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Enhancement of Growth Rates and Swimming Performance in Juvenile Marine Finfish in Aquaculture

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Project Hypotheses
We hypothesize that exercise enhances growth of hatchery-reared white seabass by reducing stress and modulating key growth-regulatory factors, and that this will be associated with positive effects on swimming performance. It is secondarily hypothesized that positive effects of exercise on growth and swimming performance will be applicable to other marine finfish, including California yellowtail and California sheephead.

Project Goals and Objectives
1. To construct and test a new raceway system for rearing juvenile marine finfish, to establish Ucrit and potential test velocities for White Seabass, California yellowtail and California sheephead juveniles; to evaluate shifts in Ucrit following exercise trials as an indicator of improved performance; to assess growth and stress endocrine factors associated with the treatments.
2. To determine the influence of exercise on stress, growth, and swimming performance in these fish, as a strategy for defining optimal exercise conditions and understanding the underlying mechanisms of the growth response.

Briefly describe project methodology
The experimental raceway system consisted of 4 fiberglass raceways, each with dimensions of 551.2 x 50.8 x 30.5 cm that allowed up to four exercise treatments to run concurrently, utilizing re-circulated water from a common filtration system to maintain consistency of water chemistry and temperature among tanks. Adjustment of the inflow and outflow rate to each raceway regulated current velocity within each raceway and a mesh screen created a laminar current flow in each fish rearing section. Current velocity was set at 20, 40, and 60% of the maximal aerobic velocity (Ucrit) of juvenile fish based on their length at the beginning of the experiment. The control raceway current flow was set at a minimal velocity necessary to maintain oxygenation and remove waste material from the swimming section. Initial Ucrit for juveniles was determined for each species based on incremental velocity tests performed at the University of San Diego in a hydrodynamic flume.

Fish for each trial were selected to have a narrow range of initial lengths and were randomly assigned to raceways. Water velocity was increased incrementally until the target current velocity was achieved and fish subsequently swam at the target velocity continuously for 28 (California sheephead) to 42 (White Seabass trial 1) days. Subsamples of fish were removed before and after transfer and at intervals throughout the experiment for mass and length determination and blood samples were taken for cortisol and insulin-like-growth factor (IGF-1) quantification. Fish were frozen at -80°C for later measurement of muscle enzyme activity and RNA extraction. At the end of the experiment, muscle samples were fixed in formalin for histology and fiber diameter measurements.

Describe progress and accomplishments toward meeting goals and objectives
Three species of California marine fish were tested in the experimental raceway to compare the effects of continuous swimming at various current velocities on fish with a wide range of natural swimming modes. Two trials with fish from different spawns were conducted for California yellowtail (a high speed, vigorous swimmer) and for White Seabass (a generalist). One trial was conducted with California sheephead, a labriform swimmer that typically swims in and out of kelp forest cover. Cortisol and IGF-1 plasma concentrations, muscle enzyme activities, red:white muscle ratios, heart ventricle size, and growth parameters have been completed for these experiments. Fiber diameter measurements to confirm effects of exercise on muscle fiber hyperplasia or hypertrophy are in process.

Project modifications
Growth rates of the California sheephead were very slow and spawning limited, so only small numbers of individuals were available for the initial experiments. The hatchery discontinued rearing of this species, so no experiments were conducted in the second year on California sheephead.

Project outcomes
All species of fish reared in the raceways demonstrated enhanced aerobic conditioning in response to faster current velocity, but the biochemical and morphological aspects of responses were species-specific.

California Yellowtail, *Seriola lalandi*, demonstrated a significant increase in growth rate in response to rearing at moderate and fast current velocity (40 and 60% Ucrit). After 34 days, juvenile yellowtail reared at high velocity had 15% greater mass than fish reared in minimal flow, primarily due to increase in muscle mass. Ucrit values expressed in body length s-1 typically decline as a function of larger size, although the larger fish are capable of sustaining higher absolute speeds. Thus, it is important to consider size-matched individuals in comparisons of aerobic capacity. Yellowtail exhibited aerobic conditioning at higher flow rates, consistently showing higher Ucrit values than size and age matched fish reared under minimal flow.

Higher Ucrit was associated with higher red:white muscle ratios in the caudal peduncle of exercise reared yellowtail, reflecting a relative increase in the proportion of slow-twitch, aerobic muscle. Exercise reared fish also demonstrated cardiac ventricle mass 49% larger ($p<0.002$) than the low flow controls. Yellowtail appeared to achieve higher aerobic performance through enhanced cardiac function and oxygen delivery to the muscles rather than through increased activity of mitochondrial enzymes in these muscles. Citrate synthase in Yellowtail red muscle was similar in fish from all raceways and significantly lower in white muscle of fast velocity reared fish. Since yellowtail citrate

synthase activity is close to the highest recorded values in fish, it is possible that their baseline mitochondrial content is so high that there is little scope for increase due to conditioning.

White Seabass (WSB), *Atractoscion nobilis*, also showed enhanced aerobic performance and Ucrit in fish reared at higher velocity, but appeared to achieve this aerobic conditioning through different mechanisms than the Yellowtail. WSB showed a significant increase in the red:white muscle ratio in the caudal peduncle indicating a selective enhancement of growth of aerobic muscle mass. Citrate synthase activity also was significantly enhanced in fish reared at higher velocity indicating an increased capacity for mitochondrial activity. Assessment of exercise effects on the activity of representative enzymes of fatty acid metabolism is in progress. In contrast to Yellowtail, WSB showed no change in ventricle mass in response to higher velocity, suggesting that aerobic conditioning of skeletal muscle alone was sufficient to support increased exercise. Enhanced aerobic conditioning should be of substantial benefit to WSB that are hatchery reared for release to the wild. Greater capacity for sustained swimming and higher swim speeds should benefit WSB in foraging and survival on release.

WSB did not show significant differences in growth rate among the exercise treatments in these two raceway experiments (2007/2008), in sharp contrast to our previous studies (fish spawned in 2000/2003). The previous raceway system used a deeper, round tank as the control condition, whereas the new system has a shallow low-flow control raceway identical in shape to the exercise raceways. Although this difference in control tank shape could potentially be a factor in different outcomes of these sets of experiments, we have reason to suspect that the response of WSB was more affected by a change in hatchery rearing conditions of larval fish. Juvenile WSB used in the current experiments were selected to be similar in length and mass to juveniles utilized in earlier studies. However, fish of this size were much younger in the early studies, indicating that the basal growth rate is much slower in fish reared more recently in the hatchery. We are investigating hatchery records to determine whether there was a change in rearing temperatures or food sources between 2000 and 2007. Early rearing temperatures of larval salmonids alter growth rates and muscle fiber number, perhaps contributing to long-term differences in the capacity for growth.

California sheephead lost mass upon transfer to the experimental raceway system and all treatment groups remained lower than pretransfer mass through the first 14 days of the exercise experiment. At 27 days, all groups displayed mass greater than the transfer mass, with the fast raceway fish significantly larger than the control and other treatment groups. Ucrit of post exercise sheephead was significantly higher than pre-exercise fish. Other than a decrease in red muscle citrate synthase in slow velocity fish there was no difference between muscle LDH or CS activity in sheephead. Muscle enzyme activities were measured in muscle from the caudal peduncle for similarity with other species, although it is likely that most routine swimming in the sheephead was powered by the pectoral muscles. Analysis of pectoral muscle metabolic enzymes should provide more insight into how sheephead were able to improve their Ucrit.

Exercise produced species-specific endocrine responses, with differences in cortisol and insulin-like growth factor seen in response to exercise in some species but not others. Plasma cortisol and IGF-1 were inversely correlated in Sheephead and Yellowtail (2007 trial). Lower plasma cortisol was correlated with higher growth rates. Sustained elevated plasma cortisol may interfere with

exercise stimulation of growth rates, as evidenced in the current WSB trials compared to previous studies and in the 2006 Yellowtail trial.

In previous studies, we had observed a decline in cortisol with exercise and a substantial enhancement of growth at higher velocity in White Seabass. WSB in our current trials did not exhibit any difference in growth rate or consistent effect of exercise on plasma cortisol, which remained relatively high and variable throughout the study. Furthermore, the WSB did not display a substantial acute stress response after transfer, which may indicate that they were chronically stressed in the hatchery in 2007/2008. WSB in these current studies were drawn from hatchery tanks with higher stocking density and exhibited baseline cortisol levels higher than previous studies. Specific growth rates (SGR) in past WSB were significantly larger than the current WSB. IGF-1 concentrations in WSB plasma did not change over time in any treatment. We suspect that changes in IGF-1 binding proteins may change the available IGF-1, even if the total IGF-1 remains the same.

Plasma cortisol levels were substantially lower prior to transfer and throughout the raceway experiments in 2007 Yellowtail compared to the 2006 experiment. Associated with lower cortisol concentrations in 2007 were higher growth rates, higher condition factor, and elevated growth in exercised yellowtail. In the 2007 yellowtail trial, plasma IGF-I levels surged in an exercise-dependent manner as the cortisol levels decreased. These increased IGF-I levels would arguably be responsible for the increased growth in mass detectable by d 27 & 34, where the fast raceways experienced the best growth. IGF-1 concentration was correlated with growth and increased over time in yellowtail. There was a negative correlation between cortisol and IGF-1, especially when the 2006 and 2007 groups are compared. Yellowtail from the 2006 spawn did not appear as healthy as the fish from 2007, experiencing lower survival and swim bladder inflation and higher cortisol levels.

Sheephead experienced an extremely large spike in cortisol on transfer to the raceway system. At day 3 cortisol was lower in exercised fish, but still above pre-transfer concentrations. Although cortisol fell below pre-transfer levels by day 7 of the raceway treatment, it increased throughout the latter weeks of the experiment in all raceways. Sheephead had been reared in tanks with PVC pipe providing structures for resting and hiding before transfer to our system. The linear, continuous flow of the raceways perhaps was more stressful for these fish. Sheephead were observed much more frequently participating in agonistic behavior and frequent changes of position within the raceways than the WSB or YT. Lack of growth in sheephead was likely correlated with the elevated cortisol seen early in the experiment. After cortisol concentrations declined, growth resumed in these fish.

Impacts of project

Particularly for aquaculture species reared for release and stock enhancement, improved physiological condition and swim capacity is highly desirable. Our project demonstrates that rearing fish under high velocity flow rates enhances their aerobic capacity and improves aerobic performance. Growth rates of some species are enhanced by the extended periods of exercise and are not compromised in other species. It should be relatively easy to apply similar raceway conditions to other species, allowing our results to be implemented at other facilities. Reduction of stress is likely to have a multitude of health benefits in addition to the aerobic conditioning documented in our study.

Benefits, commercialization and application of project results
Paul Adelizi, California Department of Fish and Game, is interested in applying the raceway rearing to Central Valley Chinook salmon in order to improve performance of released fish. He used information for submitting a 2009 SeaGrant proposal.
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Publications
Conference papers, proceedings, symposia
Title: Effects of Exercise on Endocrinology in Three Different Swim Styles: California Yellowtail, California Sheephead, and White Seabass
Authors: Peters, C.J., M.S. Lowery, K.M. Kelley, and M.A. Drawbridge.
Date: Jan 2-6 2008
Conference Title: Society for Integrative and Comparative Biology
Location: San Antonio, Texas

Title: Continuous Exercise Enhances Swimming performance and Muscle Aerobic Indicators in Juvenile White Seabass (*Atractoscion nobilis*) and California Sheephead (Semicossyphus pulcher).
Authors: Peters, C.J., M.S. Lowery, M.A. Drawbridge, and K.M. Kelley.
Date: Jan 2-6, 2008
Conference Title: Society for Integrative and Comparative Biology
Location: San Antonio, Texas

Theses, dissertations
Title: Continuous exercise enhances swim performance and alters growth rate, IGF-I, and cortisol in juvenile marine finfish in aquaculture.
Authors: Christopher Peters
Schools: University of San Diego
Date: 2009

Newsletters, periodicals
Title: Yellowtail, White Seabass, and Sheephead- Win, Place and Show. News article in the HSWRI Aquaculture Program Research Report
Authors: HSWRI (Hubbs-SeaWorld Research Institute) Staff
Date: June/July 2006

Please list any workshops/presentations given
Get in the Swim. October 14, 2006 USD. Twenty students (10-12th grade) attended a workshop on fish swimming physiology. Students were members of the BE WiSE network (Better Education for Women in Science and Engineering) and several attended both workshops. Fish dissections with comparisons of muscle fiber types and fish swimming experiments in the hydrodynamic flume were done by the students.

Fish Swimming Physiology. San Diego High School, November 7, 2006. Presentation to a Physiology Class with about 35 high school students in attendance.
Fish Aquaculture. November 11, 2006. Twenty BE WiSE students (10-12th grade) attended a workshop on aquaculture practices, including a tour of the raceway experiment at Hubbs-SeaWorld Research Institute.

Students
Christopher Peters
University of San Diego
Department: Marine Science and Environmental Studies
Degree program enrolled in: M.S.
Theses/dissertation title: Continuous exercise enhances swim performance and alters growth rate, IGF-1, and cortisol in juvenile marine finfish in aquat
Supported by Sea Grant funds? [x] yes [] no
Start date
End date

How many student volunteers were involved in the project?: 4

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Aquaculture, Exercise, Endocrine, Cortisol, Muscle Physiology, White Seabass, California Yellowtail, California Sheephead