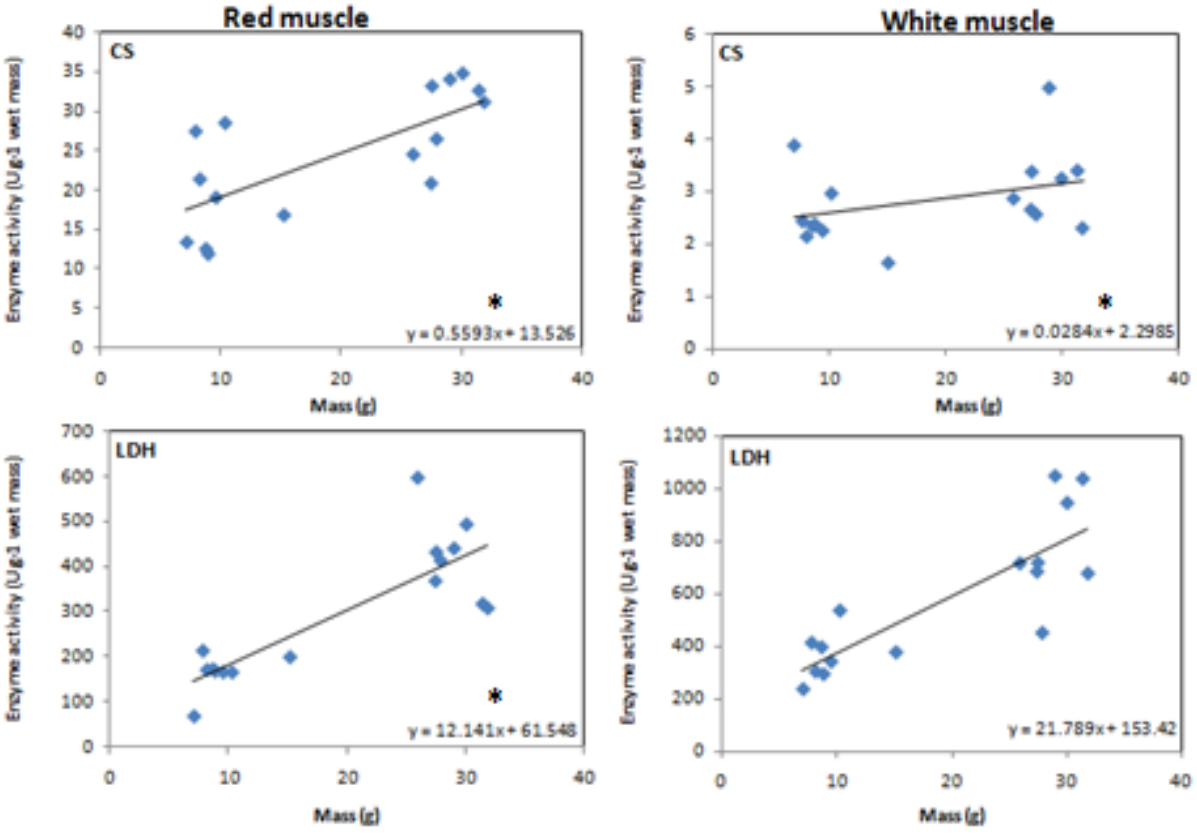

Red muscle
White muscle
CS
LDH
Enzyme activity (U g-1 wet mass)
Mass (g)
y = 0.5593x + 13.526
y = 0.0284x + 2.2985
y = 12.141x + 61.548
y = 21.789x + 153.42

Fig. 3.7. The correlation of size (g) with lactate dehydrogenase (LDH) and citrate synthase (CS) activity in juvenile California yellowtail (*Seriola lalandi).* The equations represent the linear relationship. ***** indicate a significant correlation. In the white muscle, LDH is positively correlated ($P < 0.001$) with mass. CS in the white muscle is negatively correlated ($P < 0.007$) with mass.

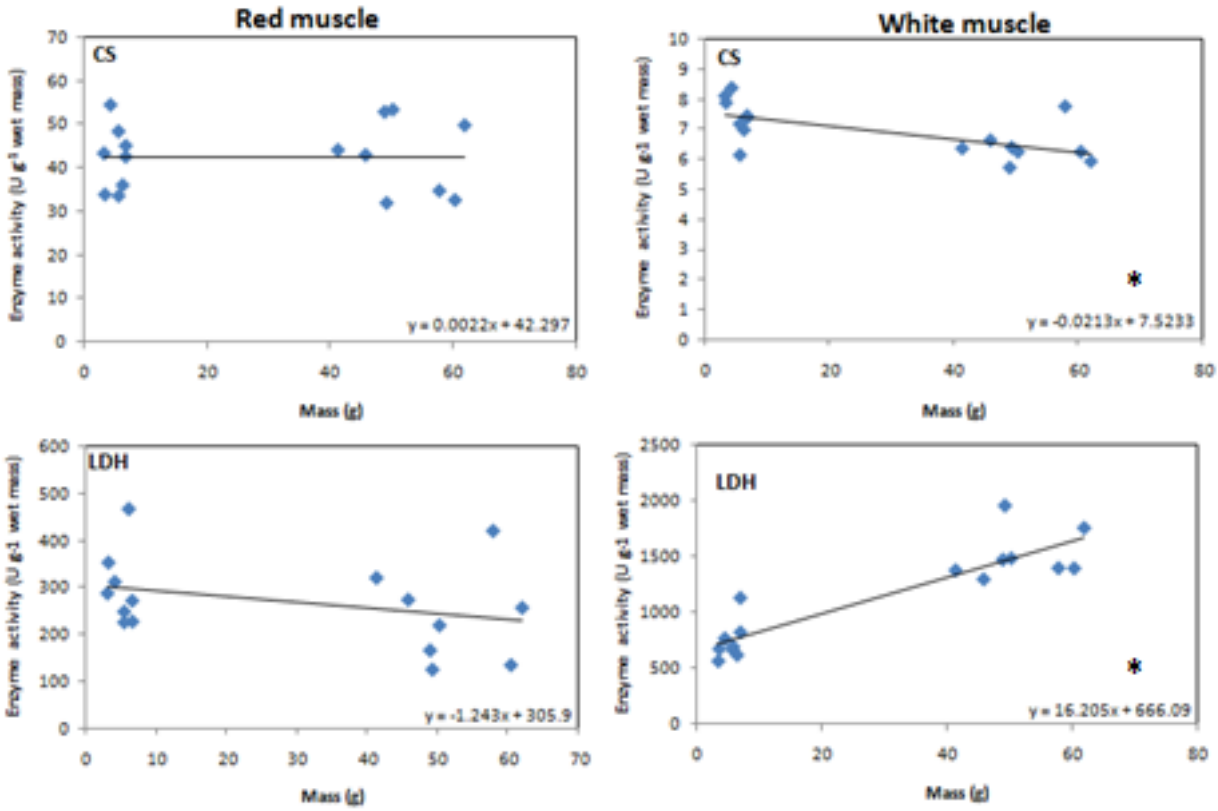

Red muscle
White muscle
CS
CS
LDH
LDH
Enzyme activity (U g-1 wet mass)
Mass (g)
y = 0.0022x + 42.297
y = -0.0213x + 7.5233
y = -1.243x + 305.9
y = 16.205x + 666.09

Fig. 3.8. The proportion of red to white muscle based on cross sectional analysis of white seabass and yellowtail after endurance training. Transverse cross sectional cuts were made posterior to the anal vents. Sheephead were excluded from analysis due to the small amount of lateral red muscle visible in this position. Values are means ± S.E.M. * indicates a significant differences ($p < 0.05$) from the Control.

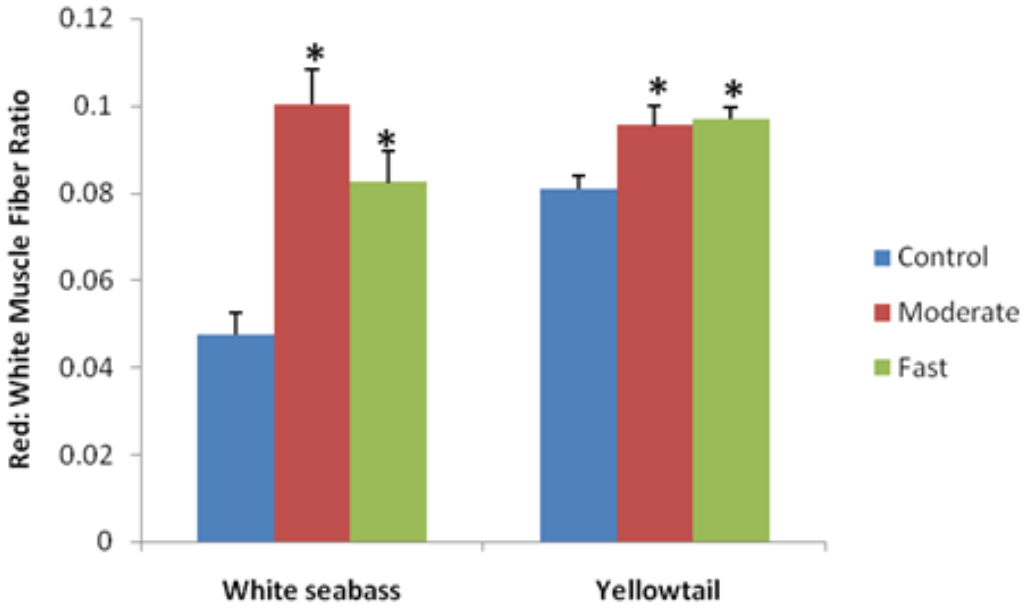

0.12
0.1
0.08
0.06
0.04
0.02
0
Red: White Muscle Fiber Ratio
*
*
*
*
White seabass
Yellowtail
Control
Moderate
Fast

Fig. 3.9. Maximum sustained swim speeds (U_{crit}) for juvenile California yellowtail (*Seriola lalandi)* and White seabass (*Atractoscion nobilis)* as a function of total length. The lines represent best fit linear regressions: y = -0.0755x + 4.6878, r^2 = 0.061, P =0.441, for trained white seabass (blue) and y = -0.0593x + 4.2355, r^2=0.18 , P = 0.169, for the control (red) white seabass. For the California yellowtail: y = -0.6319x + 17.011, r^2= 0.814. P= 0.002, for the trained (blue) and y = -0.5006x + 13.813, r^2= 0.294, P = 0.165, for the control (red) yellowtail. U_{crit} decreased with size over the size range studied.

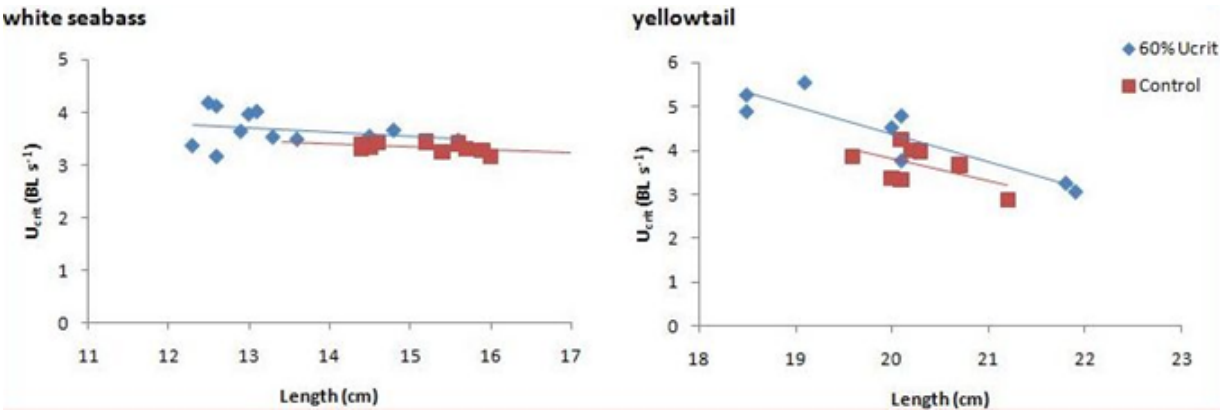

white seabass
yellowtail
60% Ucrit
Control
U_{crit} (BL s^{-1})
Length (cm)
0
1
2
3
4
5
6
11
12
13
14
15
16
17
18
19
20
21
22
23

Table 3.2. Growth performance of juvenile California sheephead, white seabass, and California yellowtail subjected to long term exercise training. Values are means ± S.E.M. N= 10 for sheephead and white seabass and 11 for the yellowtail. Pop. SGR is the specific growth rate of the population based on two separate subsamples; one taken at the beginning and one at the end of the exercise training. * indicates significant differences ($p < 0.05$) from the Control treatment. Sheephead were exercised for 27 days, white seabass 42 days, and yellowtail 34 days.

Raceway	Initial mass (g)	Final mass (g)	Initial length (cm)	Final length (cm)	Conditon factor (100 x g cm-3)	Pop. SGR (mass% day–1)
Sheephead						
Control	8.97 ± 0.22	9.96 ± 0.43	8.31 ± 0.07	8.84 ± 0.11	1.44 ± 0.03	0.50
20% U_{crit}	8.84 ± 0.28	10.33 ± 0.75	8.23 ± 0.07	8.89 ± 0.17	1.47 ± 0.04	0.58
40% U_{crit}	9.16 ± 0.23	10.15 ± 0.53	8.38 ± 0.06	8.87 ± 0.15	1.46 ± 0.04	0.38
60% U_{crit}	8.81 ± 0.21	10.84 ± 0.61	8.27 ± 0.06	9.07 ± 0.16	1.45 ± 0.01	0.77
White Seabass						
Control	10.48 ± 0.11	29.66 ± 0.90	10.75 ± 0.03	14.9 ± 0.18	0.91 ± 0.02	2.48
20% U_{crit}	10.73 ± 0.11	27.35 ± 1.64	10.76 ± 0.03	14.3 ± 0.27	0.93 ± 0.02	2.23
40% U_{crit}	10.76 ± 0.10	27.92 ± 1.97	10.79 ± 0.03	14.4 ± 0.28	0.93 ± 0.02	2.27
60% U_{crit}	10.63 ± 0.10	29.99 ± 1.22	10.77 ± 0.02	14.4 ± 0,21	1.00 ± 0.02 *	2.47
Yellowtail						
Control	6.22 ± 0.08	52.64 ± 2.19	8.41 ± 0.03	17.1 ± 0.23	1.06 ± 0.02	6.32
40% U_{crit}	6.21 ± 0.08	58.88 ± 2.38	8.40 ± 0.03	17.8 ± 0.19 *	1.04 ± 0.02	6.65
60% U_{crit}	6.00 ± 0.08	60.32 ± 1.58 *	8.39 ± 0.03	17.6 ± 0.12	1.10 ± 0.01 *	6.72

Table 3.3. Size and maximum sustainable swim speed (U_{crit}) in juvenile California yellowtail and white seabass post exercise treatment. Groups are separated by treatment; 60% U_{crit} or an unexercised control group.

Califronia yellowtail, *Seriola lalandi*

	Total length (cm)	Mass (g)	U_{crit} (BL s^{-1})	Treatment
1	20.1	102.05	4.78	60% U_{crit}
2	18.5	66.69	5.25	
3	19.1	70.39	5.53	
4	18.5	78.02	4.87	
5	20.0	97.44	4.51	
6	21.9	115.62	3.04	
7	21.8	111.66	3.24	
8	20.1	76.76	3.76	
			4.37	**Average U_{crit}**
1	19.6	75.06	3.85	Unexercised Control
2	20.1	81.82	4.24	
3	20.2	78.45	4.00	
4	20.7	90.08	3.67	
5	20.1	80.89	3.33	
6	21.2	94.19	2.89	
7	20.3	90.61	3.98	
8	20.0	84.77	3.36	
			3.66	**Average U_{crit}**

White seabass, *Atractoscion nobilis*

	Total length (cm)	Mass (g)	U_{crit} (BL s^{-1})	Treatment
1	12.6	16.31	3.16	60% U_{crit}
2	12.3	13.49	3.37	
3	13.3	19.13	3.53	
4	12.9	17.17	3.64	
5	13.6	18.37	3.49	
6	14.5	23.64	3.54	
7	13.0	18.16	3.96	
8	12.6	16.29	4.11	
9	12.5	16.93	4.18	
10	13.1	18.76	4.01	
11	14.8	29.73	3.66	
12	15.6	34.42	3.47	
			3.68	**Average U_{crit}**
1	14.4	21.16	3.30	Unexercised control
2	14.5	26.68	3.33	
3	14.5	26.34	3.34	
4	14.4	24.52	3.39	
5	14.6	24.29	3.42	
6	15.4	32.46	3.25	
7	16.0	31.92	3.16	
8	15.2	28.47	3.45	
9	15.6	30.45	3.41	
10	15.2	27.00	3.42	
11	15.9	35.02	3.27	
12	15.7	36.71	3.31	
			3.34	**Average U_{crit}**

IV. Discussion

Growth

It is generally assumed that at favorable swimming speeds ($\leq$ 1.5 bl sec^{-1}), exercise training leads to increased growth, whereas training at substantially greater speeds has distinct negative effects on growth in any fish (Davison 1997). Although the speeds vary among species and reflect differences in training methods and life styles of the species involved, exercise has been shown to improve the growth rate of Atlantic salmon *Salmo salar*, brown *Salmo trutta* and rainbow *Oncorhynchus mykiss* trout, striped bass *Morone saxatilis*, yellowtail *Seriola quinqueradiata* (Yogata and Oku 2000) all relatively active species. In contrast, growth was either negatively or unaffected by exercise training in gold fish *Carassius auratus* (Davison and Goldspink 1978), banded wrasse *Notolabrus fucicola* (Davison 1994), and Japanese flounder *Paralichthys olivaceus* (Ogata and Oku 2000), all fish with relatively inactive life styles.

In this study California yellowtail, an active pelagic fish exhibited an enhanced growth rate due to exercise while the California sheephead, a wrasse, did not over the time course examined. However, at 21 days there was some suggestion that exercise was influencing growth in the sheephead, since the daily growth rate went from negative to positive earlier in the exercised groups than the control. The white seabass did not exhibit an exercise induced growth enhancement in this study, although in other exercise studies with the white seabass, growth rate was increased with exercise training (Buhr 2002; Cepuritis 2005). The reasons for differential results between experiments with white

seabass are not entirely clear but may be due to slight variations in experimental design of the raceway systems. In the previous studies, the control fish were not held in a minimal flow raceway but rather in a square tank. The flow, although minimal, differed in direction and consistency in the square tank relative to the control raceway in our study. The hatchery conditions of the white seabass also differed between studies. Stocking density of our source group was greater relative to the other studies. High stocking densities are known to be a stressor in cultured fish contributing to lower growth rates (Montero, et al. 1999). Therefore, the fish in our study may have been stressed prior to the transfer in to the raceway potentially preventing an exercise induced growth response or producing a similar compensatory growth response in all groups including the minimal flow controls.

Aerobic Potential

In response to endurance training, skeletal muscles of fish exhibit many phenotypic responses, many of which produce a more aerobic phenotype. The responses remain species-specific relating to swimming ability and life styles of each fish (Davison 1997; Johnston 2001b). In this study, metabolic enzyme activity (CS and LDH), muscle morphology, and swimming performance were all stimulated in a species specific manner as a result of exercise training. By looking at how these parameters were affected, we can better understand how this training can contribute to the overall aerobic potential of each species.

Enzyme Activity

White seabass red muscle exhibited a great capacity for increased aerobic potential (CS and LDH activity) as a result of exercise. Comparable results were seen in rainbow trout where CS increased in the red muscle following a 28 day training bout at up to 60% U_{crit} (Farrell, et al. 1991). Exercise also significantly increased CS and LDH levels of adult zebra fish relative to controls (McClelland, et al. 2006). Previous studies have found that exercise training also increases LDH and CS activity in the white muscle (Farrel, et al. 1991; Buhr 2002). This was not seen in the white seabass and may be explained by the greater intensity (>60% U_{crit}) used in previous studies compared to the 20 – 60% U_{crit} in the current study. In rainbow trout, anaerobic metabolism is required to support swimming at speeds equal to and greater than 70 %Ucrit (Burgetz, et al. 1998). By training our fish below this level, the recruitment of white muscle may not have been required to support swimming and therefore the enzymes in that muscle were not stimulated.

The California yellowtail is a highly active fish capable of swimming at relatively fast speeds; U_{crit} of unexercised juveniles was approximately 4.5 BL sec^{-1}. The carangiform swimming mode of *S. lalandi* is comparably efficient with the thunniform swimming mode of the tunas and recent studies have shown that standard metabolic rate of the *Seriola* genus is somewhat enhanced in comparison with that of many other active species, approaching values reported for tunas (Clark and Seymour 2006). This suggests a high aerobic capacity. The

exercise induced increase of aerobic enzymes as seen in the white seabass, was not observed in the yellowtail. An explanation may be related to inter-species differences in aerobic scope. Un-exercised yellowtail exhibited high aerobic enzyme activity (42 units g^{-1}) similar to the values seen in high performance species such as tuna (55-63), marlin (≈20), and sword fish (≈24) (Dalziel, et al. 2005). It is possible that these juvenile yellowtail are near the maximum CS activity for fish and therefore have little scope for enhancement compared to the white seabass. Interestingly, the exercised white seabass exhibited CS enzyme activity approaching a similar value as the yellowtail.

The California sheephead is a labriform swimmer generally associated with rocky bottoms and kelp forests, living a relatively inactive life. Lateral red muscles may not be stimulated to the same degree in sheephead as in other species subjected to similar elevated current velocities due to their use of the labriform swimming mode and heavy reliance on pectoral fins (Westneat and Walker 1997). Lateral red muscle was examined for consistency with the other two species, but it is likely that the pectoral muscles are the site of the greatest contribution to meeting the increased demand. Training effects may be more important there and these muscles will be examined in future studies. It is also possible that due to the small amount of available red muscle in the California sheephead, the red muscle samples used in the enzyme assay contained white muscle.

Muscle Morphology

The increase in the proportion of the lateral red muscle in response to training seen in both the yellowtail and white seabass is consistent with many other exercise studies (Davison 1997). In general, training at sustainable swimming speeds ($\leq$ 2 BL sec^{-1}) leads to an increase in the red muscle proportion with an increase in both cell number and size (Young and Cech 1994; Sanger and Stoiber 2001). The aerobic muscle of the moderate (40%) and fast (60% U_{crit}) exercised yellowtail and white seabass was continually stimulated by the endurance training, resulting in an increase in the overall proportion of red muscle relative to non-exercised fish. With sustained swimming at modest velocities, the power requirements of lateral muscle would be elevated by a greater demand and that power is increased with a larger muscle mass. As the muscle mass increases so does the available power, allowing for a greater aerobic potential.

Swimming Performance

Maximum sustainable swimming tests revealed that exercise training resulted in a significant increase in absolute and relative U_{crit} in the California yellowtail but not in the white seabass. Previous studies with trained fish have shown similar results with some species exhibiting an exercise induced response, while others do not (Davison 1997). The different responses among training studies reflects differences in training regime, in terms of duration and intensity, and perhaps in this study differences in life style and swim mode among species. The ability of the yellowtail to increase the amount of muscle mass as a result of exercise is a possible explanation for the increase in U_{crit}. Aerobic swimming is

primarily powered by red muscle and if that muscle mass is increased then it is possible the relative maximum sustainable swimming speed would also be increased. It is surprising that the U_{crit} of the white seabass did not increase as a result of exercise training particularly because the seabass did exhibit an exercise induced increase in red: white ratio and citrate synthase activity.

V. Conclusion

In summary, we have shown that exercise training will improve the swimming performance of three species of juvenile fish in a species specific manner relating to the swimming mode and life-style of the fish species and it is a further example of the wide range in adaptational changes of the highly plastic muscle tissue (Sanger and Stoiber 2001). In the species with the most active lifestyle, the California yellowtail, exercise was also able to significantly improve the growth of the fish.

White seabass are currently used for fisheries stock enhancement and California sheephead are currently being considered for stocking. If the two species are exercise trained before release, then return and recapture percentages may be improved, as shown in Atlantic salmon (Wendt and Saunders 1973) and brown trout (Creswell and Williams 1983). Better swimming performance may improve feeding efficiency and the ability for the fish to escape predators once released into the wild. In a species with high commercial market potential, such as the yellowtail, more rapid growth rates would allow individuals to achieve marketable size faster. And enhancing growth rate and swimming performance without the use of drugs or genetic modification is highly beneficial and can provide techniques applicable to other finfish in aquaculture.

VI. Future Research

This research clearly shows that exercise induces an increase in the relative amount of red muscle in both the California yellowtail and the white seabass. Although somatic growth and cross sectional areas of muscle are easily measured in fish, this gives only an indirect measure of muscle growth. Postembryonic muscle growth of fish involves the increase in the number of embryonic fibers (hyperplasia) or increasing the size of each individual fiber (hypertrophy). Thus unlike in mammals and birds, and particularly in fish that reach a large ultimate size, such as the three species in this study, hyperplasia continues into the adult stages of the life cycle (Weatherley, et al. 1988). It is important to know how exactly the muscle fibers are changing as a result of exercise in order to gain insight on how the exercise may affect the ultimate size of each fish species.

Many potential factors have been used to predict the aerobic potential of fish, with many producing inconsistent results. The inconsistent results among the three species in this study confirm this. Mitochondrial density and lipid content in the muscles of trained fish have been used to indicate aerobic capacity in other studies (Sanger and Potscher 2000; Sanger and Stoiber 2001; McClelland, et al. 2006). Recent research indicates that exercising fish may use lipids as their main energy source and fuel and that lipids may play a vital role in fueling the recovery process of exercise training (Milligan 2004). By adding these two

techniques to our results, a more definitive answer to how exercise effects aerobic potential can be obtained.

In terms of the usefulness of exercise in aquaculture, long term (3-6 months) feeding studies need to be conducted. In this study, fish were fed to satiation and food conversion rates (FCR) were not obtained. If exercised fish require significantly more food then the added benefit of increased aerobic capacity or increase in size may not be worth the cost. As fish food is one of the greatest expenses to an aquaculture facility, FCR may be the most important focus for future research if exercise training is to be a feasible technique used in a grow out facility.

Chapter Four: Endocrine Study

I. Introduction

Early rearing conditions, including temperature, flow velocity, and crowding, can all influence muscle growth in fish (Johnston 2001b; Wilkes, et al. 2001; Koumoundouros, et al. 2009). Endocrine growth-regulatory factors play important roles in mediating changes in muscle growth and development, and include growth-enhancing effects of insulin-like growth factor-I (IGF-I) and growth-inhibitory effects of cortisol ((Kelley, et al. 2001; Dyer, et al. 2004b; Reinecke, et al. 2005; Davis and Peterson 2006; Wilkinson, et al. 2006). The relationships between rearing conditions, effects on endocrine growth regulators, and growth are not well understood. Defining these relationships has strong potential to help improve husbandry practices that can lead to enhanced fish growth and productivity in finfish aquaculture.

Aquaculture settings provide a variety of unavoidable stressful situations for fish. Typically, upon exposure to an acute stressor, catecholamines and corticosteroids are rapidly released from the head kidney into the blood (Wendelaar Bonga 1997). The primary corticosteroid, cortisol, is therefore often used as an indicator of stress in fish blood plasma (Barton 2002). Factors including handling, crowding, water quality, and social rank (Dyer, et al. 2004b; Wilkinson, et al. 2006) all have been shown to influence plasma cortisol levels. Gregory and Wood (1998) showed that stress-induced plasma cortisol levels are correlated with depression of feeding behavior, food conversion efficiency, condition factor, and growth rate in juvenile rainbow trout. Repeated aquaculture stressors, which increase cortisol levels, negatively impact growth related factors

in a variety of fish species including Atlantic salmon (*Salmo salar*; McCormick 1998; Basrur, et al. 2009), Eurasian perch (Perca fluviatilis; Strand, et al. 2007), and rainbow trout (*Oncorhynchus mykiss*; Wilkinson, et al. 2006).

Continuous swimming, generally at speeds of 1.5 body lengths/sec (BL s^{-1}) or less, increases growth rates in fish (Davison 1997; Yogata and Oku 2000; Martin and Johnston 2005), enhances aerobic potential (Farrell, et al. 1991; Martinez, et al. 2003), and in some cases reduces stress (Young and Cech Jr. 1993b; Davison 1997; Milligan 1997) in a variety of fish species. In some instances, exercise may enhance the metabolic recovery of a stress-induced cortisol spike (Milligan, et al. 2000; Cepuritis 2005). The rigor of the exercise regime in terms of speed and duration is an important factor in these studies, because optimal conditions vary greatly by species. Excessive exercise can result in increased stress and decreased growth rates (Davison 1997; Kieffer 2000; Merino, et al. 2007), while fish reared in still water conditions are known to be more aggressive and prone to bouts of spontaneous activity, compared with fish reared under exercised conditions (Davison 1997). If it is possible to enhance growth rates and reduce stress through exercise, it is necessary to establish optimal conditions for each species considered for aquaculture.

Little is known about the effects of exercise on the growth hormone (GH)/ IGF axis in fish. As exercise produces some of the same somatic growth enhancement effects as non-stressed optimal feeding conditions (Davison 1997; Mommsen 2001), it is conceivable that sustained swimming exercise may exert its effects through the GH-IGF axis. Past studies have demonstrated positive

correlations between plasma GH concentrations and exercise in steelhead trout (Barret and McKeown 1988) and rainbow trout (Nielsen, et al. 1994); however, GH did not appear to be good indicator for growth performance in these studies. On the other hand, exercise-stimulated GH may be expected to increase IGF-I production, leading to enhanced growth. Given the important role of IGF-I in directly stimulating somatic growth (Reinecke, et al. 2005), characterization of the integrated changes in IGF-I and cortisol in response to exercise are still needed.

The present study characterized the responses to different regimes of continuous exercise with respect to growth rates, plasma cortisol concentrations, and plasma IGF-I concentrations in three finfish species representing distinct swimming modes, including California sheephead (*Semicossyphus pulcher*), white seabass (*Atractoscion nobilis*), and California yellowtail (*Seriola lalandi*). The California sheephead is a carnivorous, temperate, rocky-reef/kelp bed fish that spends most of its life within a relatively small home range and which utilizes labriform swimming for its routine movements (Topping, et al. 2005). The white seabass, the largest of the croaker family (Sciaenidae) on the Pacific coast (Baja California to Juneau Alaska), utilizes a sub-carangiform swimming style for its routine movements (Thomas 1968). The California yellowtail, on the other hand, is the most active swimmer of the three species. They utilize a carangiform swimming mode, are facultative ram-ventilators, and share many specialized morphological adaptations with the tunas, including a fusiform body shape to reduce drag, fin grooves to increase streamlining, a high aspect-ratio tail with a

narrow caudal peduncle, and finlets along the trailing edges of the body (Dewar, et al. 1994). Comparison of these three species may provide some insight on the efficacy of exercise effects on growth and hormonal factors in fish species exhibiting different swimming adaptations.

II. Methods

All fish species used in this experiment were supplied by Hubbs-Sea World Research Institute (HSWRI). Juvenile white seabass *Atractoscion nobilis* and California sheephead *Semicossyphus pulcher* were provided by theHSWRI's Leon R. Hubbard Hatchery (Carlsbad, CA). Juvenile California yellowtail, *Seriola lalandi,* were provided by HSWRI's marine hatchery (San Diego, CA). The timing of the experiments was based upon reproductive seasonality and the availability of juveniles for each fish species. The sizes of the fish were selected in part to reflect the typical sizes of the fish when they are stocked into cages for grow-out, as well as for comparison with prior exercise studies performed on white seabass (Buhr 2002; Cepuritus 2005). Juvenile white seabass reached appropriate size (10.8 ± 0.2 cm) in March, juvenile yellowtail (8.4 ± 0.3 cm, total length) in August, and juvenile sheephead (8.3 ± 0.4 cm) in January.

Fish were sorted and randomly divided into four treatment groups: Fast (60 % U_{crit}), Moderate (40% U_{crit}), Slow (20% U_{crit}), and a minimal flow Control group. Each group contained 75 fish in the white seabass experiments and 53 fish in the sheephead experiments. In the yellowtail experiments, the Slow group was excluded due to a limited number of available fish and the lack of exercise-induced growth effects of this treatment determined in preliminary studies with this species; the remaining three groups of yellowtail each contained 80 fish. All of the exercise treatment groups were placed into their corresponding raceways and allowed to acclimate for a minimum of 24 hr under minimal flow (as in Control groups) prior to the experiments.

The experimental raceway system was designed to allow four concurrent exercise treatments. Raceways (551.2 x 50.8 x 30.5 cm; fiberglass construction) were placed on a rack creating a 2x2 stacked array. Re-circulated water from a common sump was used in order to maintain consistency of water chemistry and temperature among the raceways. At one end of each raceway, inlet chambers received water pumped by two ½ hp pumps (Speck Pumps Jacksonville, FL), with the inflow rates adjusted using ball valves fitted for each raceway. The inlet chamber was designed to remove energy from the water and create a laminar current pattern into the fish rearing section; filtration mesh (Matala USA) was used as the screening material placed between the inlet chamber and the fish chamber. A perforated plastic grid was used to separate the fish chamber from the outflow drain. Standpipes were used to maintain a constant water depth in all four raceways. With this configuration, the volume available to the white seabass and California sheephead was 236.2 x50.8x10.2 cm. To obtain higher flow velocities appropriate for the yellowtail, the cross sectional area available to the fish was reduced by inserting cinder blocks along the sides of the raceway. A thin sheet of ABS was attached to the sides of the cinder blocks to eliminate any edge effect created from the rough edge of the cinder blocks. This reduced the dimensions available to the yellowtail to 236.2 x 29.2 x 10.2 cm. To minimize any outside stress effect, the raceway system was contained within a four-sided canopy. Each individual raceway was also shielded from the side by a curtain to prevent any startling of the fish during routine maintenance, feeding, and sampling.

After the initial acclimation periods, water flow was increased gradually to appropriate velocities (as above) for each treatment group. Fish were trained continuously under natural photoperiod for 27 d in the sheephead, 34 d in the yellowtail, and 42 d in the white seabass experiments. All fish were fed to satiation daily with commercial pellet food dispensed with a continuous belt feeder during a 12 hr daylight period. During the experiments, 10 fish from each group were randomly removed from each raceway prior to daily feeding (i.e., fish had not eaten for 24 hr); Table 4.1 shows the sampling days used for each species. Fish were caught by netting and killed using an overdose of anaesthetic (MS222, tricane methanesulphonate), following an approved IACUC protocol administered through the University of San Diego. Total length and body mass were measured and in all individuals blood samples were collected within 5 min of initial handling. In smaller individuals, blood was collected using haematocrit tubes following caudal severance; once the fish grew to a size large enough, blood was collected from the caudal vein using a syringe and 22g needle. Blood was centrifuged at 3,000 rpm for 3 min and the plasma was removed, aliquoted, and stored at -80 ^{o}C for later analysis. Plasma cortisol concentrations were measured by radioimmunoassay (RIA) validated for use on fish plasma (Kelley et al. 2001; Galima, 2004), using reagents from Diagnostic Systems Laboratory (Beckman Coulter, Inc., Webster TX). Plasma concentrations of IGF-I were measured using an RIA described by Shimizu et al. (2000) and modified and validated by Galima (2004), using reagents from GroPep, Ltd. (Adelaide, Australia).

Length (cm) and mass (g) were used to calculate a condition factor [CF = (mass x total length $^{-3}$) x 100].Individual specific growth rates (SGR) were calculated using the equation:

$$Specific\ Growth\ Rate = \left(\frac{\ln(W_2/W_1)}{(t_2 - t_1)}\right) x100$$

where W_1 represents the initial mass in g or the initial length in cm, W_2 the final mass or length and *t* the number of days exercised. Statistical analyses were performed using SPSS (version 15.0; SPSS Inc, Chicago). Statistical comparisons among the four different treatment groups were made using a Kruskall-Wallis ANOVA. If a significant difference was found ($p < 0.05$), then pair-wise comparisons between treatments were made using a Mann Whitney U test. Spearman's rank correlation coefficient was used to examine relationships between the endocrine parameters and fish growth measures within and among treatment groups.

Table 4.1. Average temperature and list of sample days for each experiment.

Species	Temp (°C)	Raceway	Sample Days
White Seabas			
(*Atractoscion nobilis*)	18.0 ±1	Control	pre transfer,
		Slow (20% U_{crit})	d 1, 3, 7, 14, 28, 42
		Moderate (40% U_{crit})	
		Fast (60% U_{crit})	
California Sheepehead			
(*Semicossyphus pulcher*)	17.3 ± 0.7	Control	
		Slow (20% U_{crit})	pre transfer, post transfer,
		Moderate (40% U_{crit})	d 3, 7, 14, 27
		Fast (60% U_{crit})	
California Yellowtail 2006			
(*Seriola lalandi*)	21.1 ± 1.3	Control	pre transfer,
		Slow (20% U_{crit})	d 2, 5, 9, 15, 21
		Moderate (40% U_{crit})	
		Fast (60% U_{crit})	
California Yellowtail 2007			
(*Seriola lalandi*)	21.9 ± 1	Control	pre transfer, post transfer,
		Moderate (40% U_{crit})	d 3, 6, 13, 27, 34
		Fast (60% U_{crit})	

III. Results

California Yellowtail

Experiments on yellowtail were carried out in 2006 and then again in 2007. The experiments were repeated due to poor quality of the 2006 spawn at the Hubbs-SeaWorld Research Institute hatchery, in which the 2006 cohort experienced lower hatch rates, reduced survival to first feeding, and poor swim bladder inflation rates, as compared with typical spawns at the hatchery (Mark Drawbridge, Hubbs-SeaWorld Research Institute, personal communication). The results of these two experiments are described separately below.

2006 yellowtail experiments.

Throughout a 21 d trial, body mass (Fig. 4.1a) and length (not illustrated) increased progressively in all treatment groups. Condition factor remained relatively unchanged throughout the trial (Fig. 4.1b). Specific growth rate (SGR) showed increases in all groups over the first 10 d of the experiment, but then remained constant thereafter (Fig. 4.1c). However, there were no statistical differences among exercise treatments for any of the growth parameters.

Prior to initiating the exercise treatments, the pre-transferred yellowtail exhibited very high plasma cortisol concentrations (~100 ng mL^{-1}; day 0 in Fig. 4.2a). Two days after transfer to the raceways, cortisol concentrations in the non-exercised control, slow, and moderate exercised fish remained high (75-150 ng mL^{-1}), and concentrations in the fast-exercised group were yet higher (225 ng mL^{-1}; $p < 0.05$ vs. pre-transferred and unexercised control groups). After 5 d in the

raceways, however, plasma cortisol concentrations began to decrease to a level that was significant by 9d in the moderate and fast exercised groups (vs. pre-transfer and 2d & 5d post-transfer fish; $p < 0.05$; Fig. 4.2a). All groups exhibited cortisol concentrations <50 ng mL^{-1} for the remainder of the experiment, with the exception of a spike in cortisol concentrations in the fast-exercised group at 21d.

Plasma IGF-I concentrations progressively increased over the 21 d trial in all groups (Fig. 4.2b); however, there were no effects of the different exercise treatments. Plasma IGF-I concentrations and growth were significantly correlated over the 21 d trial, with $R^2 \geq 0.7$ (Fig. 3, $P < 0.001$) in all groups (Table 4.2). In contrast, growth was negatively correlated with plasma cortisol concentrations in the moderate and slow exercised groups ($P<0.05$). There were no correlations among the non-exercised control and fast-exercised groups (Table 4.2; Fig. 4.3). Similarly, IGF-I and cortisol were negatively correlated in the moderate and slow-exercised groups (Table 4.2; Fig. 4.3), but not in the non-exercised control and fast-exercised groups.

2007 yellowtail experiments.

Because of the poor spawn quality of the 2006 yellowtail and their high pre-transfer cortisol concentrations, a second set of experiments was conducted in 2007. This experiment was identical in design to the first trial except for two changes. First, the exercise trials were extended to 34 d to provide additional time for acclimation and potential growth differences to emerge, and secondly the slow treatment group (20% U_{crit}) was removed due to a limited number of fish

available from the hatchery for use in this study. As in the 2006 experiment, mass and length progressively increased in all groups throughout the duration of the experiment (Fig. 4.4a); however, in this experiment the fast exercised group exhibited a greater mean mass as compared with the un-exercised controls after 34 d ($p<0.05$). Condition factor (Fig. 4.4b) was also significantly increased at 27 d in the moderate exercised group and at 34 d in the fast exercised group, as compared with the corresponding un-exercised groups ($p<0.05$). In contrast to the 2006 experiments, SGR was high in all groups at the beginning of the experiment (9.5-10 mass% d^{-1}, vs. 1-3 mass % d^{-1} in 2006; $p<0.01$), and remained above 6.5 mass% d^{-1} through the remainder of the 34 d period (Fig. 4.4c).

Pre-transfer plasma cortisol concentrations in the 2007 yellowtail were ~ 9.5 ng mL^{-1} (Fig. 4.5a), in contrast to the highly elevated levels (~100 ng/mL) in the previous year. A significant increase in cortisol concentrations was observed after transfer to the raceways, to 25 ng mL^{-1} ($p<0.05$). After 3d, cortisol concentrations remained elevated in the fast-exercised group, but were significantly lower in the moderate- and non-exercised control groups. On day 6, however, cortisol levels surged in all groups over those in pre-transfer and 3d fish ($p<0.05$); in addition, the fast-exercised group had a higher mean cortisol concentration than that in the control ($P =0.002$) and moderate exercise ($P = 0.201$) groups. For the remainder of the 34d experiment, all groups remained at or near pre-transfer cortisol concentrations (Fig. 4.5a).

Plasma IGF-I concentrations in the 2007 yellowtail generally ranged between 50-80 ng mL^{-1} (Fig. 4.5b), comparable to the plasma IGF-I

concentrations reached toward the end of the 2006 experiments. At 6, 13, and 27 d, plasma IGF-I concentrations in the fast-exercised group were significantly elevated over the non-exercised control group ($p<0.05$), while at 13 d the moderate-exercised group also exhibited significantly elevated IGF-I concentrations ($p<0.05$; Fig. 4.5b). Correlation analyses indicated that plasma IGF-I concentrations were positively correlated with growth in the non-exercised control ($P= 0.004$) and moderate-exercised ($P=0.012$) groups, and exhibited a similar but non-significant trend in the fast-exercised group (Table 4.2). Cortisol exhibited a negative relationship with growth in all groups, and was significantly correlated in the moderate-exercise group ($P= 0.022$). Cortisol and IGF-I also showed an inverse relationship in the exercised groups, which was significant in the moderate-exercised group ($p<0.01$; Fig. 4.6).

Sheephead

During a 27 d experimental period, California sheephead did not exhibit any changes in mass for the first 14 days in any group; however, during the second 13 days, all groups increased in mass by approximately 12% (vs. 14 d or earlier, $p<0.05$). The fast treatment group exhibited greater mass as compared to the non-exercised control group on days 14 and 27 ($p=0.4$; Fig. 4.7a). Condition factor also did not change in most all groups throughout the 27d period (Fig. 4.7b). SGR, on the other hand, was negative in most groups during the first half of the experiment, but it progressively improved and was slightly positive in all groups (0.5-1.0 % mass/d) by the end of the 27 d (Fig. 4.7c).

Pre-transfer cortisol concentrations in sheephead were low, < 20 ng mL^{-1}, and then increased to 250 ng mL^{-1} following transfer of the fish into the raceway ($P < 0.001$). After this initial surge, all groups showed decreased cortisol levels by 3 and 7 d (Fig. 4.8a). For the remainder of the experiment, cortisol concentrations showed variable levels between 15-65 ng mL^{-1}. At 27 d, the fast exercised group exhibited slightly lower cortisol concentrations compared with that of the other groups ($p < 0.05$).

Plasma IGF-I concentrations were increased during the progression of the experiments (Fig. 4.8b). By 27d, IGF-I concentrations were greater by 10-15 ng mL^{-1} as compared with pre-transferred values ($p<0.05$). However, there were no effects of exercise on plasma IGF-I concentrations evident among all groups and times. IGF-I concentrations and growth were significantly correlated in the fast-exercised group ($p<0.05$) but not in the others (Table 4.2). Although reducing cortisol concentrations coincided with increasing IGF-I concentrations over time in all groups (Fig. 4.8), there were no significant correlations between cortisol and IGF-I. Similarly, there were no detectable correlations between cortisol and growth in any of the treatment groups.

White Seabass

Throughout a 42 d experiment in white seabass, body mass increased progressively and was significantly greater at 28 d and 42 d as compared to pre-transferred fish ($P<0.05$; Fig. 4.9a). However, there were no detectable effects of the different exercise treatments on mass, in contrast to prior findings by

Cepuritas (2005). Condition factor was also not significantly different over time, nor among the different treatment groups (Fig. 4.9b). SGR showed a slight increase over the first 7 d in all groups, but remained relatively low (2-3 %mass/d) for the remainder of the experiment (Fig. 4.9c).

Plasma cortisol concentrations in pre-transferred white seabass averaged ~48 ng mL^{-1} and were highly variable at all subsequent time-points (Fig. 4.10a). At 14, 28 and 42 d, cortisol concentrations ranged between 25-70 ng mL^{-1} and showed no consistent patterns over time or between different exercise treatment groups. Plasma IGF-I concentrations (Fig. 4.10b) were also not significantly different among the different exercise treatment groups, while there was a transient decrease in IGF-I at 3 d and 7 d (vs. pre transfer and the last two time-points, $P<0.05$). There were no significant correlations between IGF-I concentrations and growth (Table 4.2). Cortisol concentrations, which were variable across all groups and time points (Fig. 4.10a), exhibited a positive correlation with IGF-I and with growth in the un-exercised control group. There was also a positive correlation between cortisol and growth in the slow group (Table 4.2).

Fig. 4.1. Body mass (panel a), condition factor (b), and specific growth rate (c) in juvenile California yellowtail subjected to continuous swimming exercise (slow, moderate or fast water flow; see Methods) or no exercise (control) in experiments carried out an a 2006 cohort. Values shown are mean ± SE (n = 9 per group). There was not a significant exercise effect on growth during a 21 d experimental trial. Asterisk (*) indicates a significant difference ($p \leq 0.05$) from control within the sample day.

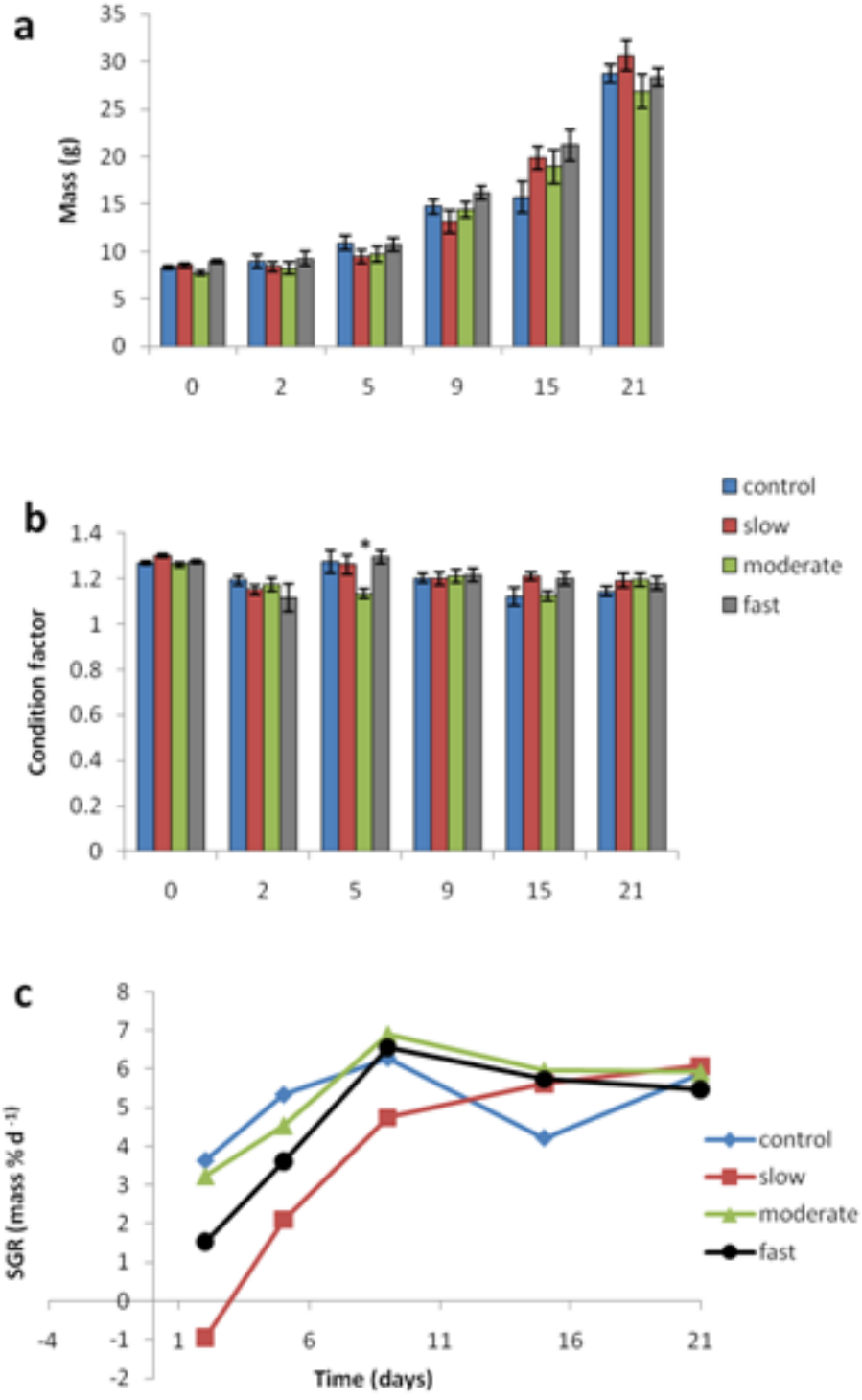

a
Mass (g)
35
30
25
20
15
10
5
0
0
2
5
9
15
21
b
Condition factor
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
control
slow
moderate
fast
c
SGR (mass % d -1)
8
7
6
5
4
3
2
1
0
-1
-2
-4
1
6
11
16
21
Time (days)
control
slow
moderate
fast

Fig. 4.2. Plasma cortisol (panel a) and IGF-I (panel b) concentrations (mean ± SE of ng/mL values) in California yellowtail subjected to continuous swimming exercise (slow, moderate or fast water flow; see Methods) or no exercise (control) in experiments carried out an a 2006 cohort. Asterisks (*) indicates a significant difference ($p \leq 0.05$) from control within each sample day. ¥ indicates that values are significantly ($p \leq 0.05$) lower from the pre-exercise value. Cortisol levels generally decreased over time and with exercise (a). IGF-I levels increased over time in all treatment groups (b).

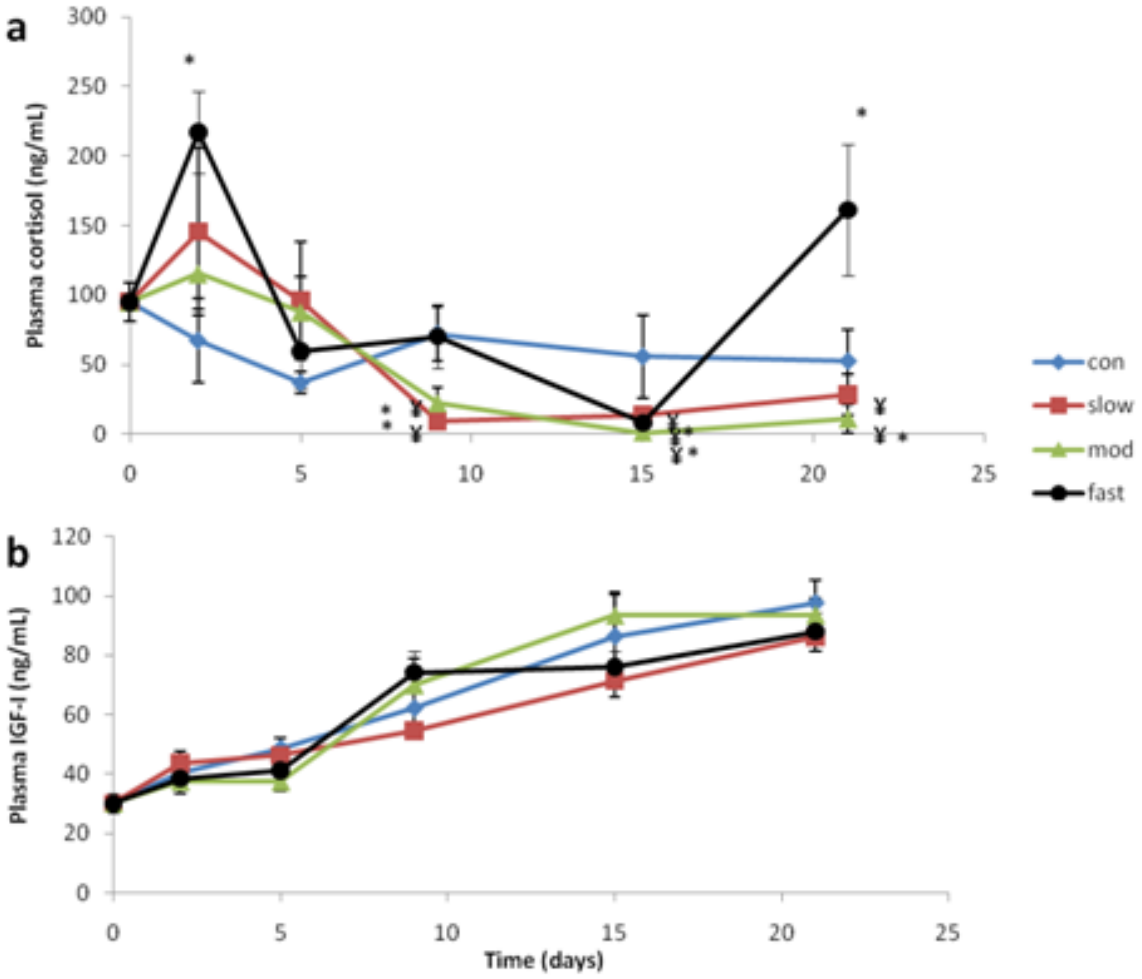

a
Plasma cortisol (ng/mL)
300
250
200
150
100
50
0
0
5
10
15
20
25
con
slow
mod
fast
b
Plasma IGF-I (ng/mL)
120
100
80
60
40
20
0
0
5
10
15
20
25
Time (days)

Fig. 4.3. Illustrations of Spearman rank correlations between plasma IGF-I and cortisol (panel a, left side) and mass and cortisol (panel b, right side) in California yellowtail subjected to moderate and slow exercise (2006 experiments). Table 2 provides R^2 and p values for all experimental groups. IGF-I concentrations showed a significant negative correlation with plasma cortisol concentrations. Growth also exhibited a significant negative correlation with plasma cortisol. R^2 values indicate the strength of the Spearman rank correlation.

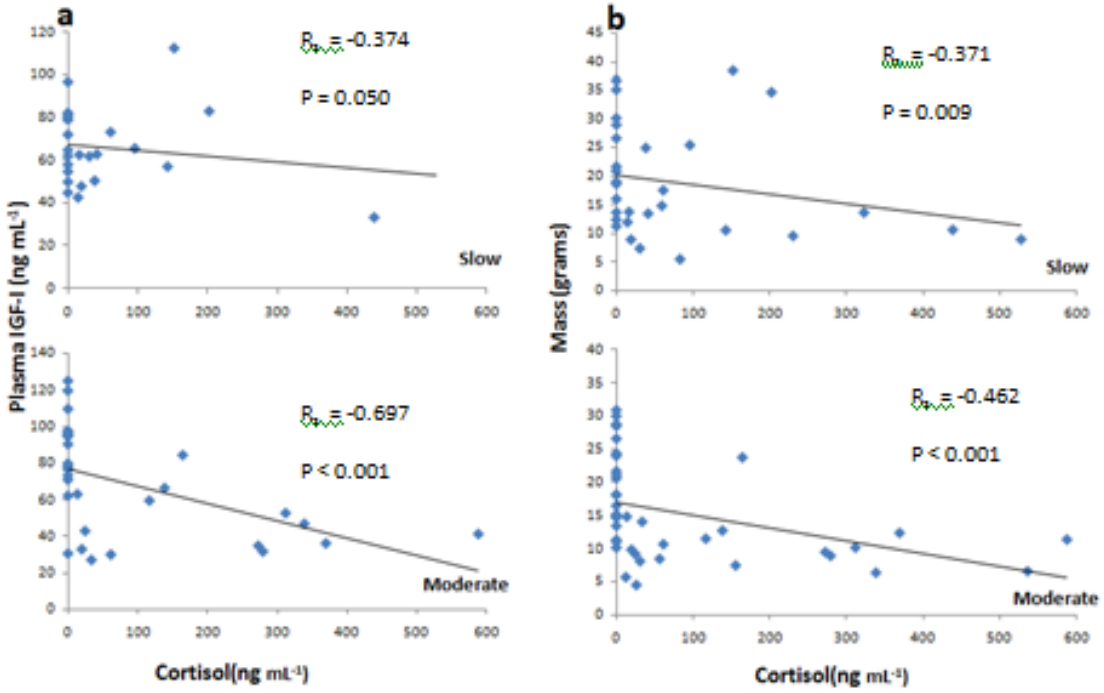

a
b
Plasma IGF-I (ng mL-1)
Mass (grams)
Slow
Moderate
P = 0.050
P = 0.009
P < 0.001
P < 0.001
Cortisol(ng mL-1)
Cortisol(ng mL-1)

Fig. 4.4. Body mass (panel a), condition factor (b), and specific growth rate (c) in juvenile California yellowtail subjected to continuous swimming exercise (moderate or fast water flow; see Methods) or no exercise (control) in experiments carried out an a 2007 cohort. Values shown are mean ± SE (n = 11 per group). Condition factor and mass of the fish exercised at 60% U_{crit} (fast) was significantly different from the non exercised group after 34d. Asterisk (*) indicates a significant difference ($p \leq 0.05$) from control within the sample day.

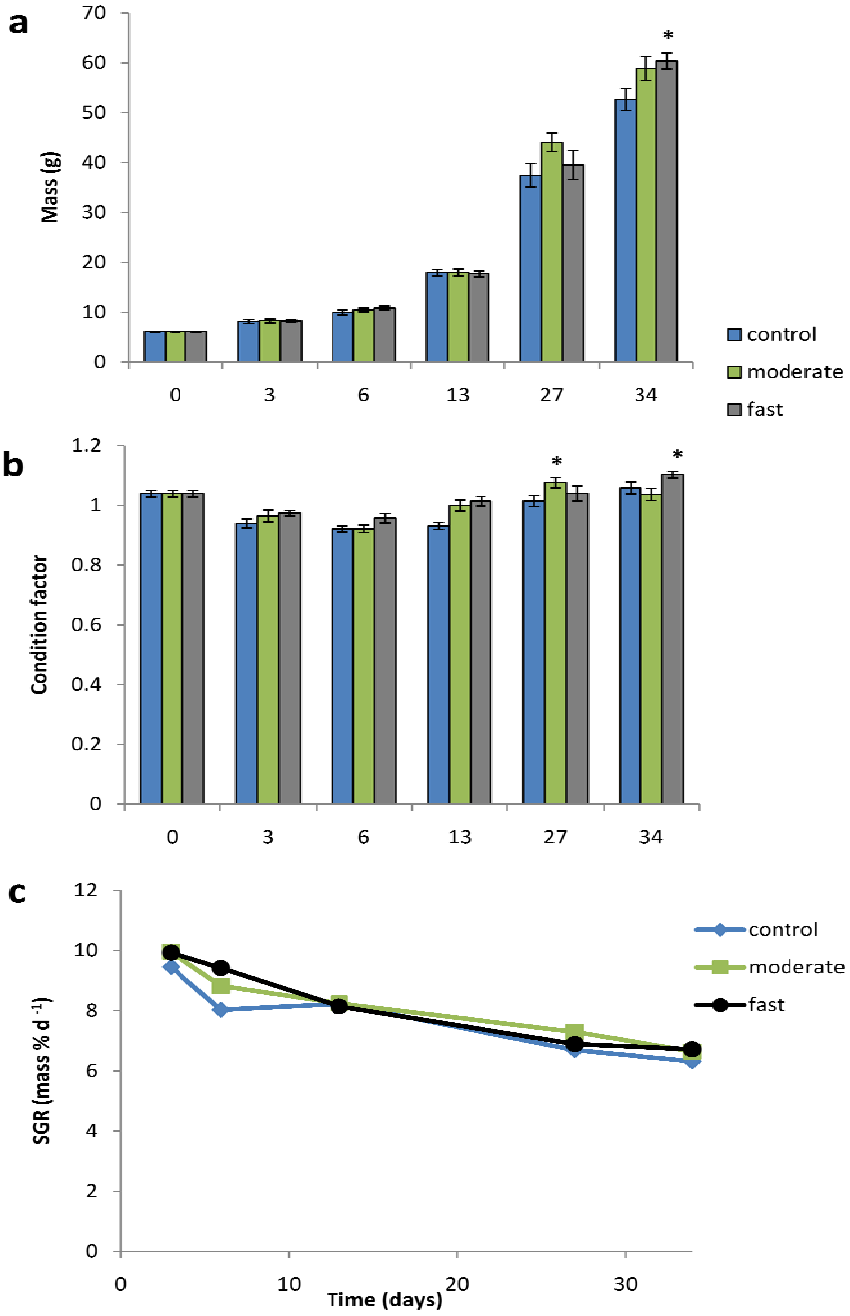

a
Mass (g)
70
60
50
40
30
20
10
0
0
3
6
13
27
34
*
control
moderate
fast
b
Condition factor
1.2
1
0.8
0.6
0.4
0.2
0
0
3
6
13
27
34
*
*
c
SGR (mass % d -1)
12
10
8
6
4
2
0
0
10
20
30
Time (days)
control
moderate
fast

Fig. 4.5. Plasma cortisol (panel a) and IGF-I (panel b) concentrations (mean ± SE of ng/mL values) in California yellowtail subjected to continuous swimming exercise (slow, moderate or fast water flow; see Methods) or no exercise (control) in experiments carried out an a 2007 cohort. Asterisks (*) indicates a significant difference ($p \leq 0.05$) from control within each sample day. ¥ indicates that values are significantly ($p \leq 0.05$) lower from the pre-exercise value. Plasma cortisol levels initially increased after transfer to the raceway in all groups (a).

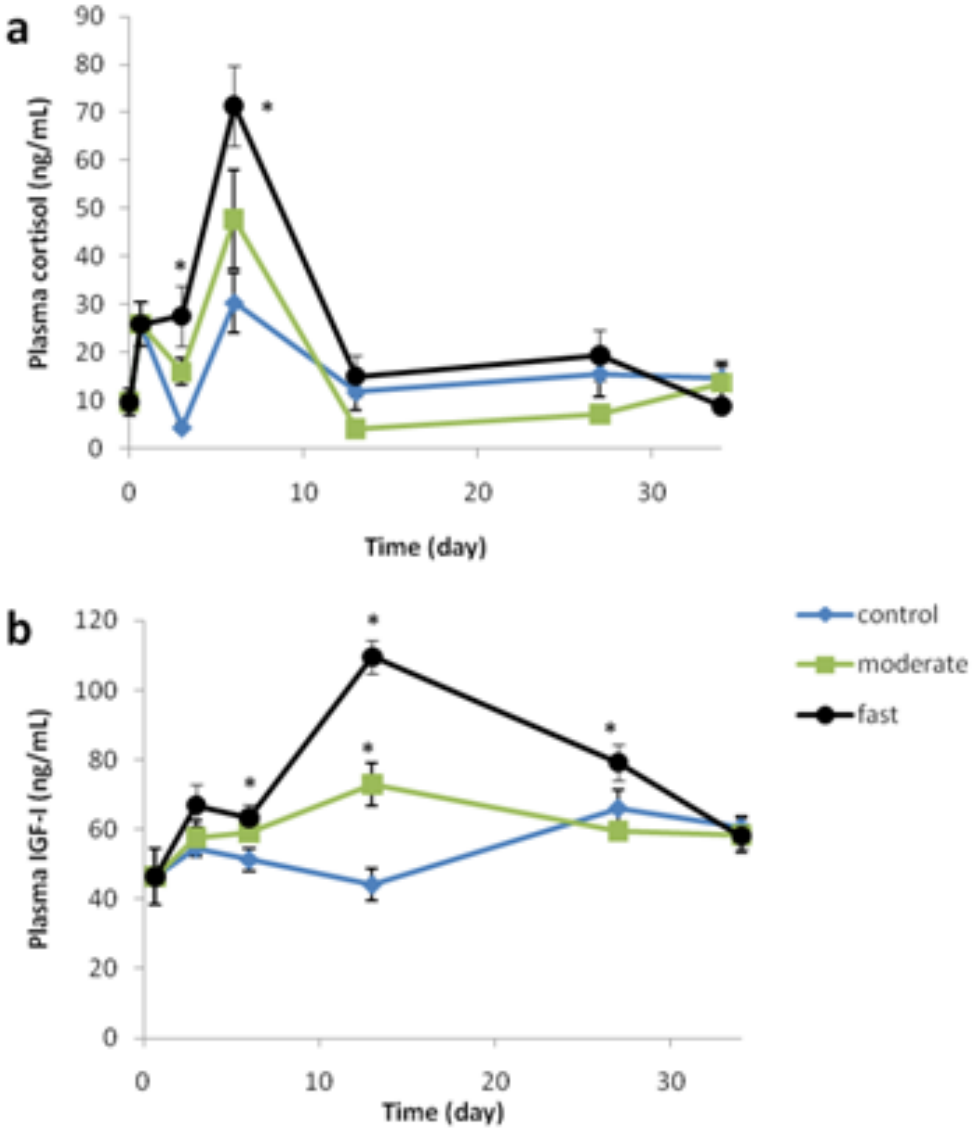

a
Plasma cortisol (ng/mL)
90
80
70
60
50
40
30
20
10
0
0
10
20
30
Time (day)
b
Plasma IGF-I (ng/mL)
120
100
80
60
40
20
0
0
10
20
30
Time (day)
control
moderate
fast

Fig. 4.6. Illustrations of Spearman rank correlations between plasma IGF-I and cortisol (panel a, left side) and mass and cortisol (panel b, right side) in California yellowtail subjected to moderate and fast exercise (2007 experiments). Table 2 provides R^2 and p values for all experimental groups. IGF-I concentrations and Growth showed significant negative correlations with plasma cortisol concentrations in the Moderate exercise group. R^2 values indicate the strength of the Spearman rank correlation.

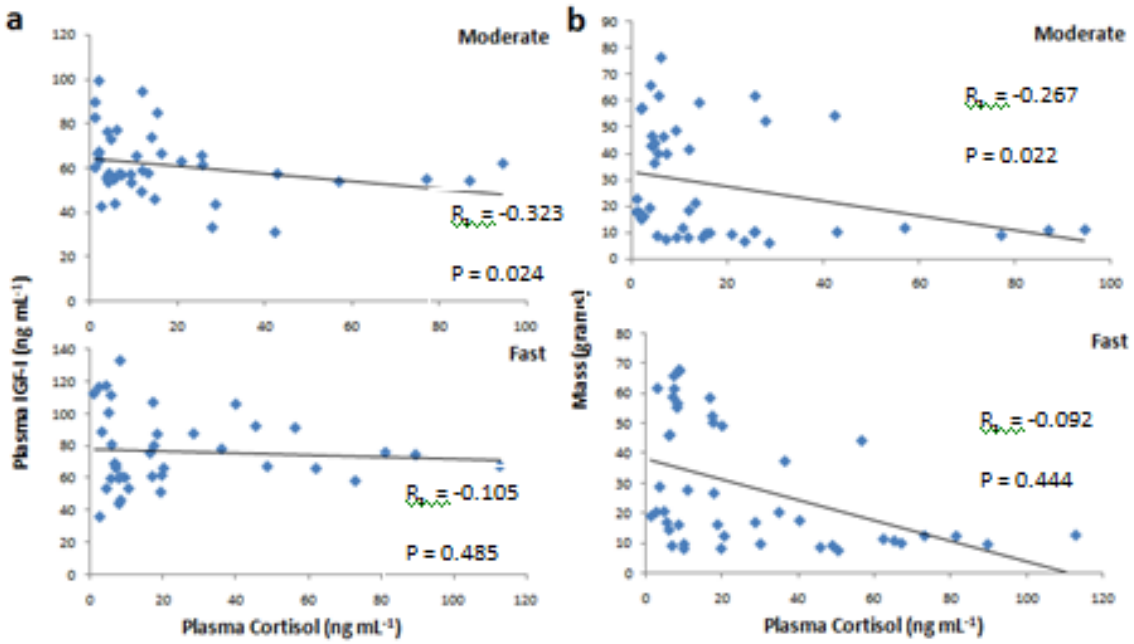

a
Moderate
R_{rs} = -0.323
P = 0.024
Fast
R_{rs} = -0.105
P = 0.485
Plasma IGF-I (ng mL⁻¹)
Plasma Cortisol (ng mL⁻¹)
b
Moderate
R_{rs} = -0.267
P = 0.022
Fast
R_{rs} = -0.092
P = 0.444
Mass (grams)
Plasma Cortisol (ng mL⁻¹)

Fig. 4.7. Body mass (panel a), condition factor (b), and specific growth rate (c) in juvenile California sheephead subjected to continuous swimming exercise (slow, moderate, or fast water flow; see Methods) or no exercise (control) in experiments carried out an a 2007 cohort. Values shown are mean ± SE (n = 10 per group). No consistent significant effect of exercise on growth was observed. Asterisk (*) indicates a significant difference ($p \leq 0.05$) from control within the sample day.

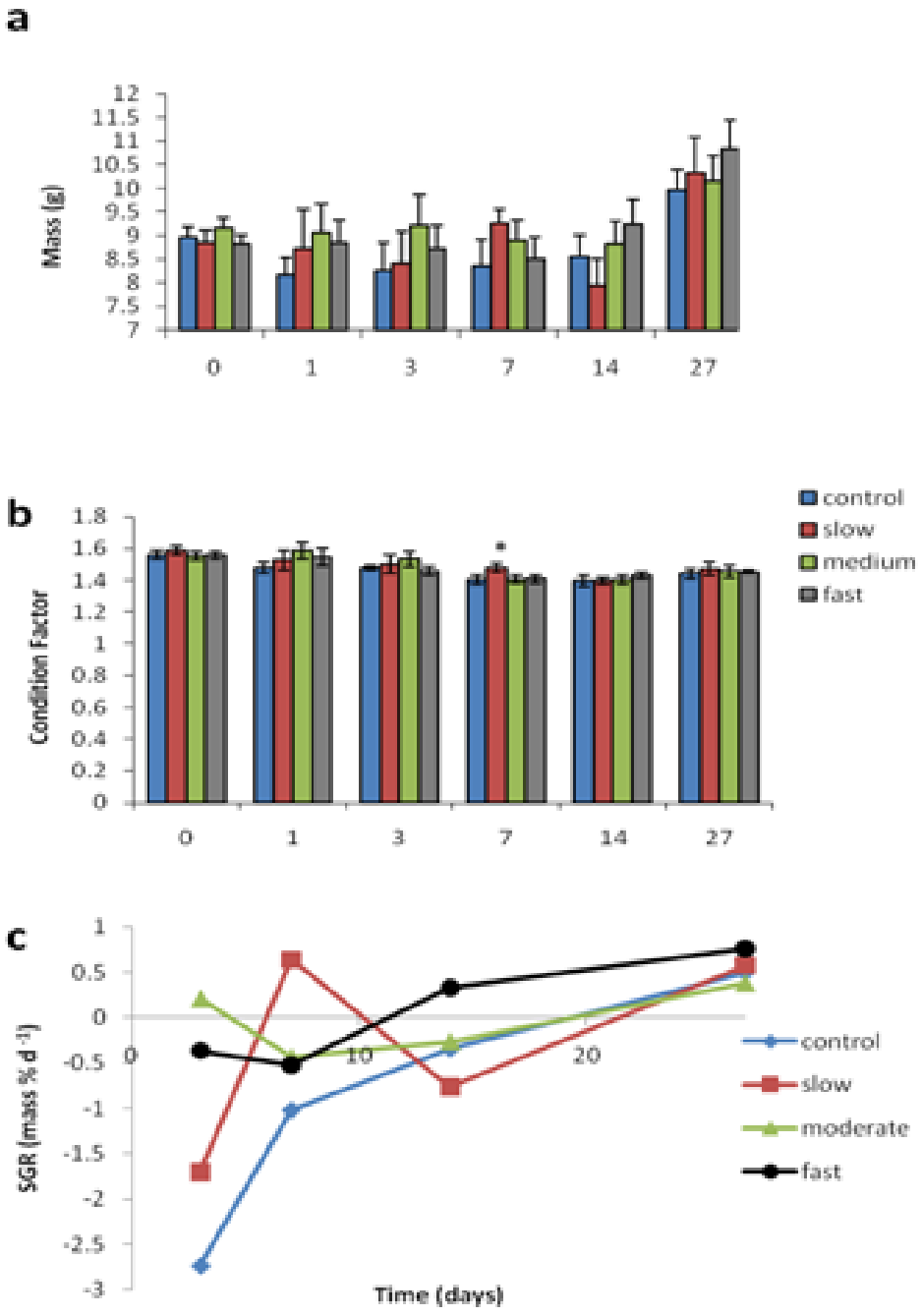

a
Mass (g)
12
11.5
11
10.5
10
9.5
9
8.5
8
7.5
7
0
1
3
7
14
27
b
Condition Factor
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
control
slow
medium
fast
*
c
SGR (mass % d -1)
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
0
10
20
Time (days)
control
slow
moderate
fast

Fig. 4.8. Plasma cortisol (panel a) and IGF-I (panel b) concentrations (mean ± SE of ng/mL values) in California sheephead subjected to continuous swimming exercise (slow, moderate or fast water flow; see Methods) or no exercise (control) in experiments carried out on a 2007 cohort. Asterisks (*) indicates a significant difference ($p \leq 0.05$) from control within each sample day. ¥ indicates that values are significantly ($p \leq 0.05$) lower from the pre-exercise value. Plasma cortisol levels initially increased after transfer to the raceway in all groups (a) corresponding to a decrease in IGF-I levels (b).

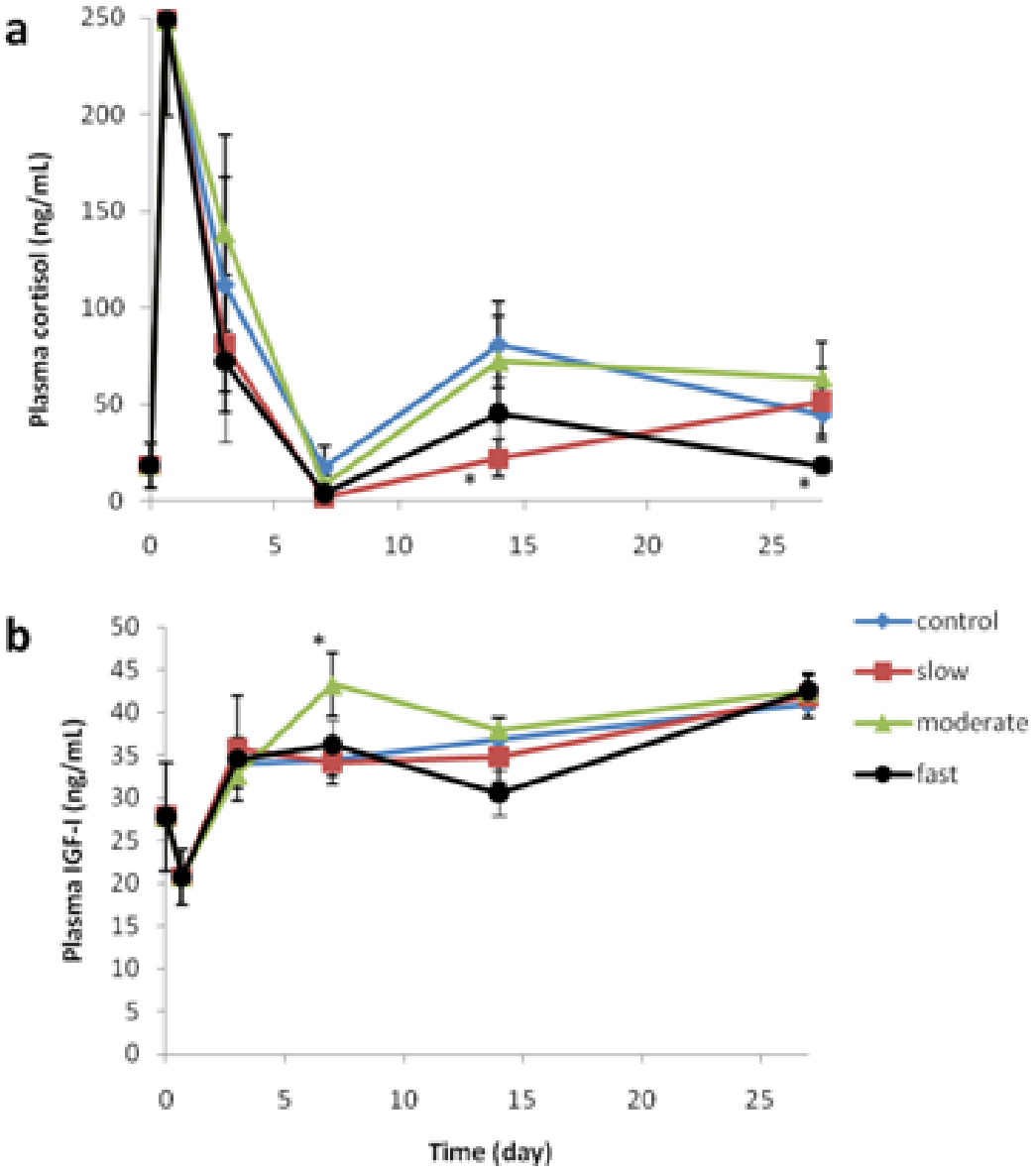

a
Plasma cortisol (ng/mL)
250
200
150
100
50
0
0
5
10
15
20
25
b
Plasma IGF-I (ng/mL)
50
45
40
35
30
25
20
15
10
5
0
0
5
10
15
20
25
Time (day)
control
slow
moderate
fast

Fig. 4.9. Body mass (panel a), condition factor (b), and specific growth rate (c) in juvenile white seabass subjected to continuous swimming exercise (slow, moderate, or fast water flow; see Methods) or no exercise (control) in experiments carried out an a 2007 cohort. Values shown are mean ± SE (n = 10 per group). Mass in the moderate and fast exercise groups was significantly lower relative to the non exercised control group after 28d. Asterisk (*) indicates a significant difference ($p \leq 0.05$) from control within the sample day.

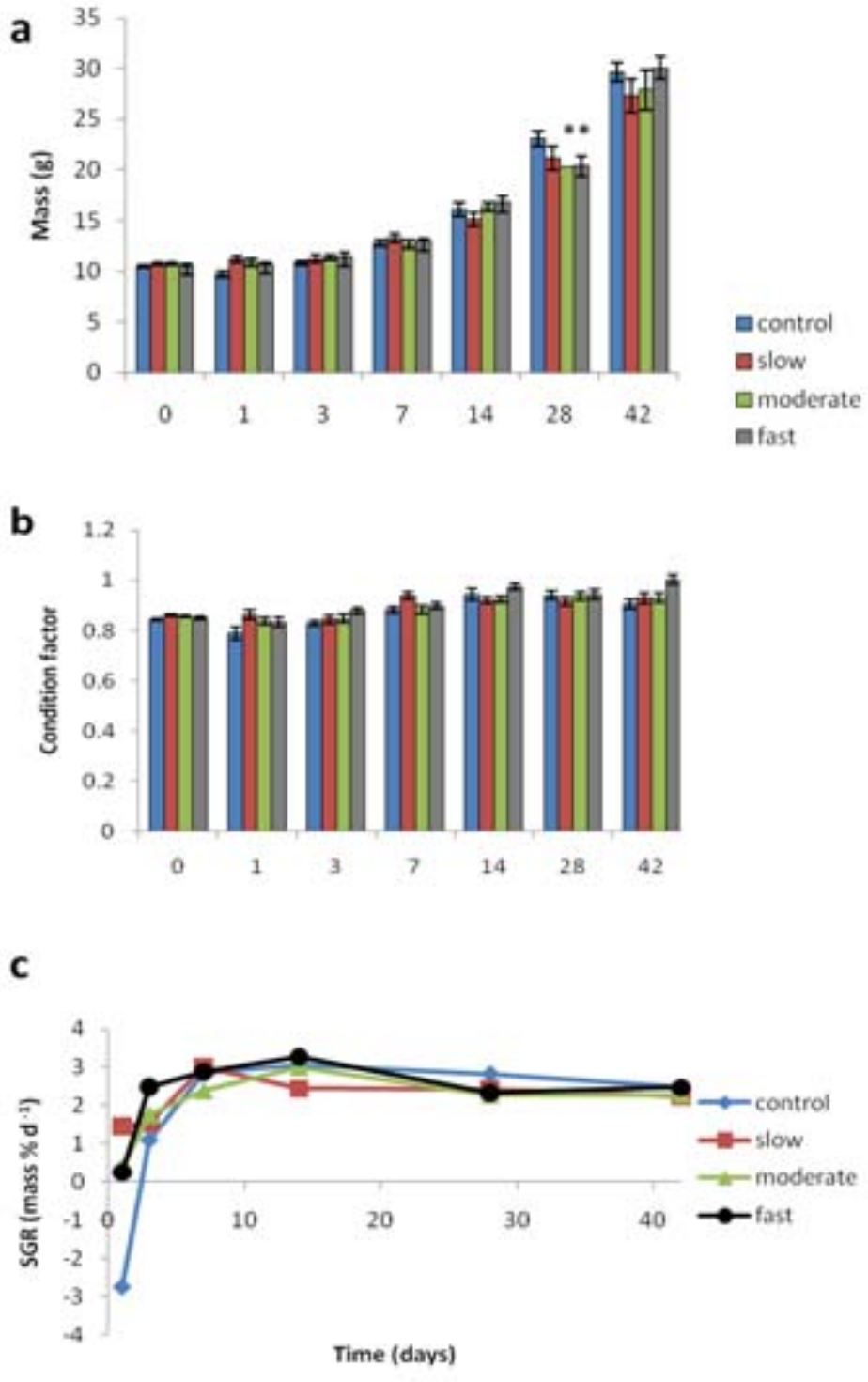

a
Mass (g)
35
30
25
20
15
10
5
0
0
1
3
7
14
28
42
**
control
slow
moderate
fast
b
Condition factor
1.2
1
0.8
0.6
0.4
0.2
0
0
1
3
7
14
28
42
c
SGR (mass % d -1)
4
3
2
1
0
-1
-2
-3
-4
0
10
20
30
40
Time (days)
control
slow
moderate
fast

Fig. 4.10. Plasma cortisol (panel a) and IGF-I (panel b) concentrations (mean ± SE of ng/mL values) in juvenile white seabass subjected to continuous swimming exercise (slow, moderate or fast water flow; see Methods) or no exercise (control) in experiments carried out on a 2007 cohort. Asterisks (*) indicates a significant difference ($p \leq 0.05$) from control within each sample day. ¥ indicates that values are significantly ($p \leq 0.05$) lower from the pre-exercise value. No significant difference in plasma cortisol concentrations (a) or IGF-I levels (b) among treatment groups throughout the experiment.

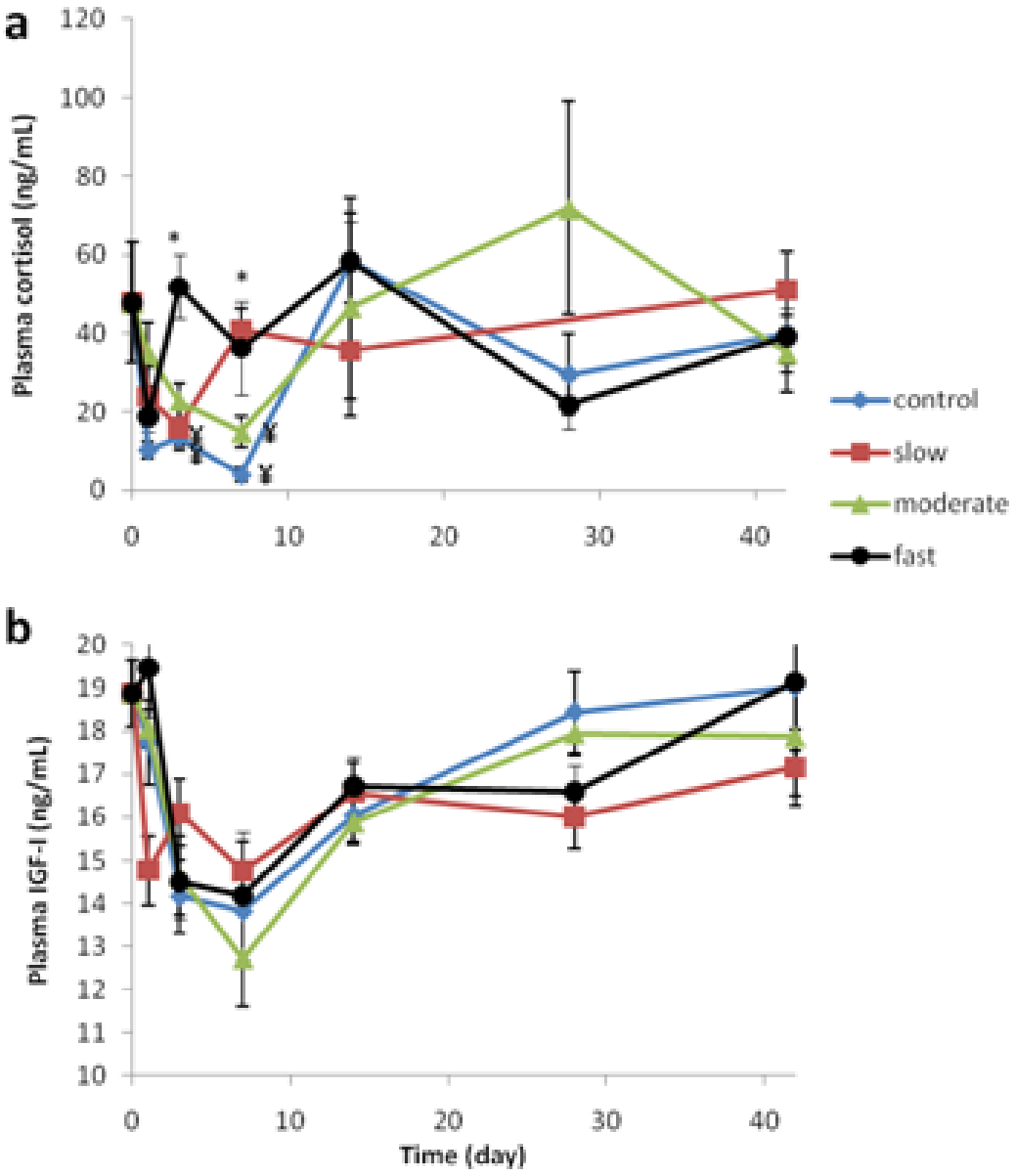

a
Plasma cortisol (ng/mL)
120
100
80
60
40
20
0
0
10
20
30
40
control
slow
moderate
fast
b
Plasma IGF-I (ng/mL)
20
19
18
17
16
15
14
13
12
11
10
0
10
20
30
40
Time (day)

Table 4.2. Results of Spearman's rank correlation analyses for all four experimental trials. Asterisk (*) indicates a significant correlation ($p \leq 0.05$).

	IGF-I and Growth (mass)			Cortisol and Growth (mass)			Cortisol and IGF-I		
	r_s	p value	n	r_s	p value	n	r_s	p value	n
Yellowtail 2006									
control	* 0.697	< 0.001	38	0.13	0.923	55	0.023	0.905	30
slow	* 0.76	< 0.001	37	* -0.371	0.009	49	* -0.374	0.050	28
moderate	* 0.733	< 0.001	39	* -0.462	< 0.001	59	* -0.697	< 0.001	35
fast	* 0.754	< 0.001	39	-0.14	0.321	52	-0.100	0.599	30
Yellowtail 2007									
control	* 0.375	0.004	58	-0.066	0.584	71	0.143	0.339	47
moderate	* 0.331	0.012	57	* -0.267	0.022	73	* -0.323	0.024	49
fast	0.253	0.065	54	-0.092	0.444	71	-0.105	0.485	46
Sheephead									
control	0.115	0.452	45	0.078	0.583	52	-0.041	0.819	33
slow	0.16	0.287	46	0.086	0.531	55	0.115	0.525	33
moderate	0.115	0.438	48	0.05	0.725	52	0.080	0.651	34
fast	* 0.286	0.044	50	0.095	0.488	56	0.163	0.329	38
White seabass									
control	0.156	0.199	69	* 0.389	0.003	55	* 0.339	0.015	51
slow	-0.055	0.655	69	* 0.403	0.007	43	-0.005	0.974	39
moderate	0.097	0.429	68	0.123	0.41	47	0.165	0.296	42
fast	-0.029	0.817	68	0.06	0.677	51	-0.210	0.157	47

IV. Discussion

The results of this thesis study indicate that effects of exercise on somatic growth and on key hormonal regulators of growth in fish may depend upon a variety of interacting factors, from species morphology to existing physiological condition. For example, a species highly adapted for active swimming that uses a relatively larger percentage of its muscle for routine movements, such as the California yellowtail, is likely to respond very differently to an exercise challenge as compared with a more sedentary species that utilizes labriform swimming, such as the California sheephead. In addition, the health and condition of a fish can strongly influence its response to a physiological challenge such as exercise. Chronic stressors such as high stocking density and disease, can all influence growth performance and are often associated with elevated plasma concentrations of the stress hormone, cortisol (Davison 1997; Mommsen, et al. 1999; Montero, et al. 1999; Basrur, et al. 2009).

It is generally assumed that at favorable swimming speeds ($\leq$ 1.5 BL sec^{-1}), exercise training can lead to increased growth rate in fish, particularly in species with relatively active lifestyles, such as salmonids (Davison 1997) and Japanese amberjack *Seriola quinqueradiata* (Yogata and Oku 2000). In agreement with this notion, in the present study it was found that exercised groups of California yellowtail in the 2007 experiments exhibited significantly greater mass and condition factor as compared with un-exercised controls. This effect was not observed in California sheephead, nor in the white seabass and 2006 yellowtail. In the latter two instances, physiological condition prior to the

experimental trials, such as larval quality and stress, appears to have been related to the lack of growth response to exercise.

Despite the positive exercise-induced growth observed in the yellowtail studied in 2007, no such effects were observed in the 2006 experiments. These differences between experiments on the same species point strongly to the important influence of prior physiological condition. In 2006, the yellowtail spawn at the hatchery experienced lower hatch rates, reduced survival to first feeding, and poor swim bladder inflation rates, as compared with the 2007 and prior cohorts (Mark Drawbridge, Hubbs SeaWorld Research Institute, personal communication). It was also observed that the 2006 fish had significantly higher plasma cortisol concentrations at the outset of the experiments, and that elevated cortisol levels persisted in many groups throughout the entire experimental period. Similarly in white seabass, which also showed no growth effects of exercise, cortisol concentrations were elevated at the outset and through the duration of the experiments. Because elevated cortisol is known to reduce growth (Wendelaar Bonga 1997; Mommsen, et al. 1999; Barton 2002; Basrur, et al. 2009) and inhibit food intake in fish (Gregory and Wood 1999), the higher levels of cortisol may be responsible for the lack of growth effects in the 2006 yellowtail and white seabass. That cortisol may exert this growth effect by inhibiting IGF-I production (Mommsen, et al. 1999; Kelley, et al. 2002b; Reinecke, et al. 2005) is supported by the present findings that IGF-I levels were often inversely related to cortisol concentrations.

Although exercise did not significantly alter growth in the California sheephead in the current study, there was a notable trend toward a positive influence. At 14 and 27 d, body mass tended to be greater in the fast exercised group as compared with the other groups. In addition, there was a turn-around in the daily growth rate from negative to positive that occurred earlier in the exercised groups than in the un-exercised controls. These findings suggest that the experimental design may not have been sufficient to detect exercise-associated effects in this species. Future study of this species should include increasing the duration of the exercise training periods to determine whether exercise-induced growth effects can be observed over longer time periods in California sheephead.

As mentioned above, the white seabass in this study did not exhibit exercise-induced growth effects. This is in contrast to prior exercise studies with this species by Buhr (2002) and Cepuritas (2005), which indicate that growth rate can be increased with exercise training. The reasons for the discrepancy between this study and the former studies are not entirely clear, but may be related to differences in the experimental raceway systems used and the prior physiological condition of the test animals. In the former studies, the control fish were held in a square tank, instead of a raceway with minimal directional flow (as in this study). The water currents therefore differed in direction and consistency in the two experimental systems, in addition to the differences in the structure of the environments (square tank vs. raceway). Physiological condition of the white seabass also differed between the studies. The stocking density of the source group was higher in the present study as compared with the former studies. High

stocking densities are known to act as stressors in cultured fish and are associated with lower growth rates (Pickering and Pottinger 1989; Montero, et al. 1999; Iguchi, et al. 2003; Basrur, et al. 2009). Therefore, the fish in our study may have been stressed prior to their transfer to the raceways, potentially preventing an exercise-induced growth response. In support of this hypothesis, cortisol concentrations in the white seabass in this study were higher and more variable than in the seabass studied by Cepuritas (2005), who showed a significant growth response of white seabass to increased exercise.

The acute stressors associated with handling and transporting of fish to the experimental raceway system resulted in significant surges in plasma cortisol concentrations in all fish tested, except for the white seabass. The stress-induced increases in cortisol are consistent with findings in a large number of studies on acute stress responses in fish (Wendelaar Bonga, 1997; Mommsen et al., 1999). With the exception of the white seabass, all species also exhibited progressively decreasing cortisol concentrations after the initial cortisol surges. Even in the 2006 yellowtail that exhibited high pre-transfer cortisol concentrations, rearing them in the raceways led to a progressive decrease in cortisol over time, which appeared to be facilitated by exercise. In the slow and moderate exercise groups, cortisol concentrations were lower than that in the un-exercised controls at 9, 15, and 21 d of the trials, suggesting that exercise improved recovery from stress. In the fast exercised group, however, cortisol concentrations were significantly lower than the controls at 15 d, but not at 9 d, while at 21 d, cortisol concentrations were significantly elevated. These latter findings suggest that the

fast exercised fish, which are exercising closest to their Ucrit, could be more prone to alterations in the stress axis and in cortisol levels than in the other groups.

In some instances, spontaneous cortisol surges were observed. As mentioned above, the 2006 yellowtail in the fast exercised group showed a significant elevation in cortisol late in the experimental trial (21 d). In the yellowtail in 2007, all experimental groups exhibited a >3-fold increase in cortisol at 6 d, as compared with fish at 3 d, which was then followed by low cortisol concentrations (<20 ng mL^{-1}) for the rest of the experimental trial. It is conceivable that these isolated increases in cortisol were due to responses to temporary stressors at the hatchery, including sounds or vibration, or visual disturbance by people.

The lack of a discernable cortisol response in the white seabass differs from that observed in other fish under controlled conditions. In addition, in the study of white seabass by Cepuritas (2005), handling and transport resulted in significant elevations in cortisol concentrations to >150 ng mL^{-1}. In the present study, pre-transfer cortisol levels in white seabass were already relatively elevated (~48 ng mL^{-1}), suggesting that the fish may have been chronically stressed prior to the experimental trials. Published studies of other fish species indicates that prolonged exposure to stressors (e.g., confinement, handling, crowding) can reduce the ability of the fish to produce normal elevations in plasma cortisol in response to new acute stressors (Barton, et al. 1987; Pickering and Pottinger 1989; Basrur, et al. 2009). Therefore, it is suggested that chronic stress in the white

seabass in this study may have resulted in their inability to produce a cortisol response to the handling and transport at the beginning of the experimental trials. In addition, cortisol levels were highly variable and fluctuated around 45 ng mL^{-1} throughout the experimental period, in contrast to the study by Cepuritus (2005) in which white seabass had generally lower plasma cortisol levels throughout the experiment (~20 ng mL^{-1}) and faster growth rates. Furthermore, plasma IGF-I levels were relatively low in this study as compared with that by Cepuritus (2005), suggesting that chronically elevated cortisol may lead to reduced IGF-I levels, which can lead to growth retardation. Suppressed growth rates are commonly observed in a fish that are chronically stressed or treated with exogenous cortisol (Barton, et al. 1987; McCormick, et al. 1998; Gregory and Wood 1999; De Boeck, et al. 2001; Bernier, et al. 2004; Peterson and Small 2005; Basrur, et al. 2009).

In further support of a negative influence of cortisol on growth, the present study found that cortisol concentrations in yellowtail were typically inversely related to IGF-I concentrations and to growth, while IGF-I concentrations showed strong positive correlations with growth (R^2>0.7). The inverse relationship of cortisol and IGF-I has also been demonstrated in tilapia injected with cortisol (Kajimura, et al. 2003) and in sunshine bass subjected to an acute confinement stress (Davis and Small 2006). Interestingly, in the 2007 experiments on yellowtail, exercise appeared to be associated with improved recovery from stress and enhanced growth. After cortisol concentrations had subsided to <20 ng mL^{-1} in the fast exercised group (by 13 d), IGF-I concentrations increased to >80 ng

mL^{-1} (at 13 and 27 d), significantly higher than concentrations in the un-exercised control and moderate exercised groups. These increases in IGF-I were associated with a significant increase in growth by 34 d. Enhanced growth rate often corresponds with increased concentrations of IGF-I in fish plasma (Beckman, et al. 2004a; Dyer, et al. 2004a; Davis and Peterson 2006) and exogenous IGF-I treatment stimulates growth in fish (McCormick, et al. 1992).

V. Conclusions

The present study demonstrated that plasma cortisol concentrations of California yellowtail and California sheephead, increased in response to environmental stressors, such as handling and transport, and that cortisol levels were often inversely correlated with IGF-I concentrations and somatic growth. In California yellowtail, exercise could improve recovery from stress, increase plasma IGF-I concentrations, and lead to enhanced growth, although prior physiological condition appeared to play a major role in the efficacy of the exercise effects. An effect of exercise on plasma IGF-I concentrations in fish is a novel finding that has not yet been reported in the scientific literature. Analysis of a third species in this study, the white seabass, also suggested that chronic stress may be associated with alterations in cortisol and poor growth performance, which exercise did not improve during a 30 d period.

VI. Future research

The data obtained during this investigation provide a basis upon which to further investigate the effects of exercise on the endocrine system in fish. Recent research has shown that stress and in particular elevated levels of cortisol often corresponds to increased levels of low molecular IGF-binding proteins (≤31 kDa IGFBPs; Kelley et al. 2001; Kajimura et al. 2003; Peterson and Small 2004;2005). In some of these studies, plasma IGF-I concentrations have not changed in response to certain treatments or stressors, yet IGFBPs may be substantially altered. Mammalian IGFBP-1 is known to increase several-fold in response to stress or cortisol treatment, and it acts as a growth inhibitor by preventing IGFs from reaching their target receptors. IGFBPs are an important consideration in future studies.

In each of the experiments in this study, flow velocities were based on the initial lengths and aerobic capacities of the fish as they entered the raceway. The velocities were constant throughout the experimental trials, which mean that as the fish grew they experienced lower relative velocities and potentially reduced training effects. Future studies may consider progressively increasing flow velocities relative to fish size and aerobic capacity, to maintain an identical training intensity throughout the experimental period.

Literature Cited

Adams, C. E., F. A. Huntingford, J. Krpal, M. Jobling and S. J. Burnett. 1995. Exercise, agnostic behaviour and food acquisition in Arctic charr, *Salvelinus alpinus*. . Environmental Biology of Fishes 43: 213-218.

Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts and P. Walter. 2002. Molecular Biology of The Cell, Fourth Edition. Pp. *in* eds.). Garland Science, New York.

Barret, B. A. and B. A. McKeown. 1988. Growth hormone response to sustained swimming in exercise-acclimated steelhead trout, *Salmo gairdneri*. Journal of Fish Biology 32: 799-800.

Barry, T. P., J. A. Malison, J. A. Held and J. J. Parrish. 1995. Ontogeny of the cortisol stress response in larval rainbow trout. Gen Comp Endocrinol 97: 57-65.

Barton, B. A., C. B. Schreck and L. D. Barton. 1987. Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. Diseases of Aquatic Organisms 2: 173-185.

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.

Basrur, T. V., R. Longland and R. J. Wilkinson. 2009. Effects of repeated crowding on the stress response and growth performance in Atlantic salmon (*Salmo salar*). Fish Physiology and Biochemistry Published online: 15 March 2009.

Beamish, F. W. H. 1978. Swimming capacity. Pp. *in* Fish Physiology, vol. VII (D. J. R. S. Hoar, eds.). Academic Press, New York.

Beckman, B. R., M. Shimizu, B. A. Gadberry and K. A. Cooper. 2004a. Response of the somatotropic axis of juvenile coho salmon to alterations in plane of nutrition with an analysis of the relationships among growth rate and circulating IGF-I and 41 kDa IGFBP. Gen Comp Endocrinol 135: 334-344.

Beckman, B. R., M. Shimizu, B. A. Gadberry, P. J. Parkins and K. A. Cooper. 2004b. The effect of temperature change on the relations among plasma IGF-I, 41-kDa IGFBP and growth rate in postsmolt coho salmon. Aquaculture 241: 601-619.

Bernier, N. J., N. Bedard and R. E. Peter. 2004. Effects of cortisol on food intake, growth, and forebrain neuropeptide Y and corticotropin-releasing factor gene expression in goldfish. Gen Comp Endocrinol 135: 230-240.

Berwert, L., H. Segner and M. Reinecke. 1995. Ontogeny of IGF-1 and the classical islet hormones in the turbot, Scophthalmus maximus. Peptides 16: 113-122.

Biga, P. R., K. D. Cain, R. W. Hardy, G. T. Schelling, K. Overturf, S. B. Roberts, F. W. Goetz and T. L. Ott. 2004. Growth hormone differentially regulates muscle myostatin1 and -2 and increases circulating cortisol in rainbow trout (Oncorhynchus mykiss). Gen Comp Endocrinol 138: 32-41.

Bjornevik, M., O. Karlsen, I. A. Johnston and A. Kiessling. 2003. Effects of sustained exercise on white muscle structure and flesh quality in farmed cod. . Aquaculture Research 34: 55-64.

Black, E. C., N. J. Bosomworth and G. E. Docherty. 1966. Combined effects of starvation and severe exercise on glycogen metabolism of rainbow trout, *Salmo gairdneri.* Journal of Fisheries Research Board of Canada 23: 1461-1463.

Brett, J. R. 1964. The respiratory metabolism and swimming performance of youngsockeye salmon. Journal of Fisheries Research Board of Canada 21: 1183-1226.

Buhr, G. J. 2002. The effects of exercise conditioning on the growth and muscle development of juvenile white seabass (*Atractoscion nobilis)*. M.S. Thesis, University of San Diego, California.

Burgetz, I. J., A. Rojas-Vargas, S. G. Hinch and D. J. Randall. 1998. Initial recruitment of anaerobic metabolism during sub-maximal swimming in rainbow trout (Oncorhynchus mykiss). Journal of Experimental Biology 201 (Pt 19): 2711-2721.

Caelers, A., G. Berishvili, M. L. Meli, E. Eppler and M. Reinecke. 2004. Establishment of a real-time RT-PCR for the determination of absolute amounts of IGF-I and IGF-II gene expression in liver and extrahepatic sites of the tilapia. Gen Comp Endocrinol 137: 196-204.

Cepuritis, A. 2005. The effects of exercise conditioning on the growth and IGF axis of juvenile white seabass (*Atractoscion nobilis*). M.S. Thesis, University of San Diego, California

Chauvigne, F., J. C. Gabillard, C. Weil and P. Y. Rescan. 2003. Effect of refeeding on IGFI, IGFII, IGF receptors, FGF2, FGF6, and myostatin mRNA expression in rainbow trout myotomal muscle. General and Comparative Endocrinology 132: 209-215.

Cheema, U., R. Brown, V. Mudera, S. Y. Yang, G. McGrouther and G. Goldspink. 2005. Mechanical signals and IGF-I gene splicing in vitro in relation to development of skeletal muscle. J Cell Physiol 202: 67-75.

Chen, M. H., G. Lin, H. Gong, C. Lee, C. Chang, T. Chen and J. Wu. 1998. Cloning and characterization of insulin-like growth factor I cDNA from black seabream (*Acanthopagrus schlegeli*). Zoological Studies 37: 213-221.

Claireaux, G., C. Couturier and A. L. Groison. 2006. Effect of temperature on maximum swimming speed and cost of transport in juvenile European sea bass (Dicentrarchus labrax). J Exp Biol 209: 3420-3428.

Clark, T. D. and R. S. Seymour. 2006. Cardiorespiratory physiology and swimming energetics of a high-energy-demand teleost, the yellowtail kingfish (Seriola lalandi). J Exp Biol 209: 3940-3951.

Cowen, R. K. 1990. Sex change and life history patterns of the labrid, *Semicossyphus pulcher*, across an enviormental gradient. Copeia 3: 787-795.

Creswell, R. C. and R. Williams. 1983. Post-stocking movements and recapture of hatchery-reared trout released into flowing water-effect of prior acclimation to flow. Aquaculture Research 23: 97-103.

Dalziel, A. C., S. E. Moore and C. D. Moyes. 2005. Mitochondrial enzyme content in the muscles of high-performance fish: evolution and variation among fiber types. Am J Physiol Regul Integr Comp Physiol 288: R163-172.

Davis, K. B. and B. C. Peterson. 2006. The effect of temperature, stress, and cortisol on plasma IGF-I and IGFBPs in sunshine bass. Gen Comp Endocrinol 149: 219-225.

Davis, K. B. and B. C. Small. 2006. Rates of cortisol increase and decrease in channel catfish and sunshine bass exposed to an acute confinement stressor. Comp Biochem Physiol C Toxicol Pharmacol 143: 134-139.

Davison, W. and G. Goldspink. 1978. The effect of training on the swimming muscles of the goldfish (Carassius auratus). J Exp Biol 74: 115-122.

Davison, W. 1989. Training and its effects on teleost fish. Comp Biochem Physiol 94A: 1-10.

Davison, W. 1994. Exercise training in the banded wrasse *Notolabrus fucicola* affects muscle fibre diameter but not muscle mitochondrial morphology. New Zealand Journal of Natural Science 21: 11-16.

Davison, W. 1997. The effects of exercise training on teleost fish, a review of recent literature. Comp Biochem Physiol 117A: 67-75.

Dayton, P. K., M. J. Tegner, P. B. Edwards and K. L. Riser. 1998. Sliding baselines, ghosts, and reduced expectations in kelp forest communities. Ecol Appl 8: 309-322.

De Boeck, G., D. Alsop and C. Wood. 2001. Cortisol effects on aerobic and anaerobic metabolism, nitrogen excretion, and whole-body composition in juvenile rainbow trout. Physiol Biochem Zool 74: 858-868.

Dewar, H. and J. Graham. 1994. Studies of Tropical Tuna Swimming Performance in a Large Water Tunnel - Kinematics. J Exp Biol 192: 45-59.

Dewar, H., J. Graham and R. Brill. 1994. Studies of Tropical Tuna Swimming Performance in a Large Water Tunnel - Thermoregulation. J Exp Biol 192: 33-44.

Dickson, K. A., M. O. Gregorio, S. J. Gruber, K. L. Loefler, M. Tran and C. Terrel. 1993. Biochemical indices of aerobic and anaerobic capacity in muscle tissues of California elasmobranch fishes differing in typical activity level Marine Biology 117: 185-193.

Duan, C., S. Duguay and E. M. Plisetskaya. 1993. Insulin-like growth factor I mRNA in coho salmon, Oncorhynchus kisutch.: Tissue distribution and effects of growth hormone/prolactin family proteins. . Fish Physiology and Biochemistry 11: 371-379.

Duan, C., S. Duguay, P. Swanson, W. W. Dickhoff and E. M. Plisetskaya. 1994. Tissue-specific expression of insulin-like growth factor I mRNAs in salmonids: developmental, hormonal, and nutritional regulation. Pp. *in* Comparative Endocrinology (K. G. Davey, S. S. Tobe and D. E. Peter, eds.). National Research Council of Canada, Toronto.

Duan, C. 1997. The insulin-like growth factor system and its biological actions in fish. American Zoologist 37: 491-503.

Duan, C. 1998. Nutritional and developmental regulation of insulin-like growth factors in fish. J Nutr 128: 306S-314S.

Duan, C., J. Ding, Q. Li, W. Tsai and K. Pozios. 1999. Insulin-like growth factor binding protein 2 is a growth inhibitory protein conserved in zebrafish. Proc Natl Acad Sci U S A 96: 15274-15279.

Duan, C. and Q. Xu. 2005. Roles of insulin-like growth factor (IGF) binding proteins in regulating IGF actions. Gen Comp Endocrinol 142: 44-52.

Duguay, S. J., L. K. Park, M. Samadpour and W. W. Dickhoff. 1992. Nucleotide sequence and tissue distribution of three insulin-like growth factor I prohormones in salmon. Mol Endocrinol 6: 1202-1210.

Duguay, S. J., P. Swanson and W. W. Dickhoff. 1994. Differential expression and hormonal regulation of alternatively spliced IGF-I mRNA transcripts in salmon. J Mol Endocrinol 12: 25-37.

Duguay, S. J., J. Lai-Zhang, D. F. Steiner, B. Funkenstein and S. J. Chan. 1996. Developmental and tissue-regulated expression of IGF-I and IGF-II mRNAs in Sparus aurata. J Mol Endocrinol 16: 123-132.

Dyer, A. R., C. G. Barlow, M. P. Bransden, C. G. Carter, B. D. Glencross, N. Richardson, P. M. Thomas, K. C. Williams and J. F. Carragher. 2004a. Correlation of plasma IGF-I concentrations and growth rate in aquacultured finfish: a tool for assessing the potential of new diets. Aquaculture 236: 583-592.

Dyer, A. R., Z. Upton, D. Stone, P. M. Thomas, K. L. Soole, N. Higgs, K. Quinn and J. F. Carragher. 2004b. Development and validation of a radioimmunoassay for fish insulin-like growth factor I (IGF-I) and the effect of aquaculture related stressors on circulating IGF-I levels. Gen Comp Endocrinol 135: 268-275.

Evans, D. H. 1998. The Physiology of Fishes, 2nd Edition. CRC Press LLC.

Farrell, A. P., J. A. Johansen and R. K. Suarez. 1991. effects of exercise-training on cardiac perfromance and muscle enzymes in rainbow trout, *Onccorhynchus mykiss*. Fish Physiology and Biochemistry 9: 303-312.

Fauconneau, B. and G. Paboeuf. 2001. Muscle satellite cells in fish. Pp. *in* Muscle Development and Growth (I. A. Johnston, eds.). Academic Press, San Diego.

Fukazawa, Y., K. Siharath, T. Iguchi and H. A. Bern. 1995. In vitro secretion of insulin-like growth factor-binding proteins from liver of striped bass, Morone saxatilis. Gen Comp Endocrinol 99: 239-247.

Gabillard, J. C., C. Weil, P. Y. Rescan, I. Navarro, J. Gutierrez and P. Y. Le Bail. 2003. Effects of environmental temperature on IGF1, IGF2, and IGF type I receptor expression in rainbow trout (Oncorhynchus mykiss). Gen Comp Endocrinol 133: 233-242.

Galima, M. M. 2004. Catch-and-release stress: impacts in the endocrine physiology of the California sheephead, *Semicossyphus pulcher*. M.S. Thesis, California State Univeristy, Long Beach, CA.

Geist, D. R., R. S. Brown, V. I. Cullinan, M. G. Mesa, S. P. Vanderkooi and C. A. McKinstry. 2003. Relationships between metabolic rate, muscle electromyograms and swim performance of adult chinook salom. Journal of Fish Biology 63: 970-989.

Gibb, A. C. and K. A. Dickson. 2002. Functional Morphology and Biochemical Indices of Performance: Is there a Correlation Between Metabolic Enzyme Activity and Swimming Performance? Integ. and Comp. Biol. 42: 199-207.

Gillanders, B. M., D. J. Ferrell and N. L. Andrew. 2001. Estimates of movement and life-history parameters of the yellowtail kingfish (*Seriola lalandi*): how useful are data from cooperative tagging programme? Marine and Freshwater Research 52: 179-192.

Goldspink, G. 2006. Impairment of IGF-I gene splicing and MGF expression associated with muscle wasting. Int J Biochem Cell Biol 38: 481-489.

Goos, H. J. and D. Consten. 2002. Stress adaptation, cortisol and pubertal development in the male common carp, Cyprinus carpio. Mol Cell Endocrinol 197: 105-116.

Greene, M. W., M. J. Shamblott and T. T. Chen. 1999. Presence of GH-dependent IGF-II mRNA in the diffuse pancreatic tissue of a teleost Comp Biochem Physiol B Biochem Mol Biol 122: 287-292.

Gregory, T. R. and C. M. Wood. 1999. The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. Physiol Biochem Zool 72: 286-295.

Guderley, H., P. H. Leroy and A. Gagne. 2001. Thermal acclimation, growth, and burst swimming of threespine stickleback: enzymatic correlates and influence of photoperiod. Physiol Biochem Zool 74: 66-74.

Hammer, C. 1995. Fatigue and exercise tests with fish. Comp Biochem Physiol 112A: 1-20.

Hill, M. and G. Goldspink. 2003. Expression and splicing of the insulin-like growth factor gene in rodent muscle is associated with muscle satellite (stem) cell activation following local tissue damage. J Physiol 549: 409-418.

Houlihan, D. F. and P. Laurent. 1987. Effects of exercise training on the performance, growth, and protein turnover of rainbow trout (*Salmo gairdneri*). Canadian Journal of Fisheries and Aquatic Sciences 44: 1614-1621.

Iguchi, K., K. Ogawa, M. Nagae and F. Ito. 2003. The influence of rearing density on stress response and disease susceptibility of ayu (*Plecoglossus altivelis*) Aquaculture 220: 515-523.

Johnson, J., J. Silverstein, W. R. Wolters, M. Shimizu, W. W. Dickhoff and B. S. Shepherd. 2003. Disparate regulation of insulin-like growth factor-binding proteins in a primitive, ictalurid, teleost (Ictalurus punctatus). Gen Comp Endocrinol 134: 122-130.

Johnston, I. A. 1999. Muscle development and growth: potential implications for flesh quality in fish. Aquaculture 117: 99-115.

Johnston, I. A. 2001a. Genetic and environmental determinants of muscle growth patterns. Pp. *in* Muscle Development and Growth (I. A. Johnston, eds.). Academic Press, San Diego.

Johnston, I. A. 2001b. Muscle Development and Growth. Pp. *in* Fish Physiology Series (W. S. Hoar and A. P. Farrell, eds.). Academic Press, San Diego.

Jonsson, E., J. I. Johnsson and B. T. Bjornsson. 1998. Growth hormone increases aggressive behavior in juvenile rainbow trout. Horm Behav 33: 9-15.

Kajimura, S., T. Hirano, N. Visitacion, S. Moriyama, K. Aida and E. G. Grau. 2003. Dual mode of cortisol action on GH/IGF-I/IGF binding proteins in the tilapia, Oreochromis mossambicus. J Endocrinol 178: 91-99.

Kajimura, S., K. Aida and C. Duan. 2005. Insulin-like growth factor-binding protein-1 (IGFBP-1) mediates hypoxia-induced embryonic growth and developmental retardation. Proc Natl Acad Sci U S A 102: 1240-1245.

Kajimura, S., K. Aida and C. Duan. 2006. Understanding hypoxia-induced gene expression in early development: in vitro and in vivo analysis of hypoxia-inducible factor 1-regulated zebra fish insulin-like growth factor binding protein 1 gene expression. Mol Cell Biol 26: 1142-1155.

Kajimura, S. and C. Duan. 2007. Insulin-like growth factor-binding protein-1: an evolutionarily conserved dine turner of insulin-like growth factor action under catabolic and stressful conditions. Journal of Fish Biology 71: 309-325.

Kelley, K. M., K. Siharath and H. A. Bern. 1992. Identification of insulin-like growth factor-binding proteins in the circulation of four teleost fish species. J Exp Zool 263: 220-224.

Kelley, K. M., Y. Oh, S. E. Gargosky, Z. Gucev, T. Matsumoto, V. Hwa, L. Ng, D. M. Simpson and R. G. Rosenfeld. 1996. Insulin-like growth factor-binding proteins (IGFBPs) and their regulatory dynamics. Int J Biochem Cell Biol 28: 619-637.

Kelley, K. M., P. Desai, J. T. Roth, J. T. Haigwood, S. A. Arope, R. M. Flores, K. E. Schmidt, M. Perez, G. S. Nicholson and W. W. Song. 2000. Evolution of endocrine growth regulation: The insulin-like growth factors (IGFs), their regulatory binding proteins (IGFBPs), and IGF receptors in fishes and other ectothermic vertebrates. Pp. 190-228 *in* Recent Advances in Marine Biotechnology, Volume 4: Aquaculture, Part B Fishes (M. Fingerman and R. Nagabhushanam, eds.). Science Publishers, Plymouth, UK.

Kelley, K. M., J. T. Haigwood, M. Perez and M. M. Galima. 2001. Serum insulin-like growth factor binding proteins (IGFBPs) as markers for anabolic/catabolic condition in fishes. Comp Biochem Physiol B Biochem Mol Biol 129: 229-236.

Kelley, K. M., M. M. Galima, K. Sak, A. Hawayek, J. A. Reyes, M. Jamison, T. Price, L. Berg, L. Balogh, C. Gillespie, A. Gavrilla and C. G. Lowe. 2002a. What we know and don't yet know about the growth-modulating roles of IGFBPs in fishes. Pp. 73-76 *in* Developments in Understanding Fish Growth (B. C. Small and D. MacKinlay, eds.). Proceedings of the American Fisheries Society, Bethesda, MD.

Kelley, K. M., K. E. Schmidt, L. Berg, K. Sak, M. M. Galima, C. Gillespie, L. Balogh, A. Hawayek, J. A. Reyes and M. Jamison. 2002b. Comparative endocrinology of the insulin-like growth factor-binding protein. J Endocrinol 175: 3-18.

Kieffer, J. D. 2000. Limits to exhaustive exercise in fish. Comp Biochem Physiol A Mol Integr Physiol 126: 161-179.

Kieffer, J. D., A. M. Wakefield and M. K. Litvak. 2001. Juvenile sturgeon exhibit reduced physiological responses to exercise. J Exp Biol 204: 4281-4289.

Kiessling, A., D. Higgs, B. Dosanjh and J. Eales. 1994. Influence of sustained exercise at two ration levels on growth and thyroid function of all female Chinook salmon (*Oncorhynchus tshawytscha*) in seawater. Canadian Journal of Fisheries and Aquatic Sciences 51: 1975-1984.

Kolkovski, S. and Y. Sakakura. 2004. Yellowtail kingfish, from larvae to mature fish - problems and oppurtunities. Pp. 16-19 *in* Advances in Aquaculture Nutrition (L. E. Suarez, D. Marie, M. G. Lopez, U. Scholz and M. Gonzalez, eds.). VII International Symposium on Aquaculture Nutrition, Hermosillo, Sonora, Mexico.

Koumoundouros, G., C. Ashton, D. G. Sfakianakis, P. Divanach, m. Kentouris, N. Anthwal and N. C. Stickland. 2009. Thermally induced phenotypic plasticity of swimming performance in European sea bass *Dicentrarchus labrax* juveniles. Journal of Fish Biology 74: 1309-1322.

Larsen, D. A., B. R. Beckman and W. W. Dickhoff. 2001. The effect of low temperature and fasting during the winter on metabolic stores and endocrine physiology (insulin, insulin-like growth factor-I, and thyroxine) of coho salmon, Oncorhynchus kisutch. Gen Comp Endocrinol 123: 308-323.

Larsen, D. A., M. Shimizu, K. A. Cooper, P. Swanson and W. W. Dickhoff. 2004. Androgen effects on plasma GH, IGF-I, and 41-kDa IGFBP in coho salmon (Oncorhynchus kisutch). Gen Comp Endocrinol 139: 29-37.

Levesque, H. M., M. A. Shears, G. L. Fletcher and T. W. Moon. 2008. Myogenesis and muscle metabolism in juvenile Atlantic salmon (Salmo salar) made transgenic for growth hormone. J Exp Biol 211: 128-137.

Luckenbach, J. A., R. Murashige, H. V. Daniels, J. Godwin and R. J. Borski. 2007. Temperature affects insulin-like growth factor I and growth of juvenile southern flounder, Paralichthys lethostigma. Comp Biochem Physiol A Mol Integr Physiol 146: 95-104.

Mancera, J. M. and S. D. McCormick. 1998. Osmoregulatory actions of the GH/IGF axis in non-salmonid teleosts. Comp Biochem Physiol B Biochem Mol Biol 121: 43-48.

Martin, C. I. and I. A. Johnston. 2005. The role of myostatin and the calcineurin-signalling pathway in regulating muscle mass in response to exercise training in the rainbow trout Oncorhynchus mykiss Walbaum. J Exp Biol 208: 2083-2090.

Martin, C. I. and I. A. Johnston. 2006. Endurance exercise training in common carp Cyprinus carpio L. induces proliferation of myonuclei in fast muscle fibres and slow muscle fibre hypertrophy. Journal of Fish Biology 69: 1221-1227.

Martinez, M., H. Guderley, J. D. Dutil, P. D. Winger, P. He and S. J. Walsh. 2003. Condition, prolonged swimming performance and muscle metabolic capacities of cod Gadus morhua. J Exp Biol 206: 503-511.

Maures, T., S. J. Chan, B. Xu, H. Sun, J. Ding and C. Duan. 2002. Structural, biochemical, and expression analysis of two distinct insulin-like growth factor I receptors and their ligands in zebrafish. Endocrinology 143: 1858-1871.

Maures, T. J. and C. Duan. 2002. Structure, developmental expression, and physiological regulation of zebrafish IGF binding protein-1. Endocrinology 143: 2722-2731.

Mazik, P. M., B. A. Simco and N. C. Parker. 1991. Influence of water hardness and salrs on survival and physiological characteristics of striped bass during and after transport. Transactions of the American Fisheries Society 120: 121-126.

McClelland, G. B., P. M. Craig, K. Dhekney and S. Dipardo. 2006. Temperature- and exercise-induced gene expression and metabolic enzyme changes in skeletal muscle of adult zebrafish (Danio rerio). J Physiol 577: 739-751.

McCormick, M. I. 1998. Behaviorally induced maternal stress in a fish influences progeny quality by a hormonal mechanism. Ecology 79: 1873-1883.

McCormick, S. D., K. M. Kelley, G. Young, R. S. Nishioka and H. A. Bern. 1992. Stimulation of coho salmon growth by insulin-like growth factor I. Gen Comp Endocrinol 86: 398-406.

McCormick, S. D. 1996. Effects of growth hormone and insulin-like growth factor I on salinity tolerance and gill Na+, K+-ATPase in Atlantic salmon (Salmo salar): interaction with cortisol. Gen Comp Endocrinol 101: 3-11.

McCormick, S. D., J. M. Shrimpton, J. B. Carey, M. F. O'Dea, K. E. Sloan, S. Moriyama and B. T. Bjornsson. 1998. Repeated acute stress reduces growth rate of Atlantic salmon parr and alters plasma levels of growth hormone, insulin-like growth factor I and cortisol. Aquaculture 168: 221-235.

McCormick, S. D., S. Moriyama and B. T. Bjornsson. 2000. Low temperature limits photoperiod control of smolting in atlantic salmon through endocrine mechanisms. Am J Physiol Regul Integr Comp Physiol 278: R1352-1361.

Merino, G. E., R. H. Piedrahita and D. E. Conklin. 2007. Effect of water velocity on the growth of California halibut (*Paralichthys californicus*) juveniles. Aquaculture 271: 206-215.

Meton, I., A. Caseras, E. Canto, F. Fernandez and I. V. Baanante. 2000. Liver insulin-like growth factor-I mRNA is not affected by diet composition or ration size but shows diurnal variations in regularly-fed gilthead sea bream (Sparus aurata). J Nutr 130: 757-760.

Milligan, C. L. 1996. Metabolic recovery from exhaustive exercise in rainbow trout. Comp Biochem Physiol 113A: 51-60.

Milligan, C. L. 1997. The role of cortisol in amino acid mobilization and metabolism following exhaustive exercise in rainbow trout (Oncorhynchus mykiss Walbaum). Fish Physiology and Biochemistry 16: 119-128.

Milligan, C. L., G. B. Hooke and C. Johnson. 2000. Sustained swimming at low velocity following a bout of exhaustive exercise enhances metabolic recovery in rainbow trout. J Exp Biol 203: 921-926.

Milligan, C. L. 2003. A regulatory role for cortisol in muscle glycogen metabolism in rainbow trout Oncorhynchus mykiss Walbaum. J Exp Biol 206: 3167-3173.

Milligan, C. L. 2004. Of fish, fat, and fuel: fatty acid transport in trout muscle. Am J Physiol Regul Integr Comp Physiol 286: 23-24.

Moerland, T. S. and S. Egginton. 1998. Intracellular pH of muscle and temperature: Insight from *in vivo* ^{31}P NMR measurements in a stenothermal Antarctic teleost (*Harpagifer antarcticus*). Journal of Thermal Biology 23: 275-282.

Mommsen, T. P., M. M. Vijayan and T. W. Moon. 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries 9: 211-268.

Mommsen, T. P. 2001. Paradigms of growth in fish. Comp Biochem Physiol B Biochem Mol Biol 129: 207-219.

Mommsen, T. P. and T. W. Moon. 2001. Hormonal regulation of muscle growth. Pp. 251-308 *in* Muscle Development and Growth (I. A. Johnston, eds.). Academic Press, San Diego.

Montero, D., M. S. Izquierdo, L. Tort, L. Robaina and J. M. Vergara. 1999. High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead seabream, Sparus aurata, juveniles. Fish Physiology and Biochemistry 20: 53-60.

Montserrat, N., J. C. Gabillard, E. Capilla, M. I. Navarro and J. Gutierrez. 2007a. Role of insulin, insulin-like growth factors, and muscle regulatory factors in the compensatory growth of the trout (Oncorhynchus mykiss). Gen Comp Endocrinol 150: 462-472.

Montserrat, N., P. Gomez-Requeni, G. Bellini, E. Capilla, J. Perez-Sanchez, I. Navarro and J. Gutierrez. 2007b. Distinct role of insulin and IGF-I and its receptors in the white skeletal muscle during the compensatory growth of gilthead sea bream (Sparus aurata). Aquaculture 267: 188-198.

Moriyama, S., P. Swanson, M. Nishii, A. Takahashi, H. Kawauchi, W. W. Dickhoff and E. M. Plisetskaya. 1994. Development of a homologous radioimmunoassay for coho salmon insulin-like growth factor-I. Gen Comp Endocrinol 96: 149-161.

Moriyama, S., F. G. Ayson and H. Kawauchi. 2000. Growth regulation by insulin-like growth factor-I in fish. Biosci Biotechnol Biochem 64: 1553-1562.

Nakada, M. 2002. Yellowtail culture develoment and solutions for the future. Reviews in Fisheries Science 10: 559-575.

Negatu, Z. and A. H. Meier. 1995. In vitro incorporation of [14C]glycine into muscle protein of gulf killifish (Fundulus grandis) in response to insulin-like growth factor-I. Gen Comp Endocrinol 98: 193-201.

Nielsen, M. E., L. Boesgaard, R. M. Sweeting, B. A. McKeown and P. Rosenkilde. 1994. Plasma levels of lactate, potassium, glucose, cortisol, growth hormone and triiodo-L-thyronine in rainbow trout (Oncorhynchus mykiss) during exercise at various levels for 24h Canadian Journal of Zoology 72: 1643-1647.

Norton, S. F., A. Eppley and B. D. Sidell. 2000. Allometric scaling of maximal enzyme activities in the axial musculature of striped bass, *Morone saxatilis* (Walbaum). Physiol Biochem Zool 73: 819-828.

Ogata, H. Y. and H. Oku. 2000. Effects of Water Velocity on Growth Performance of Juvenile Japanese Flounder Paralichthys olivaceus. Journal of the World Aquaculture Society 31: 225-231.

Park, R., B. S. Shepherd, R. S. Nishioka, E. G. Grau and H. A. Bern. 2000. Effects of homologous pituitary hormone treatment on serum insulin-like growth-factor-binding proteins (IGFBPs) in hypophysectomized tilapia, Oreochromis mossambicus, with special reference to a novel 20-kDa IGFBP. Gen Comp Endocrinol 117: 404-412.

Parrizas, M., E. M. Plisetskaya, J. Planas and J. Gutierrez. 1995. Abundant insulin-like growth factor-1 (IGF-1) receptor binding in fish skeletal muscle. Gen Comp Endocrinol 98: 16-25.

Pelletier, D., P. Blier, J. D. Dutil and H. Guderley. 1995. How should enzyme activities be used in fish growth studies? J Exp Biol 198: 1493-1497.

Perez-Sanchez, J. and P. Y. Le Bail. 1999. Growth hormone axis as marker of nutritional status and growth performance in fish. Aquaculture 177: 117-128.

Peterson, B. C. and B. C. Small. 2004. Effects of fasting on circulating IGF-binding proteins, glucose, and cortisol in channel catfish (Ictalurus punctatus). Domest Anim Endocrinol 26: 231-240.

Peterson, B. C., G. C. Waldbieser and L. Bilodeau. 2004. IGF-I and IGF-II mRNA expression in slow and fast growing families of USDA103 channel catfish (Ictalurus punctatus). Comp Biochem Physiol A Mol Integr Physiol 139: 317-323.

Peterson, B. C. and B. C. Small. 2005. Effects of exogenous cortisol on the GH/IGF-I/IGFBP network in channel catfish. Domest Anim Endocrinol 28: 391-404.

Pickering, A. D. and T. G. Pottinger. 1989. Stress responses and disease resistance in salmonid fish: Effects of chronic elevation of plasma cortisol. Fish Physiology and Biochemistry 7: 253-258.

Pierce, A. L., B. R. Beckman, K. D. Shearer, D. A. Larsen and W. W. Dickhoff. 2001. Effects of ration on somatotropic hormones and growth in coho salmon. Comp Biochem Physiol B Biochem Mol Biol 128: 255-264.

Pierce, A. L., M. Shimizu, B. R. Beckman, D. M. Baker and W. W. Dickhoff. 2005. Time course of the GH/IGF axis response to fasting and increased ration in chinook salmon (Oncorhynchus tshawytscha). Gen Comp Endocrinol 140: 192-202.

Planas, J. V., E. Mendez, N. Banos, E. Capilla, J. Castillo, I. Navarro and J. Gutierrez. 2000. Fish insulin, IGF-I and IGF-II receptors: A phylogenetic approach. American Zoology 40: 223-233.

Plisetskaya, E. M. 1998. Some of my not so favorite things about insulin and insulin-like growth factors in fish. Comp Biochem Physiol B Biochem Mol Biol 121: 3-11.

Randall, D. J. and C. Brauner. 1991. Effects of environmental factors on exercise in fish. J Exp Biol 160: 113-126.

Reid, S. G. and S. F. Perry. 1994. Storage and differential release of catecholamines in rainbow trout (*Oncorhynchus mykiss*) and American eel (*Anguilla rostrata*). Physiological Zoology 67: 216-237.

Reinecke, M., B. T. Bjornsson, W. W. Dickhoff, S. D. McCormick, I. Navarro, D. M. Power and J. Gutierrez. 2005. Growth hormone and insulin-like growth factors in fish: where we are and where to go. Gen Comp Endocrinol 142: 20-24.

Rescan, P. Y., I. Jutel and C. Ralliere. 2001. Two myostatin genes are differentially expressed in myotomal muscle of the trout (*Oncorhynchus mykiss*). Journal of Experimental Biology 204: 3523-3529.

Sakakura, Y., M. Tagawa and K. Tsukamoto. 1998. Whole-body cortisol concentrations and ontogeny of aggressive behavior in yellowtail (Seriola quinqueradiata Temminck & Schlegel; Carangidae). Gen Comp Endocrinol 109: 286-292.

Sanger, A. M. and U. Potscher. 2000. Endurance exercise training affects fast white axial muscle in the Cyprinid species *Chalcalburnus Chalcoides mento* (Agassiz, 1832), Cyprinidae, Teleostei. Basic Appl Myol 10: 297-300.

Sanger, A. M. and W. Stoiber. 2001. Muscle fiber diversity and plasticity. Pp. *in* Muscle Development and Growth (I. A. Johnston, eds.). Academic Press, San Diego.

Sepulveda, C. and K. A. Dickson. 2000. Maximum sustainable speeds and cost of swimming in juvenile kawakawa tuna (Euthynnus affinis) and chub mackerel (Scomber japonicus). J Exp Biol 203: 3089-3101.

Shamblott, M. J. and T. T. Chen. 1992. Identification of a second insulin-like growth factor in a fish species. Proc Natl Acad Sci U S A 89: 8913-8917.

Shimizu, M., P. Swanson and W. W. Dickhoff. 1999. Free and protein-bound insulin-like growth factor-I (IGF-I) and IGF-binding proteins in plasma of coho salmon, Oncorhynchus kisutch. Gen Comp Endocrinol 115: 398-405.

Shimizu, M., P. Swanson, H. Fukada, A. Hara and W. W. Dickhoff. 2000. Comparison of extraction methods and assay validation for salmon insulin-like growth factor-I using commercially available components. Gen Comp Endocrinol 119: 26-36.

Shimizu, M., J. T. Dickey, H. Fukada and W. W. Dickhoff. 2005. Salmon serum 22 kDa insulin-like growth factor-binding protein (IGFBP) is IGFBP-1. J Endocrinol 184: 267-276.

Sidell, B. D. 1998. Intracellular oxygen diffusion: the roles of myoglobin and lipid at cold body temperature. J Exp Biol 201: 1119-1128.

Siharath, K., K. M. Kelley and H. A. Bern. 1996. A low-molecular-weight (25-kDa) IGF-binding protein is increased with growth inhibition in the fasting striped bass, Morone saxatilis. Gen Comp Endocrinol 102: 307-316.

Silverstein, J. T., W. R. Wolters, M. Shimizu and W. W. Dickhoff. 2000. Bovine growth hormone treatment of channel catfish: strain and temperature effects on growth, plasma IGF-I levels, feed intake and efficiency and body composition. Aquaculture 190: 77-88.

Sjogren, K., J. L. Liu, K. Blad, S. Skrtic, O. Vidal, V. Wallenius, D. LeRoith, J. Tornell, O. G. Isaksson, J. O. Jansson and C. Ohlsson. 1999. Liver-derived insulin-like growth factor I (IGF-I) is the principal source of IGF-I in blood but is not required for postnatal body growth in mice. Proc Natl Acad Sci U S A 96: 7088-7092.

Somero, G. N. and J. J. Childress. 1980. A violation of the metabolism-size scaling paradigm: Activities of glycolytic enzymes in muscle increase in large-size fishes. Physiol Zool 53: 322-337.

Somero, G. N. and J. J. Childress. 1990. Scaling of ATP-supplying enzymes, myofibrillar proteins and buffering capacity in fish muscle: Relationship to locomotory habit J Exp Biol 149: 319-333.

Strand, A., C. Magnhagen and A. Alanara. 2007. Effects of repeated disturbances on feed intake, growth rates and energy expenditures of juvenile perch, *Perca fluviatilis*. Aquaculture 265: 163-168.

Sullivan, K. M. and G. N. Somero. 1983. Size- and diet- related variations on enzymic activity and tissue composition in the sable fish, *Anolopoma fimbria.* Biol. Bull. 164: 315-326.

Thomas, J. 1968. Management of the white seabass (*Cynoscion nobilis*) in California waters. . California Department of Fish and Game Fish Bulletin 142: 1-42.

Topping, D. T., C. G. Lowe and J. E. Caselle. 2005. Home range and habitat utilization of adult California sheephead, *Semicossyphus pulcher* (Labridae), in a temperate no-take marine reserve. Marine Biology 147: 301-311.

Tripathi, G. and P. Verma. 2003. Pathway-specific response to cortisol in the metabolism of catfish. Comp Biochem Physiol B Biochem Mol Biol 136: 463-471.

Tripathi, G. and P. Verma. 2004. Scaling effects on metabolism of a teleost. J Exp Zoolog A Comp Exp Biol 301: 718-726.

Vijayan, M. M., J. S. Ballantyne and J. F. Leatherland. 1991. Cortisol-induced changes in some aspects of the intermediary metabolism of Salvelinus fontinalis. Gen Comp Endocrinol 82: 476-486.

Vojkoich, M. and J. R. Reed. 1983. White seabass, *Atractoscion nobilis*, in Californai-Mexican waters: Status of the fishery. CalCOFI Reports 24: 79-83.

Wallin, J. E. and M. J. Van Den Ayle. 1995. Interactive effects of stocking site salinity and handling stress on survival of striped bass fingerlings. Transactions of the American Fisheries Society 124: 736-745.

Weatherley, A. H., H. S. Gill and A. F. Lobo. 1988. Recruitment and maximal diameter of axial muscle fibres in teleosts and their relationship to somatic growth and ultimate size. Journal of Fish Biology 33: 851-859.

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

Wendt, C. A. G. and R. L. Saunders. 1973. Changes in the carbohydrate metabolism in young Atlantic salmon in response to various forms of stress. Pp. 55-82 *in* Proceedings of the International Symposium on the Atlantic Salmon: managment, biology, and survival of the species (M. W. Smith and W. M. Carter, eds.). Unipress, Fredericton, N.B.

West, T., P. Schulte and P. Hochachka. 1994. Implications of Hyperglycemia for Post-Exercise Resynthesis of Glycogen in Trout Skeletal Muscle. J Exp Biol 189: 69-84.

Westneat, M. and J. Walker. 1997. Motor patterns of labriform locomotion: kinematic and electromyographic analysis of pectoral fin swimming in the labrid fish Gomphosus varius. J Exp Biol 200: 1881-1893.

Wilkes, D., S. Q. Xie, N. C. Stickland, H. Alami-Durante, M. Kentouri, A. Sterioti, G. Koumoundouros, B. Fauconneau and G. Goldspink. 2001. Temperature and myogenic factor transcript levels during early development determines muscle growth potential in rainbow trout (Oncorhynchus mykiss) and sea bass (Dicentrarchus labrax). J Exp Biol 204: 2763-2771.

Wilkinson, R. J., M. Porter, H. Woolcott, R. Longland and J. F. Carragher. 2006. Effects of aquaculture related stressors and nutritional restriction on circulating growth factors (GH, IGF-I and IGF-II) in Atlantic salmon and rainbow trout. Comp Biochem Physiol A Mol Integr Physiol 145: 214-224.

Wood, A. W., C. Duan and H. A. Bern. 2005. Insulin-like growth factor signaling in fish. Int Rev Cytol 243: 215-285.

Xu, C., G. Wu, Y. Zohar and S. J. Du. 2003. Analysis of myostatin gene structure, expression and function in zebrafish. J Exp Biol 206: 4067-4079.

Yakar, S., J. L. Liu, B. Stannard, A. Butler, D. Accili, B. Sauer and D. LeRoith. 1999. Normal growth and development in the absence of hepatic insulin-like growth factor I. Proc Natl Acad Sci U S A 96: 7324-7329.

Yang, T. H. and G. N. Somero. 1993. Effects of feeding and food deprivation on oxygen consumption, muscle portien concentration and activites of energy metabolism enzymes in muscle and brain of shallow-living (*Scorpana guttata*) and deep-living (*Sebastolobus alascannus*) scorpaenud fishes. J Exp Biol 181: 213-232.

Yogata, H. and H. Oku. 2000. The effects of swimming exercise on growth and whole-body protein and fat contents of fed and unfed fingerling yellowtail. Fisheries Science 66: 1100-1105.

Young, G. 1993. Effects of hypophysectomy on coho salmon interrenal: maintenance of steroidogenic pathway and restoration of in vitro responsiveness to adrenocorticotropin after handling. Gen Comp Endocrinol 92: 428-438.

Young, P. S. and J. J. Cech Jr. 1993a. Improved growth, swimming performance, and muscular development in exercise-conditioned young-of-the-year striped bass (*Morone saxatilis*). Canadian Journal of Fisheries and Aquatic Sciences 50: 703-707.

Young, P. S. and J. J. Cech Jr. 1993b. Effects of exercise conditioning on stress responses and recovery in cultured and wild young-of-the-year striped bass (*Morone saxatilis*). Canadian Journal of Fisheries and Aquatic Sciences 50: 2094-2099.

Young, P. S. and J. J. Cech Jr. 1994a. Optimum exercise conditioning velocity for growth, muscular development, and swimming performance in young-of-the-year striped bass (*Morone saxatilis*). Canadian Journal of Fisheries and Aquatic Sciences 51: 1519-1527.

Young, P. S. and J. J. Cech Jr. 1994b. Effects of different exercise conditioning velocities on the energy reserves and swimming stress responses in young-of-the-year striped bass (*Morone saxatilus*). Canadian Journal of Fisheries and Aquatic Sciences 51: 1528-1534.

Zimmerman, A. M. and M. S. Lowery. 1999. Hyperplastic development and hypertrophic growth of muscle fibers in the white seabass (Atractoscion nobilis). J Exp Zool 284: 299-308.