Exercise duration and cohort affect variability and longevity of the response to exercise training in California Yellowtail (*Seriola dorsalis*)

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Abstract

Five cohorts of cultured California Yellowtail (*Seriola dorsalis*) were used in exercise training experiments to assess the duration of exercise necessary to induce a positive growth and fitness response, quantify the variability and replicability of this response between cohorts, and track the longevity (persistence) of exercise-induced benefits following removal from the exercise stimulus. Custom-designed raceways were used to continuously exercise juvenile yellowtail at their optimal swimming speed for two, three, or four weeks following which several fitness metrics including measures of somatic growth, white muscle fiber area, metabolic rate, and feed conversion were tracked for up to 20 weeks post exercise in comparison to non-exercised controls. Within a cohort, the longest duration of exercise (4 weeks) generally had the largest and longest-lasting impact on growth, followed by the 3-week, and then the 2-week exercise regimes. However, all exercise treatments showed substantial variability in the magnitude and longevity of the response between cohorts. For example, the positive growth response (increase in mass above that of controls) of the 4-week swimming group ranged from 9.8% to 37.8% between cohorts. This variability in the exercise response between cohorts is similar in magnitude to that associated with other experimentally manipulated variables in the exercise regimes of previous studies, and thus highlights the need for additional species-specific

experiments to quantify replicability of positive exercise results. In addition, the longevity of exercise-induced benefits was highly variable between cohorts and generally not retained for prolonged periods post exercise. Most notably, the exercise-induced growth response which may result from muscle hypertrophy (increase in white muscle fiber size) during exercise, subsided within weeks. Taken together, these results indicate that exercise can play an important role in the growth and fitness of *S. dorsalis* and other species, however the duration of the exercise, as well as the timing of exercise in the rearing process likely have important implications for optimizing exercise training for aquaculture enhancement.

1. Introduction

A main goal of aquaculture research is to improve rearing protocols and growout efficiency to enhance animal health, minimize operating costs, and maximize profits. Tracking metrics of fish physiological fitness such as growth rate, feed conversion ratio, and metabolic rate can help identify areas for targeted, species-specific improvements in these protocols (Davison, 1997; Palstra and Planas, 2011). Exercise training has emerged as a promising non-invasive and non-hormonal treatment that has been shown to stimulate growth and muscle development (Brown et al., 2011; Christiansen et al., 1989; Davison and Goldspink, 1977; Palstra et al., 2015; Totland et al., 1987; Yogata and Oku, 2000), improve feed conversion (Christiansen et al., 1992; Davison and Goldspink, 1977; East and Magnan, 1987; Jørgensen and Jobling, 1993; Yogata and Oku, 2000), and potentially affect metabolism (Bagatto et al., 2001; Brown et al., 2011; Skov et al., 2011) in many active fish species. However, the presence and magnitude of exercise-induced physiological changes appear to be largely species-specific and dependent on the exercise regime and other experimental conditions.

Previous studies involving exercise of cultured fish have largely focused on examining the intensity or duration of exercise required to induce the most favorable response. Several studies on salmonids (see review by Davison and Herbert, 2013) and recent work on the Yellowtail Jack, *Seriola lalandi* (Palstra et al., 2015) have generally shown that the most promising growth responses occurred when fish were exercised at their optimal swimming speeds (U_{opt}). This is likely because the optimal swimming speed represents the lowest cost of transport or most efficient swimming speed in terms of energy expenditure per work performed (distance traveled), thus maximizing the positive benefits of exercise such as growth and muscle development (see reviews by Davison, 1997; Davison and Herbert, 2013; Palstra and Planas, 2011). However, consensus on the duration of exercise needed to provide optimal growth and fitness benefits has not been reached. There has been large variation in exercise duration used across studies, with the majority of previous work focusing on periods of sustained exercise on the order of four weeks to several months (Brown et al., 2011; Herbert et al., 2011; Totland et al., 1987). However, recent work on *S. lalandi* by Palstra et al. (2015) demonstrated that a mere 18-day exercise regime resulted in enhanced growth comparable to longer exercise studies, suggesting that equal exercise benefits for some species may be realized in shorter durations than previously recognized. However, it remains unclear if such short durations of sustained swimming can consistently elicit such benefits.

A major shortcoming of previous exercise studies has been the lack of replicates used to examine the potential variability across cohorts given a consistent exercise regime. In addition, the longevity or persistence of any exercise-induced growth or other physiological response post exercise is poorly understood since most previous work has only examined responses immediately following completion of exercise training. Understanding the consistency

(replicability) and longevity (persistence post exercise) of positive physiological responses to exercise is critical in determining the application of exercise training in finfish aquaculture. Thus, we sought to address these knowledge gaps through exercise training experiments on the California Yellowtail, *Seriola dorsalis*, by examining: 1.) how duration of exercise at the optimal swimming speed impacts the response to exercise, 2.) the consistency of positive growth to exercise across multiple cohorts, and 3.) the longevity of exercise-induced benefits post exercise. These goals were tested through tracking growth and fitness metrics of juvenile *S. dorsalis* subjected to exercise of equal intensity but varying duration across five separate cohorts.

S. dorsalis makes an excellent test species in which to address these questions as it has been the subject of recent physiological studies establishing baseline fitness metrics that can be used to evaluate the success of exercise (Schwebel et al., 2018; Parish et al., 2020; Wegner et al., 2018). In general, species of the carangid genus *Seriola* are high priority targets for continued aquaculture development due to their fast growth rates and high market value (Nakada, 2008). In recent years, there has also been a push to optimize rearing protocols to reduce dependence on wild seed to meet the growing demand and expansion of culturing this desirable genus (Kolkovski and Sakakura, 2004; Moran et al., 2007; Stuart and Drawbridge, 2013; Yang et al., 2016). Recent work on *S. dorsalis* indicates that although this species grows well in land-based aquaculture (Stuart and Drawbridge, 2013), cultured fish often show reduced fitness in comparison to wild-caught conspecifics. In particular, cultured fish have been shown to have higher standard metabolic rates, slower critical swimming speeds (Wegner et al., 2018), and less efficient feed conversion ratios (Schwebel et al. 2018). Such reduced fitness and efficiency have been hypothesized to be from a lack of exercise in captivity (Schwebel et al., 2018; Wegner et al., 2018) and can have significant negative impacts on production when scaled up to a

commercial level. Results from previous exercise studies on *S. lalandi* and the Japanese Amberjack, *S. quinqueradiata*, have shown multiple measurable benefits in response to exercise training (Brown et al., 2011; Palstra et al., 2015; Yogata and Oku, 2000), which demonstrates the feasibility of this treatment to improve fitness for these highly active species. Thus, in addition to testing critical questions on the variability and longevity of exercise on fish in general, this study also provides specific insight into *S. dorsalis* and other *Seriola* species, for which rearing protocols are still being optimized and improvements are in high demand.

2. Methods

2.1 Experimental Fish

This study conducted separate exercise-training and subsequent growout trials on each of five cohorts of cultured California Yellowtail, *S. dorsalis*. Each cohort was derived from fertilized eggs resulting from separate spawning events of either established wild-caught or F1 generation broodstock held at Hubbs-SeaWorld Research Institute (San Diego, CA) (Table 1). Resulting larvae and fingerlings were reared following established methods (Stuart and Drawbridge, 2013). Up to 600 individuals from a given cohort were transferred to the Southwest Fisheries Science Center (SWFSC, La Jolla, CA) as fingerlings for experimentation. Upon arrival, fish were kept indoors under natural day/night light cycles in a 3200 L oval tank (304 x 154 x 80 cm *l* x *w* x *h*) supplied with flow-through and temperature-controlled (22 °C) filtered seawater drawn from the end of the Scripps Institution of Oceanography pier. Following acclimation for a minimum of three days, fish were lightly anesthetized using MS-222 (tricaine methanesulfonate, 79 mg L^{-1}) and sorted for size and to eliminate fish with obvious deformities. Fish in Cohorts 2-5 were also individually tagged in the visceral cavity with 8 mm passive

integrated transponder (PIT) tags (Oregon RFID, Portland, OR) in order to more effectively track individual fitness metrics during the study (PIT tags were not available for tagging Cohort 1). Growth and feed conversion ratio were measured for all cohorts, while metabolic rate and muscle fiber cross-sectional area were measured for select cohorts opportunistically due to the labor and time intensive nature of these processes (Table 1). Fish were allowed to recover from sorting and tagging stress for 2-4 days (fish typically demonstrated normal swimming and feeding behavior within hours of PIT tagging) before exercise trials began. All fish care, handling, and experimentation was done according to the SWFSC Institutional Animal Care and Use Committee approved protocol #SW1401.

2.2 Fish Raceways

Yellowtail exercise training occurred in four custom-designed raceways (Fig. 1, Oceans Design, Colorado Springs, CO). Each raceway consisted of a linear working section (169 x 20 x 22 cm) with a clear acrylic viewing window to allow observation of fish swimming behavior, constructed on a metal frame above its own individual sump (approx. sump volume 510 L, total system volume including raceway, approx. 680 L). A 3-phase, 3 horsepower induction motor (Teco Westinghouse Motor Company, Round Rock, TX) circulated water from the sump up to the raceway, and a Matala filter pad and flow straighteners helped to streamline water flow that subsequently traveled through the working section to a standpipe at the opposite end leading back to the sump. Mesh fencing at the front and back of the working section kept the fish contained and prevented fish from traveling down into the sump. The adjustable-speed motor and interchangeable standpipes allowed for manipulation of water flow speed and volume in each raceway. Fish were placed in the raceways at the lowest flow speed allowed by the pumps (under 4 BL s^{-1}), which was then gradually increased over several hours until reaching the size-specific

optimal swimming speed (U_{opt}). This optimal speed was calculated based on combined data from Palstra et al. (2015), Clark and Seymour (2006) and Brown et al. (2011) for *S. lalandi* and Schwebel et al. (2018) for *S. dorsalis* in order to establish a U_{opt} vs. body length regression. Flow speed within each raceway was checked twice daily using a cylindrical vane wheel flowmeter (Höntzsch Gmbh, Waiblingen, Germany) placed in the middle of the raceway approximately 20 cm from the front of the working section. Water flow speed within the raceway was adjusted accordingly to maintain U_{opt} as the fish grew based on the estimated body length of the fish. If an adjustment of speed was needed, the motor speed would be increased in increments of 1 Hz and the flow speed rechecked until the desired speed was reached. Fresh seawater was supplied to each sump at a minimum rate of 5 L min^{-1} to help maintain water quality and temperature within the raceways, and excess water was removed from each system via a standpipe in the sump. Fish waste was collected by four 100-micron mesh filter socks in each sump, which were changed regularly. The mean water temperature across raceways and cohorts was 22.1 ± 0.6 °C, and mean dissolved oxygen was 101.2 ± 2.5 % (7.18 ± 0.17 mgO_2 L^{-1}).

2.3 Exercise Protocol

In order to understand how exercise duration affects growth and other physiological parameters, fish from a given cohort were exercised in the raceways for different durations [two weeks (2W), three weeks (3W), or four weeks (4W)] in comparison to non-exercised controls. Because each cohort was from a different spawning event (Table 1), cohorts were spread across time and no cohorts were run concurrently in the raceways. Immediately preceding a cohort's exercise trials, fish were lightly anaesthetized using MS-222 (79 mg L^{-1}) and measured for body length (BL = total length), fork length (FL), and body mass (M) (Table 1). One quarter of the fish were then placed into a 3200 L oval growout tank to comprise the non-exercised control

group, while the remaining three quarters were distributed into 3-4 raceways (depending on raceway availability and appropriate stocking density) for exercise training (see sampling schematic Fig. 2). After two weeks of exercise, all fish (both controls and fish exercising in the raceways) were systematically removed from their tank / raceway, lightly anesthetized as described above and remeasured (BL, FL, mass). Following measurement, controls were replaced in their tank for continued growout, while one-third of the exercised fish were divided out and placed into a 3200 L oval growout tank (identical to that holding the control fish) to comprise the 2-week (2W) exercise group (Fig. 2). The remaining two-thirds of the exercised fish were replaced in the raceways to continue their exercise training. The separation of exercised fish into the growout tank (to form the 2W group) or back to the raceways (to continue exercise) was done in a manner to ensure even distribution by size (mean body mass ± standard deviation) across groups. This process was then repeated at three weeks in which all fish were lightly anesthetized, remeasured, and half of the remaining exercise fish were moved to another oval growout tank to comprise the 3-week (3W) exercise group (Fig. 2). The remaining fish were then replaced in the raceways for a final week of exercise, following which all fish were again remeasured, and the fish remaining in the raceways were moved to an oval growout tank to form the 4-week (4W) exercise group (Fig. 2). This sampling process was the same between cohorts with the exception of Cohort 1 (which necessitated a much larger *n* due to respirometry and muscle tissue sampling, Table 1). For Cohort 1, 30 fish from each treatment group (instead of all individuals) were randomly selected at the end of each exercise regime and anesthetized for somatic growth measurements, but protocols were otherwise the same. There was some variability in raceway stocking density between cohorts depending on cohort number and starting size (Table 1); however, average tank densities during exercise were all kept low (< 4 kg/m^3),

187 which is well below the recommended maximum stocking density (< 8 kg/m^3) for the closely
188 related *S. dumerili* to prevent impacts on growth and plasma cortisol levels (Fernández-Montero
189 et al., 2020).

190 *2.4 Feed Conversion*

191 To evaluate feed conversion ratio, fish were hand fed to apparent satiation three to five
192 times daily, seven days a week during the exercise period (first four weeks of the growout), and
193 six days a week thereafter (reduced to accommodate staffing since fish were no longer expending
194 as much energy). Feeding was monitored closely, and apparent satiation was reached once fish
195 were no longer interested in food. Fish were fed pellets of increasing size of Otohime marine fish
196 larval and weaning feed (Marubeni Nisshin Feed Co., Ltd, Tokyo, Japan) followed by EWOS
197 commercial pellets (EWOS, Surrey, BC, Canada) as they grew. The feed conversion ratio (FCR)
198 was calculated using:

199 $$FCR = \frac{total\ dry\ feed\ consumed\ (g)}{total\ weight\ gained\ (g)} \quad (1)$$

200 It is important to note that during exercise trials with Cohort 1, it was noticed that the flow
201 speeds in the raceways caused some feed to pass by the fish during feedings into the sump where
202 it was captured by the filter socks. Unfortunately, uneaten food in the filter socks could not easily
203 be separated from solid waste for accurate measurement, thus FCR could not be calculated for
204 Cohort 1 during the exercise period. For all subsequent cohorts, nets were placed at the back of
205 the working section of the raceways during feeding to collect uneaten food, which was then
206 dehydrated, weighed, and subtracted from the amount of food consumed for that day. Thus, FCR
207 was determined only for Cohorts 2-5 during the exercise period, but for all cohorts during the
208 post-exercise growout period.

209 *2.5 Growth and White Muscle Histology*

210 Following the completion of exercise trials, fish from each group (controls, 2W, 3W,
211 4W) were retained for up to an additional 20 weeks to monitor growth and other fitness metrics
212 post exercise. As with the raceways, water temperature (22.0 ± 0.3 °C) and other parameters
213 (e.g., dissolved oxygen, stocking density, and water quality) were monitored and kept consistent
214 within the four 3200 L oval growout tanks within a given cohort. Water was supplied to each
215 growout tank at a rate of approximately 22 L min^{-1} through spray bars that promoted water
216 mixing and some directional flow. For all cohorts, somatic measurements (BL, FL, and M) were
217 taken at the "start" immediately preceding exercise in the raceways, and again after two weeks,
218 three weeks, and four weeks (when each exercise regime was completed), and every two weeks
219 thereafter until the end of the growout period. For four of the five cohorts, measurements were
220 taken for 20 weeks post exercise (for a total of 24 weeks of growth measurements), while timing
221 and space limitations only allowed for four weeks of post-exercise growout for Cohort 2 (for a
222 total of eight weeks of growth measurements). For Cohort 1, at each time point a subset of 30
223 fish from each group were randomly selected, lightly anaesthetized using MS-222 (79 mg L^{-1})
224 and measured. For all other cohorts, all fish were anaesthetized and measured at each time point.
225 Following measurements, fish recovered quickly and generally resumed normal swimming
226 within five minutes and were feeding normally within a few hours. Minor mortality occurred in
227 each group of each cohort throughout the growout trials usually associated with wall strikes,
228 jumping out of the growout tank, and occasionally being held under anesthesia for too long
229 during measurement. Fish mortalities were recorded, and somatic growth measurements were
230 taken to account for group growth dynamics and feed conversion calculations.

231 Mass data were used to calculate the specific growth rate (SGR) for each treatment (2W,
232 3W, 4W, control) in each cohort using:

233 $$SGR = \frac{ln(M_2) - ln(M_1)}{t_2 - t_1} \, x \, 100 \quad (2)$$

234 where M_2 is the mean mass (g) of a given group at time t_2 (days), and M_1 is the mean mass (g) of
235 that group at time t_1 (days).

236 To assess if growth associated with exercise is a result of muscle hypertrophy or
237 hyperplasia, samples of white muscle were collected at regular intervals following exercise and
238 throughout the growout period from each exercised group (2W, 3W, 4W) and non-exercised
239 controls from Cohorts 1 and 4 (sampling was only done for these cohorts due to limitations in the
240 number of fish available for growout and the time and labor-intensive process of sample
241 collection, processing, and analysis). At each sampling point, 3-5 randomly selected individuals
242 from each experimental group were sacrificed using an overdose of MS-222 and white muscle
243 tissue was sampled from the axial musculature immediately anterior and ventral to the first
244 dorsal spine. This location was chosen as a representative location on the fish fillet. Each sample
245 was fixed in 10% neutral buffered formalin and subsequently embedded in paraffin, sliced in
246 semi-thin cross-sections (5 μm), mounted on slides, and stained with hematoxylin and eosin.

247 White muscle fiber cross-sections were viewed and photographed using a light
248 microscope (Leica DM 2500 M, Leica Microsystems, Wetzlar, Germany) with attached digital
249 camera (Leica MC170 HD). Fiber cross-sectional area was measured for 35 muscle fibers per
250 fish using ImageJ software (National Institutes of Health, Bethesda, MD, USA; http://
251 rsb.info.nih.gov/ij/). The mean white muscle fiber cross-sectional area was then plotted against
252 the mass of each individual and a power law regression was used to calculate the scaling
253 exponent of muscle fiber cross-sectional area with growth. Because the randomly sampled

individuals for muscle collection were not necessarily representative of the mean of their entire group (2W, 3W, 4W, control), and because muscle fiber cross-sectional area was found to increase with size, the fiber cross-sectional area of each individual was scaled to the mean mass of its corresponding group. Mean fiber cross-sectional areas were then compared between groups at each sampling time point. In addition to determining mean fiber area, white muscle fibers with a cross-sectional area <1000 μm^2 were counted as potential newly recruited fibers representing hyperplasia.

2.6 Respirometry

In order to examine the effect of exercise on metabolic performance, oxygen consumption ($\dot{M}O_2$) was measured at three points during the post-exercise growout period in randomly selected individuals from each experimental group (2W, 3W, 4W, and control) in Cohort 1. These respirometry trials were conducted through incremental velocity tests using two Brett-style swim tunnel respirometers (Loligo Systems, Viborg, Denmark) at: Size A, within a week following completion of exercise when the mean size of all fish was 53.7 ± 25.2 g and 15.9 ± 2.4 cm BL; Size B when fish were 204.3 ± 53.8 g and 25.1 ± 2.0 cm BL; Size C, when fish were 409.1 ± 62.0 g and 31.9 ± 1.4 cm BL. Trials at Size A were conducted using a 5.4 L variable speed swim tunnel respirometer with a 30 x 7.5 x 7.5 cm working section, and trials at Size B and C were conducted using a 29.6 L respirometer with a 55 x 14 x 14 cm working section. Trials were run on eight individuals from each of the four experimental groups at each of the three time points (total n=96). Because of the time and labor-intensive nature of swim tunnel respirometry trials, and the number of replicates for each experimental group, these measurements could only be conducted on Cohort 1.

276 Respirometry trials were conducted according to methods of Wegner et al. (2018) and
277 Schwebel et al. (2018) for *S. dorsalis* of this size range. The swim tunnel respirometer was
278 submerged in a buffer tank supplied with aerated seawater at approximately 22.0 °C (consistent
279 with the rearing and exercise temperature), which was used to help maintain a stable temperature
280 and to flush the respirometer with fresh aerated seawater between $\dot{M}_{O_2}$ measurements. Prior to
281 each trial, water velocity within the swim tunnel was calibrated with a cylindrical vane wheel
282 flow meter probe (Höntzsch GmbH, Waiblingen, Germany). Each fish was fasted for
283 approximately 24 hours prior to placement in the swim tunnel respirometer. Once the fish was in
284 the respirometer and swimming steadily with a regular gait, it was acclimated for a minimum of
285 one hour at a preferred low flow speed (typically under 20 cm s^{-1} in the small respirometer and
286 under 40 cm s^{-1} in the large respirometer). Following acclimation, fish were made to swim
287 against a previously calibrated flow speed for approximately 30 minutes before the flow speed
288 was increased by 10 cm s^{-1} in a stepwise fashion for a minimum of eight speeds. Approximately
289 two minutes after an increase in speed, an inlet and outlet valve were manually closed to seal the
290 system from the surrounding buffer tank water to measure the dissolved oxygen level using a
291 Fibox 3 fiber optic oxygen transmitter and temperature probe (PreSens Precision Sensing GmbH,
292 Regensburg, Germany) which logged the oxygen saturation every five seconds using PreSens
293 software version PST3v602. The oxygen level within the closed swim tunnel respirometer was
294 allowed to be drawn down to ~80% saturation before the inlet and outlet valves were opened and
295 the system was manually flushed with fresh seawater from the surrounding buffer tank to restore
296 respirometer water to full oxygen saturation. If time allowed within the 30-minute swimming
297 speed step, the system was resealed and dissolved oxygen measurements from 100% to 80%
298 saturation were duplicated and the resulting traces were averaged to find the mean oxygen

consumption rate ($\dot{M}_{O_2}$) at that speed. After each swimming trial was concluded, the fish was removed, and the respirometer was resealed to measure background respiration, which was subtracted from the fish's calculated $\dot{M}_{O_2}$. Fish were then sacrificed using an overdose of MS-222 (800 mg L^{-1}) and measured (BL, FL, and M).

Following each trial, measured swimming speeds were corrected for the effect of the flow meter used for calibration using the continuity equation:

$$A_1V_1=A_2V_2 \quad (3)$$

Where A_1 is the cross-sectional area of the swim tunnel minus the cross-sectional area of the flow meter, V_1 is the velocity of the water within that area, A_2 is the cross-sectional area of the swim tunnel (without the flow meter), and V_2 is the velocity of the water without the flow meter (the variable we solved for). Additionally, we corrected for the size-specific blocking effect of each fish (according to Bell and Terhune, 1970). Mean water temperature for all swim tunnel respirometry trials was 22.1 ± 0.3 °C; however, temperature fluctuated slightly during each trial due to the process of temperature adjusting ambient seawater from the ocean. The mean minimum temperature reached during any $\dot{M}_{O_2}$ measurement was 20.8 °C and the maximum was 23.4 °C, so each $\dot{M}_{O_2}$ measurement was corrected to a temperature of 22.0 °C using $Q_{10} = 2$ (Pirozzi and Booth, 2009) in order to eliminate any affect of temperature within or between trials. In addition, to eliminate the effect of body mass on oxygen consumption rate for more accurate comparison between experimental groups at a given point, metabolic data for each fish were scaled to a common mean body mass of 55 g at Size A, 205 g at Size B, and 410 g at Size C using $\text{mass}^{0.80}$ (Brett and Groves, 1979; Chabot et al., 2016; Schwebel et al., 2018; Wegner et al., 2018) using the equation:

$$\dot{M}_{O_2(c)} = (\dot{M}_{O_2(i)} / M_{(i)}^{0.80})\, M_{(c)}^{0.80} \quad (4)$$

where $\dot{M}_{O_2(c)}$ is the corrected oxygen consumption rate ($mgO_2\ min^{-1}$) scaled to a common mass ($M_{(c)}$, either 55 g, 205 g, or 410 g), and $\dot{M}_{O_2(i)}$ is the originally calculated oxygen consumption rate for that individual ($mgO_2\ min^{-1}$) at its measured mass ($M_{(i)}$).

The size and temperature corrected relationship of oxygen consumption versus swimming speed for each fish typically resulted in a check-mark shaped curve (characteristic of *S. dorsalis* and many other pelagic fishes), in which higher oxygen consumption rates at low swimming speeds are thought to be associated with the increased energetic costs required to maintain hydrostatic equilibrium at low speeds (Schwebel et al., 2018; Sepulveda et al., 2003; Webb, 1998; Wegner et al., 2018). Data points from the low swimming speeds that were higher than the vertex of the curve were therefore removed for estimates of standard metabolic rate, which are made by extrapolating the linear regression of the $\dot{M}_{O_2}$-swimming speed relationship to a speed of 0 m/s (Schwebel et al., 2018; Sepulveda et al., 2003; Wegner et al., 2018).

2.7 Statistical Analysis

Somatic growth and specific growth rate data were compared between the four treatment groups within each cohort. Prior to determining statistical differences, data were filtered to remove fish that lost weight between measurements, as this was seen to be a result of behavioral interactions or mouth deformities that developed (occasionally observed at later growout stages) and not as a result of any exercise treatment. In addition, boxplots were constructed to find outliers in the mass data (± 1.5*IQR), which were removed from further analysis (Bayliff, 1988; Francis, 1988). Normality was evaluated using a Shapiro-Wilk test. For normally distributed data, a single-factor ANOVA was conducted, followed by a Tukey post-hoc test if $p \leq 0.05$, and

for non-normally distributed data a Kruskall-Wallis ANOVA was used followed by a Dunn's comparison test if $p \leq 0.05$. To evaluate mean muscle fiber area, a one-way ANOVA was used to determine significant differences between groups at each sampling point. The effect of exercise duration and cohort on the growth response to exercise was examined by comparing the percent difference in mass above the controls for each exercise group from each cohort using a 2-way ANOVA with exercise duration (2W, 3W, 4W) and cohort as variables.

In order to examine the effects of exercise on feed conversion ratio (FCR), data for each treatment group were combined for all five cohorts and plotted against mass for both the exercise and post-exercise periods. A bootstrap analysis was conducted, in which 10,000 linear regression replicates of the relationship between mass and FCR were created for exercised fish as compared to the non-exercised controls both during and post exercise. Statistical significance between groups was determined if less than 5% of resultant regression lines overlapped at a given value.

Scaled metabolic data were similarly compared using a bootstrap analysis in which 10,000 exponential regression replicates of the relationship between oxygen consumption and swimming speed were created from all individuals from each group (2W, 3W, 4W, control) at each size (A, B, C) (Schwebel et al., 2018). Each regression line was then extrapolated to a swimming speed of 0 cm s^{-1} and results for each group were averaged to estimate the standard metabolic rate. Statistical significance in SMR between groups was determined if less than 5% of resultant regression lines overlapped at a swimming speed of 0 m s^{-1}.

For all data, significance was determined if $p \leq 0.05$. Data are presented as the mean ± standard deviation, unless otherwise specified.

3. Results

365 *3.1 Growth and White Muscle Histology*

366 Somatic growth measures taken immediately post exercise showed an increase in body
367 mass for all exercise regimes in all five cohorts in comparison to the non-exercised controls
368 (Table 2); however, no significant differences were seen in mean BL or FL for any cohort ($p >$
369 0.05). When averaged across cohorts, the largest increase in body mass occurred within the 4W
370 exercise groups averaging 16.3 ± 11.4% larger than the controls immediately post-exercise,
371 followed by 10.4 ± 5.1% larger in the 3W exercise groups, and 8.3 ± 3.7% larger in the 2W
372 groups (Fig. 3). The magnitude of the difference in mass associated with exercise was highly
373 variable between cohorts and durations of exercise as seen in Table 2. A 2-way ANOVA
374 examining the percent difference in mass of all exercised fish above the controls showed that
375 both exercise duration ($p = 0.002$) and cohort ($p < 0.001$) had a significant effect on the positive
376 growth response. Specifically, the 4W exercise regime showed a significantly greater difference
377 in mass than the 2W ($p = 0.001$) and 3W regimes ($p = 0.017$). In addition, Cohort 1 showed a
378 significantly larger growth response than Cohort 2 ($p = 0.001$) and Cohort 3 ($p < 0.001$), and
379 Cohort 5 had a significantly greater difference in mass than Cohort 3 ($p = 0.005$) immediately
380 post exercise. The results of the 2-way ANOVA also showed a significant ($p = 0.019$) interaction
381 between the duration of exercise and the cohort. This suggests that the relationship between
382 exercise duration and the difference in mass is dependent on the cohort.

383 While the mean mass of all exercised groups was greater than the controls immediately
384 following exercise, there was a clear declining trend in this size advantage once fish were
385 removed from the exercise regime, indicating that the positive growth response was not retained
386 over time (Fig. 3). When averaged between cohorts, the 4W exercise groups retained their mass
387 advantage the longest post exercise, followed by the 3W and 2W groups (Fig. 3). However, the

388 duration or longevity of the mass advantage post exercise varied between cohorts, with all
389 exercise groups in Cohort 1 having significantly larger masses than the controls for several
390 weeks post-exercise, while two other cohorts showed no retention of the mass advantage in any
391 of the exercised groups.

392 The increased mass of the exercise groups resulted from significantly higher specific
393 growth rates (SGR) than the controls during and/or immediately following the exercise period.
394 For example, of the 12 exercise groups that could be evaluated statistically (i.e. Cohorts 2-5, in
395 which fish were individually PIT tagged), eight showed significantly higher SGRs during the
396 exercise period (Table 2) and four groups showed significantly elevated SGRs for 1-2 weeks post
397 exercise ($p < 0.05$). However, the observed increase in SGR ended shortly after the fish were
398 removed from the exercise stimulus. Beyond two weeks post exercise, the SGRs of the exercise
399 groups were either no longer significantly different than the control or had variable differences
400 throughout the remainder of the growout period with no clear pattern.

401 Both of the cohorts that were sampled for white-muscle tissue showed larger mean white
402 muscle fiber cross-sectional areas when measured immediately post exercise (the white muscle
403 fibers of the 4W swimming group were 20.0% larger than the controls for Cohort 1, and 23.2%
404 larger for Cohort 4) though the differences were not significant ($p > 0.05$). This increase in white
405 muscle fiber cross-sectional area appeared to diminish over time post-exercise for Cohort 4 (Fig.
406 4A) returning to control size, while Cohort 1 did not show a clear pattern and was highly variable
407 during the post exercise growout period (Fig. 4B). Very few small white muscle fibers (area
408 $<1000\ \mu m^2$) were observed, with no distinguishable pattern between exercise treatments and
409 control fish.

410 *3.2 Feed Conversion*

411 Feed conversion ratios (FCR) determined at the end of each exercise regime in Cohorts 2-
412 5 are shown in Table 2. During exercise, the mean FCR of the 4W groups across these cohorts
413 was on average 5.2 ± 2.6% greater than that of the controls, the 3W exercise group mean FCR
414 was 6.4 ± 1.7% larger, and the 2W group was 4.0 ± 5.4% larger. Figure 5 illustrates the
415 relationship between FCR and mean mass of pooled data for all exercise treatments in
416 comparison to the control fish during both the exercise period (Cohorts 2-5) and the post-
417 exercise growout period (all cohorts). The bootstrap analysis of the FCR-body mass relationship
418 during exercise (Fig. 5A) showed that exercised fish had significantly higher FCRs than the
419 controls when fish were 35 grams and larger, while during post-exercise growout (Fig. 5B), the
420 exercise groups showed significantly higher FCRs above 375 grams (less than 5% of bootstrap
421 regression lines overlapped at those points).

422 *3.3 Metabolic Data*

423 The standard metabolic rate (SMR) of randomly selected individuals from Cohort 1
424 estimated from oxygen consumption data are summarized in Table 3. Although not statistically
425 different ($p > 0.05$), the SMRs at Size A (fish mass scaled to 55 g) for 4W (4.78 ± 0.64 $mgO_2\,kg^{-1}\,min^{-1}$), 3W (4.96 ± 0.61 $mgO_2\,kg^{-1}\,min^{-1}$), and 2W (5.05 ± 0.83 $mgO_2\,kg^{-1}\,min^{-1}$) groups were
427 14.5%, 11.3%, and 9.6% lower than the control (5.59 ± 0.30 $mgO_2\,kg^{-1}\,min^{-1}$), respectively. At
428 Size B, when fish mass was scaled to 205 g, no significant differences were observed ($p > 0.05$),
429 and only the SMR of the 4W group remained lower (approx. 6.2%) than the control SMR (3.02 ±
430 0.24 $mgO_2\,kg^{-1}\,min^{-1}$ vs. 3.22 ± 0.38 $mgO_2\,kg^{-1}\,min^{-1}$). Finally, at Size C, when fish mass was
431 scaled to 410 g, the 2W group had a significantly higher SMR than the control group (3.36 ±
432 0.40 $mgO_2\,kg^{-1}\,min^{-1}$ vs. 2.00 ± 0.40 $mgO_2\,kg^{-1}\,min^{-1}$, $p < 0.001$).

4. Discussion

This study tracked several fitness metrics across five cohorts of *S. dorsalis* for up to 20 weeks post-exercise to better understand the duration of exercise needed to elicit a positive physiological response, as well as the variability and longevity in the observed response. We found that *S. dorsalis* that swam continuously for two, three, or four weeks at optimal speeds generally showed improved growth (increased mass) and slightly higher feed conversion ratios during exercise. Cohorts that were sampled for white muscle tissue (Cohorts 1 and 4) showed a general increase in mean fiber diameter when measured immediately post exercise, although the pattern and magnitude of the response was variable. Respirometry results for Cohort 1 demonstrated a trend toward increased metabolic efficiency (lower standard metabolic rates) immediately following exercise training, and although not statistically significant, these differences could have biological significance and warrants further investigation. The growth response to exercise appeared highly variable between cohorts and the observed differences in growth and in other metrics were not retained over time once fish were removed from the exercise stimulus. Generally, a longer exercise period improved both the magnitude and longevity of the enhanced fitness response. While numerous other studies have examined the effects of exercise on growth and feed conversion ratios, including other *Seriola* species, this study represents one of the first quantitative attempts to examine variability of these traits across multiple cohorts within a single species as well as the longevity of the response over an extended period following exercise.

The exercise-induced growth response measured in this study immediately following each exercise regime fell within the range of what has been observed in previous exercise studies of other *Seriola* species (Table 2), with exercised groups showing a 5.5% to 37.8% improvement

456 in growth (percent increase in mass) associated with a 4.0% to 12.0% increase in the specific
457 growth rate over the controls. It is important to note that the "growth" response associated with
458 fish exercise is often described using different terms in comparison to controls, including the
459 difference in overall mass, the difference in growth (difference in the increase in mass, as mostly
460 discussed in this manuscript), and the difference in specific growth rate. All three measures are
461 presented for comparison in Table 2 for this and previous *Seriola* exercise studies. Several other
462 species have demonstrated improved growth of a similar magnitude when measured immediately
463 post exercise training (see reviews by Davison, 1997; Jobling et al., 1993; and Zeng et al., 2017),
464 including Arctic Char (*Salvelinus alpinus* (Christiansen et al., 1989; Grünbaum et al., 2008)),
465 Atlantic Salmon (*Salmo salar* (Totland et al., 1987)), Brook Trout (*Salvelinus fontinalis* (Leon,
466 1986)), Gilthead Sea Bream (*Sparus aurata* L. (Blasco et al., 2015; Ibarz et al., 2011)), Striped
467 Bass (*Morone saxatilis* (Young and Cech Jr, 1993), and Qingbo (*Spinibarbus sinensis* (Li et al.,
468 2016)).

469 Variability in the exercise-induced growth response between studies has been primarily
470 explained by differences in experimental design or species-specific differences (active species
471 are more likely to show a positive growth response than less active species). In most cases, the
472 effect of exercise on growth appears closely tied to the exercise regime, including flow speed and
473 duration. For example, Palstra et al (2015) found the best exercise-induced growth response for
474 *S. lalandi* occurs at the optimal swimming speed (U_{opt}, which was used in all exercise treatments
475 in the current study and adjusted as fish grew), while other studies have used a variety of
476 constant flow speeds ranging from 0.75 – 4.00 BL s^{-1} (Brown et al., 2011, *Seriola lalandi*; Li et
477 al., 2016, *Spinibarbus sinensis*; Yogata and Oku, 2000, *Seriola quinqueradiata*). Similarly,
478 studies have ranged widely in the duration of exercise from 18 days (Palstra et al., 2015) up to 60

479 days (Young and Cech Jr, 1994, *Morone saxatilis*) with varied outcomes. The results of the
480 current study indicate that while shorter durations of exercise can have a positive physiological
481 effect, the magnitude of the response is generally positively correlated with exercise duration.

482 Previous exercise studies of *Seriola* have examined fish with an initial mass of 4.3 -
483 1591 g (Brown et al., 2011; Palstra et al., 2015; Yogata and Oku, 2000), so the cohorts examined
484 in this study (starting size: 4.35 - 39.60 g) were relatively small. Although the cohorts used in
485 these exercise trials had some variability in starting size, the best growth performance of each
486 cohort was near or slightly below reported values for similar sized fish (Yogata and Oku, 2000),
487 and below that previously observed in much larger *S. lalandi* exercised using a similar protocol
488 (46% increase in growth of 500 g fish; Palstra et al., 2015) (Table 2). Additionally, when
489 comparing the three cohorts that were most similar in size (Cohorts 1, 4, 5; starting mass range
490 4.35 – 5.39 g) the growth improvement in the 4W group varied from 12.1% to 37.8% (Table 2).
491 Thus, despite the similar starting size of these three cohorts, the exercise induced growth
492 response was highly variable. This suggests the likely influence of inherent differences in
493 individual fish and cohort fitness and potential phenotypic predisposition for an enhanced growth
494 response associated with exercise. While numerous experimental variables can affect the growth
495 response to exercise, this study shows that the variability in that response between cohorts is
496 similar in magnitude to that driven by other experimentally manipulated conditions. It is thus
497 critical to consider the impact that underlying variability in cohort fitness may have on the
498 magnitude and repeatability of the exercise response in order to refine best rearing practices for a
499 given species.

500 In addition to documenting the high variability in the magnitude of the growth response
501 between cohorts, this study is also novel in its attempt to thoroughly characterize the longevity of

this response once exercise has ceased. In general, the fish that exercised the longest (4 weeks) showed the longest retention of the mass increase (Fig. 3), although, like the magnitude, the longevity of the response was highly variable between cohorts. For example, all exercise treatments in Cohort 1 had significantly larger masses than the controls for several weeks post-exercise while two of the other cohorts showed no retention of the mass advantage in any of the exercised groups after being removed from exercise. Overall, the fish that swam for four weeks showed the greatest longevity of the growth response which varied from 0 to 16 weeks (0-112 days), while the impact of three weeks of exercise training lasted 0-12 weeks (0-84 days), and, in most cohorts, there was no persistence of the growth advantage after only two weeks of exercise. The only other study known to the authors to examine the longevity of a growth response to exercise, showed improvements lasting at least 56 days post-exercise (measurements were not conducted beyond that point) in young-of-the-year Striped Bass, *Morone saxatilis,* that were exercised for 60 days (Young and Cech, 1994). Other exercise studies have typically treated the end of the exercise regime as the endpoint of the study. Understanding the longevity of a positive growth response is helpful in determining the potential timing of exercise in the aquaculture rearing process. Specifically, the results of the current study indicate that exercise may only be effective in increasing fish mass immediately pre-harvest, as the growth benefits of exercise appear to diminish fairly quickly post exercise.

In the context of aquaculture growout, the growth of the fast-twitch (white) axial musculature is of particular importance as it comprises the most valuable end product, the fillet. Since no significant increase in body length was seen in any of the *S. dorsalis* cohorts examined in this study, the observed exercise-induced growth (increase in mass) appears associated with "bulking up" of the muscle during exercise. For the cohorts sampled (Cohorts 1 and 4),

525 hypertrophy was observed through an increase in mean white-muscle fiber cross-sectional area,
526 with the exercised fish showing upwards of 20% larger white muscle fibers than the controls
527 upon completion of the exercise regime. Several studies have observed similar white muscle
528 growth in response to sustained exercise training (Ibarz et al., 2011; Totland et al., 1987; Young
529 and Cech, 1993). For Cohort 4, these hypertrophic white muscle fibers appeared to reduce in size
530 once removed from the exercise stimulus (Fig. 4A), which appears to correlate with the reduction
531 in the exercise-induced growth response over time (Fig. 3). However, for Cohort 1, white muscle
532 fiber diameter showed high variability post exercise (Fig 4B). For both cohorts, no trends were
533 apparent in the presence of small muscle fibers (area <1000 μm^2), suggesting the observed
534 growth response was not a result of white muscle hyperplasia. Although these results provide
535 some evidence to support the idea that these Cohorts "bulked-up" during exercise, additional
536 sampling of exercised individuals and at different locations on the fish fillet are needed to get a
537 better idea of the magnitude and variability of this response across cohorts. In order to better
538 understand the dynamics leading to white muscle growth in response to exercise, future studies
539 could also examine protein synthesis in muscle development during and post exercise.
540 Techniques such as measurement of protein synthesis using deuterium oxide can potentially
541 further our understanding of this process over longer time frames (Gasier et al., 2009).

542 In addition to improved growth, exercise also showed potential to enhance metabolic
543 efficiency in this species. Although not significantly different, the lower standard metabolic rates
544 of the 4W, 3W, and 2W groups in Cohort 1 (14.5%, 11.3%, and 9.6% lower respectively)
545 measured immediately post-exercise in comparison to the controls may have contributed to the
546 exercise enhanced growth seen in this cohort. Specifically, having reduced energy needs for
547 basic metabolic functions (i.e., a lower standard metabolic rate) can free up energy for other

functions such as growth (Fry, 1947; Warren and Davis, 1967). Similar results were seen in exercised *S. lalandi* from Brown et al. (2011) that had approximately 7% lower standard metabolic rates than non-exercised controls, which mirrored a growth improvement of similar magnitude (10%). These findings are also consistent with previous work on *S. dorsalis* in which wild-captured yellowtail (which presumably had more exercise stimulation in the wild to avoid predators, find prey, and swim against currents) had lower standard metabolic rates than aquaculture-reared conspecifics (Schwebel et al 2018, Wegner et al 2018). Consistent with the pattern seen in the growth and muscle data, the increase in metabolic efficiency seen for the exercised fish in Cohort 1 was not retained over time post exercise. This parallels the trend of decreasing metabolic fitness over time observed for wild-caught fish retained in captivity in Schwebel et al. (2018), presumably as wild-caught fish were removed from natural exercise upon arrival and continued growout in a less-active captive environment. Further examination of this metric across additional cohorts would provide insight on the repeatability and variability of this response. Future studies should also examine if enhanced metabolic fitness can be maintained for a longer duration through sustained or periodic exercise, which may have a larger and more prolonged effect on growth and other fitness metrics over time.

Despite the potential for exercise to increase metabolic efficiency, the feed conversion ratio of 11 out of 12 exercised groups in this study was slightly higher (lower feed conversion efficiency) compared to controls during the exercise period (Table 2). This likely represents the increased energy required during exercise. Previous work has shown similar limited or negative impacts to FCR during sustained exercise for several species (Castro et al., 2011; Jørgensen and Jobling, 1993; Li et al., 2013; Totland et al., 1987), while other studies have shown improved FCRs (Herbert et al., 2011; Ibarz et al., 2011; Palstra et al., 2015; Yogata and Oku, 2000).

571 Studies involving *Seriola*, have shown large variability in the FCRs of exercised fish, from 0.62
572 (Yogata et al., 2000) to 2.18 (Brown et al., 2011) with the FCRs of the exercised fish in the
573 current study (0.67-0.92) falling within that range. In those same studies, changes in FCR of
574 exercised fish as compared to a control, have varied greatly (Table 2). FCR is a difficult metric
575 to directly compare between studies because it is largely species specific, and even within
576 species, is influenced by numerous factors including fish size (as seen in Figure 5), temperature
577 (Brown et al., 2011), feeding regime (Leon, 1986), and diet (Moran et al., 2009). For example,
578 Árnason et al. (2009) found that in general, feed conversion ratio was lower (more efficient) for
579 smaller sized Turbot (*Schophthalmus maximus*) at a given temperature than larger Turbot, and
580 also that the optimal temperature for a low FCR changed with fish size. Furthermore, they found
581 that optimal temperature for FCR was different than the optimal temperature for growth, and the
582 relationship of these two variables changed with fish size. In contrast, Handeland et al (2008)
583 found that as Atlantic Salmon grew, feed conversion efficiency generally increased, meaning the
584 fish became more efficient at larger sizes, although the magnitude of the response was also
585 related to temperature. It's clear that FCR is dependent on complex interactions between the fish
586 and its environment. The current study indicates that for *S. dorsalis* FCR may be somewhat
587 negatively affected by exercise; however, the actual impact of this difference on production in an
588 applied setting remains to be determined.

589 **5. Conclusions**

590 This study showed that exercise training generally has a positive effect on *S. dorsalis*
591 growth likely associated with the hypertrophy of white muscle fibers, and that the magnitude and
592 longevity of this response generally increases with exercise duration. However, both the
593 magnitude and longevity of this positive response to exercise were highly variable across

cohorts, and this variability was similar in extent to that of other experimentally manipulated variables in this and previous studies. This suggests that future work should focus not only on the interplay between the multiple variables involved in crafting an optimal exercise regime (such as duration and intensity of exercise, temperature, fish size / life stage, diet, and feeding regime), but also the potential genetic and phenotypic predisposition of specific individuals and cohorts to an enhanced growth response. In addition, this study highlights that the benefits of sustained exercise training for 2, 3, or 4 weeks are not permanent, suggesting that the timing, duration, and intensity of the exercise in relation to harvest, should be an important consideration for application in industry.

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References

Árnason, T., Björnsson, B., Steinarsson, A., Oddgeirsson, M., 2009. Effects of temperature and body weight on growth rate and feed conversion ratio in turbot (*Scophthalmus maximus*). Aquaculture 295, 218-225.

Bagatto, B., Pelster, B., Burggren, W.W., 2001. Growth and metabolism of larval zebrafish: effects of swim training. J. of Exp. Biol. 204, 4335-4343.

615 Bayliff, W.H., 1988. Growth of Skipjack, *Katsuwonus pelamis,* and Yellowfin *Thunnus*
616 *albacares,* Tunas in the Eastern Pacific Ocean, as Estimated From Tagging Data. Inter-
617 American Tropical Tuna Commission Bulletin.
618 Bell, W.H., Terhune, L.D.B., 1970. Water tunnel design for fisheries research. Fisheries
619 Research Board of Canada Technical Report No, pp. 195 (69 pp).
620 Blasco, J., Moya, A., Millán-Cubillo, A., Vélez, E.J., Capilla, E., Pérez-Sánchez, J., Gutiérrez, J.,
621 Fernández-Borrás, J., 2015. Growth-promoting effects of sustained swimming in
622 fingerlings of gilthead sea bream (*Sparus aurata* L.) J. Comp. Physiol. 185B, 859-868.
623 Brett, J.R., and Groves, T.D.D., 1979. Physiological energetics. In: Hoar, W. S., Randall, D. J.,
624 Brett, J. R. (Eds.), Fish Physiology. Bioenergetics and Growth Vol. 8. Academic Press,
625 New York, pp. 279-352.
626 Brown, E.J., Bruce, M., Pether, S., Herbert, N.A., 2011. Do swimming fish always grow fast?
627 Investigating the magnitude and physiological basis of exercise-induced growth in
628 juvenile New Zealand yellowtail kingfish, *Seriola lalandi*. Fish Physiol. Biochem. 37,
629 327-336.
630 Castro, V., Grisdale-Helland, B., Helland, S.J., Kristensen, T., Jørgensen, S.M., Helgerud, J.,
631 Claireaux, G., Farrell, A.P., Krasnov, A., Takle, H., 2011. Aerobic training stimulates
632 growth and promotes disease resistance in Atlantic salmon (*Salmo salar*). Comp.
633 Biochem. Physiol. 160A, 278-290.
634 Chabot, D., Steffensen, J. F., Farrell, A. P., 2016. The determination of standard metabolic rate
635 in fishes. Journal of Fish Biology 88, 81-121.

636 Christiansen, J.S., Ringø, E., Jobling, M., 1989. Effects of sustained exercise on growth and
637 body composition of first-feeding fry of Arctic charr, *Salvelinus alpinus* (L.).
638 Aquaculture 79, 329-335.
639 Christiansen, J.S., Svendsen, Y.S., Jobling, M., 1992. The combined effects of stocking density
640 and sustained exercise on the behaviour, food intake, and growth of juvenile Arctic charr
641 (*Salvelinus alpinus* L.). Can. J. Zool. 70, 115-122.
642 Davison, W., 1997. The effects of exercise training on teleost fish, a review of recent literature.
643 Comp. Biochem. Physiol. 117A, 67-75.
644 Davison, W., Goldspink, G., 1977. The effect of prolonged exercise on the lateral musculature of
645 the brown trout (*Salmo trutta*). J. Exp. Biol. 70, 1-12.
646 Davison, W., Herbert, N.A., 2013. Swimming-enhanced growth. Swimming Physiology of Fish
647 (pp. 177-202): Springer.
648 East, P., Magnan, P., 1987. The effect of locomotor activity on the growth of brook charr,
649 *Salvelinus fontinalis* Mitchill. Can. J. Zool. 65, 843-846.
650 Fernández-Montero, A., Torrecillas, S., Tort, L., Ginés, R., Acosta, F., Izquierdo, M. S.,
651 Montero, D., 2020. Stress response and skin mucus production of greater amberjack
652 (*Seriola dumerili*) under different rearing conditions. Aquaculture 520, 735005.
653 Francis, R.I.C.C., 1988. Maximum likelihood estimation of growth and growth variability from
654 tagging data. New Zeal. J. Mar. Fresh. 22, 22-51.
655 Fry, F.E.J., 1947. Effects of the environment on animal activity. Univ. of Toronto Press.
656 Gasier, H.G., Previs, S.F., Pohlenz, C., Fluckey, J.D., Gatlin, D.M., 3rd, and Buentello, J.A.
657 2009. A novel approach for assessing protein synthesis in channel catfish, *Ictalurus*
658 *punctatus*. Comp. Biochem. Physiol. 154B, 235-238.

659 Grünbaum, T., Cloutier, R., Le François, N.R., 2008. Positive effects of exposure to increased
660 water velocity on growth of newly hatched Arctic charr, *Salvelinus alpinus.* L. Aquac.
661 Res. 39, 106-110.
662 Handeland, S.O., Imsland, A.K., Stefansson, S.O., 2008. The effect of temperature and fish size
663 on growth, feed intake, food conversion efficiency and stomach evacuation rate of
664 Atlantic salmon post-smolts. Aquaculture 283, 36-42.
665 Herbert, N.A., Kadri, S., Huntingford, F.A., 2011. A moving light stimulus elicits a sustained
666 swimming response in farmed Atlantic salmon, *Salmo salar* L. Fish Physiol. Biochem.
667 37, 317-325.
668 Ibarz, A., Felip, O., Fernández-Borràs, J., Martín-Pérez, M., Blasco, J., Torrella, J.R., 2011.
669 Sustained swimming improves muscle growth and cellularity in gilthead sea bream. J.
670 Comp. Physiol. 181B, 209-217.
671 Jobling, M., Baardvik, B.M., Christiansen, J.S., Jørgensen, E.H., 1993. The effects of prolonged
672 exercise training on growth performance and production parameters in fish. Aquaculture
673 International 1, 95-111.
674 Jørgensen, E.H., Jobling, M., 1993. The effects of exercise on growth, food utilisation and
675 osmoregulatory capacity of juvenile Atlantic salmon, *Salmo salar*. Aquaculture 116, 233-
676 246.
677 Kolkovski, S., Sakakura, Y., 2004. Yellowtail kingfish, from larvae to mature fish – problems
678 and opportunities. In: Cruz Suárez, L.E., Ricque Marie, D., Nieto López, M.G.,
679 Villarreal, D., Scholz, U. y González, M. Avances en Nutrición Acuícola VII. Memorias
680 del VII Simposium Internacional de Nutrición Acuícola.16-19 November, 2004.
681 Hermosillo, Sonora, México.

682 Leon, K.A., 1986. Effect of exercise on feed consumption, growth, food conversion, and stamina
683 of brook trout. The Prog. Fish-Cult. 48, 43-46.
684 Li, X.-M., Yu, L.-J., Wang, C., Zeng, L.-Q., Cao, Z.-D., Fu, S.-J., Zhang, Y.-G., 2013. The effect
685 of aerobic exercise training on growth performance, digestive enzyme activities and
686 postprandial metabolic response in juvenile qingbo (*Spinibarbus sinensis*). Comp.
687 Biochem. Physiol. 166A, 8-16.
688 Li, X.-M., Yuan, J.-M., Fu, S.-J., Zhang, Y.-G., 2016. The effect of sustained swimming exercise
689 on the growth performance, muscle cellularity and flesh quality of juvenile qingbo
690 (*Spinibarbus sinensis*). Aquaculture 465, 287-295.
691 Moran, D., Pether, S.J., Lee, P.S., 2009. Growth, feed conversion and faecal discharge of
692 yellowtail kingfish (*Seriola lalandi*) fed three commercial diets. New Zeal. J. Mar. Fresh.
693 43, 917-927.
694 Moran, D., Smith, C.K., Gara, B., Poortenaar, C.W., 2007. Reproductive behaviour and early
695 development in yellowtail kingfish *(Seriola lalandi* Valenciennes 1833). Aquaculture
696 262, 95-104.
697 Nakada, M., 2008. Capture-based aquaculture of yellowtail. In: Lovatelli, A., Holthus, P.F.
698 (Eds.), Capture-based aquaculture. Global overview. FAO Fisheries Technical Paper. No.
699 508. Rome, FAO. pp. 199–215.
700 Palstra, A.P., Mes, D., Kusters, K., Roques, J.A., Flik, G., Kloet, K., Blonk, R.J.W., 2015.
701 Forced sustained swimming exercise at optimal speed enhances growth of juvenile
702 yellowtail kingfish (*Seriola lalandi*). Front. Physiol. 5, 506.
703 Palstra, A.P., Planas, J.V., 2011. Fish under exercise. Fish Physiol. Biochem. 37, 259-272.

Parish, G.R., Rowbotham, I., Mendoza, N.E., Norton, A.G., Klinger, D.H., Dale, J.D., Hopkins, K.D., Garcia-Ortega, A., Block, B.A., Farwell, C., Gardner, L.D., 2020. Energetic savings when switching from a whole-fish type diet to a commercial pelleted diet in California yellowtail (*Seriola dorsalis*). Aquaculture 516, 734496.

Pirozzi, I., Booth, M.A., 2009. The routine metabolic rate of mulloway (*Argyrosomus japonicus*: Sciaenidae) and yellowtail kingfish (*Seriola lalandi*: Carangidae) acclimated to six different temperatures. Comp. Biochem. Physiol. 152A, 586-592.

Schwebel, L.N., Stuart, K., Lowery, M.S., Wegner, N.C., 2018. Swim bladder inflation failure affects energy allocation, growth, and feed conversion of California Yellowtail (*Seriola dorsalis*) in aquaculture. Aquaculture 497, 117-124.

Sepulveda, C.A., Dickson, K.A., Graham, J.B., 2003. Swimming performance studies on the eastern Pacific bonito *Sarda chiliensis*, a close relative of the tunas (family Scombridae) I. Energetics. J. Exp. Biol. 206, 2739-2748.

Skov, P.V., Larsen, B.K., Frisk, M., Jokumsen, A., 2011. Effects of rearing density and water current on the respiratory physiology and haematology in rainbow trout, *Oncorhynchus mykiss* at high temperature. Aquaculture 319, 446-452.

Stuart, K.R., Drawbridge, M.A., 2013. Captive spawning and larval rearing of California yellowtail (*Seriola lalandi*). Aquac. Res. 44, 728-737.

Totland, G.K., Kryvi, H., Jødestøl, K.A., Christiansen, E.N., Tangerås, A., Slinde, E., 1987. Growth and composition of the swimming muscle of adult Atlantic salmon (*Salmo salar* L.) during long-term sustained swimming. Aquaculture 66, 299-313.

Warren, C.E., Davis, G.E., 1967. Laboratory studies on the feeding, bioenergetics, and growth of fish. Agricultural Experiment Station, Oregon State University, Corvalis, OR.

727 Webb, P.W., 1998. Swimming. In: Evans, D.H. (Ed.), The Physiology of Fishes, Second Edition.
728 CRC Press, Boca Raton, pp. 3-24.
729 Wegner, N.C., Drawbridge, M.A., Hyde, J.R., 2018. Reduced swimming and metabolic fitness of
730 aquaculture-reared California Yellowtail (*Seriola dorsalis*) in comparison to wild-caught
731 conspecifics. Aquaculture 486, 51-56.
732 Yang, S.G., Hur, S.W., Ji, S.C., Lim, S.G., Kim, B.S., Jeong, M., Lee, C.H., Lee, Y.-D., 2016.
733 Morphological development of embryo, larvae and juvenile in yellowtail kingfish,
734 *Seriola lalandi*. Dev. Reprod. 20, 131-140.
735 Yogata, H., Oku, H., 2000. The effects of swimming exercise on growth and whole-body protein
736 and fat contents of fed and unfed fingerling yellowtail. Fish. Sci. 66, 1100-1105.
737 Young, P.S., Cech Jr., J.J., 1993. Improved growth, swimming performance, and muscular
738 development in exercise-conditioned young-of-the-year striped bass (*Morone saxatilis*).
739 Can. J. Fish. Aqua. Sci. 50, 703-707.
740 Young, P.S., Cech Jr., J.J., 1994. Optimum exercise conditioning velocity for growth, muscular
741 development, and swimming performance in young-of-the-year striped bass (*Morone*
742 *saxatilis*). Can. J. Fish. Aqua. Sci. 51, 1519-1527.
743 Zeng, P., Lin, X., Li, L., Tu, Q., Xu, Z., 2017. Physiological mechanism and application of fish
744 exercise training in aquaculture. J. Fish. Livest. Prod. 5, 257.

Figure 1. (A) Schematic of custom-built dual raceway unit used for sustained exercise-training trials on the California Yellowtail, *Seriola dorsalis* (two of these units were built for a total of four raceways, each with its own individual sump, motor, working section, filtration, and water input). **(B)** Side view of the working section of one raceway showing yellowtail fingerlings swimming at their optimal swimming speed during exercise training.

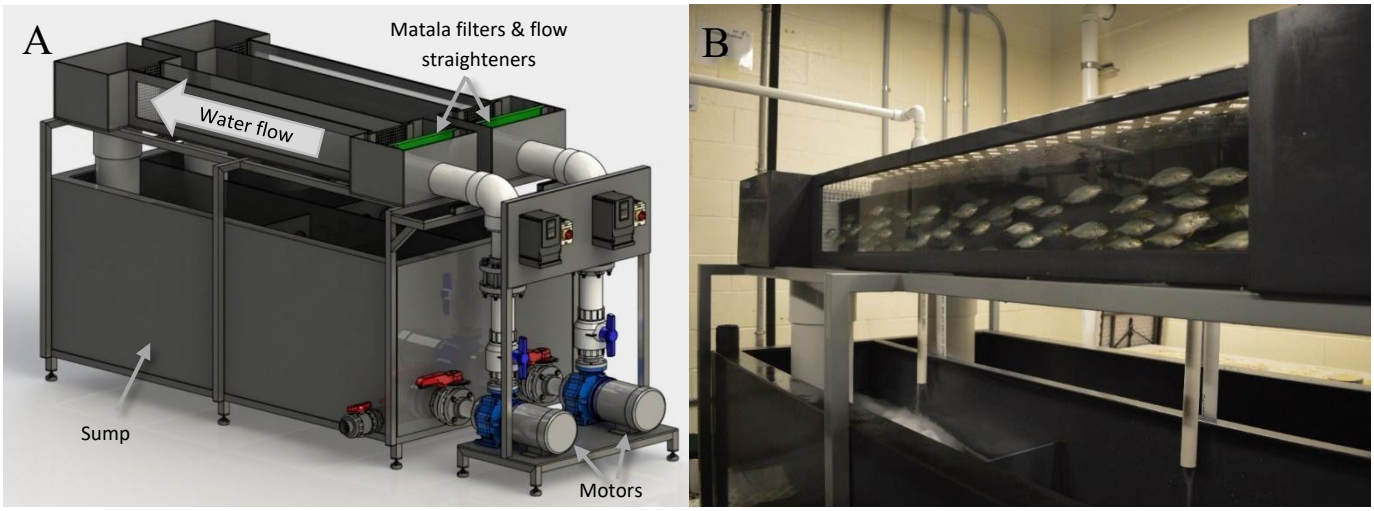

Figure 2. Schematic showing the treatment and measurement sampling of experimental fish during the exercise protocol using Cohort 3 as an example. Fish were measured at the start of exercise and then divided between the raceways and a non-exercised control tank. Subsequent measurements and fish division occurred at the end of each exercise duration.

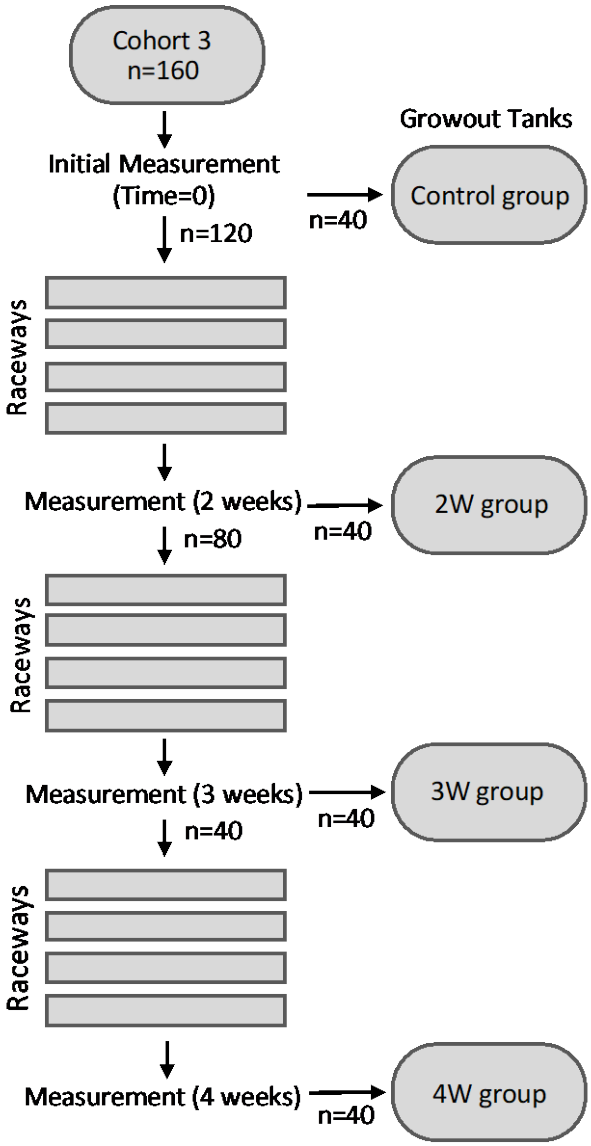

Table 1. Number of individuals (n) at the start of exercise training, parentage (broodstock origin), hatch date, mean stocking density of the raceways during the exercise period, initial body size, and metrics evaluated during and post exercise for each cohort of *S. dorsalis*.

Cohort	Initial *n*	Parentage	Hatch Date	Mean Stocking Density (kg/m^3)	Starting Mass (g)	Starting FL (cm)	Starting BL (cm)	Growth	Feed Conversion	Muscle Histology	Respirometry
Cohort 1	580	Wild	6/17/2016	3.76 ± 0.04	4.35 ± 1.27	6.39 ± 0.68	7.05 ± 0.71	✓	✓	✓	✓
Cohort 2	114	Wild	4/23/2017	1.55 ± 0.05	29.14 ± 8.56	12.65 ± 1.19	13.60 ± 1.30	✓	✓		
Cohort 3	160	F1	6/12/2017	2.01 ± 0.06	39.60 ± 5.37	13.94 ± 0.65	15.04 ± 0.68	✓	✓		
Cohort 4	150	Wild	5/27/2018	0.62 ± 0.02	5.39 ± 0.68	6.93 ± 0.31	7.71 ± 0.35	✓	✓	✓	
Cohort 5	101	Wild	7/12/2018	0.68 ± 0.03	4.47 ± 0.49	6.88 ± 0.26	7.50 ± 0.28	✓	✓		

Values are mean ± standard deviation. Note: Due to respirometry trials and muscle histology samples for Cohort 1, many more fish were removed and sacrificed throughout the trials, necessitating a larger starting number of fish.

Table 2. Morphometric, growth, and feed conversion ratio (FCR) data measured immediately following exercise for five cohorts of *S. dorsalis* in comparison to other recent exercise studies for other *Seriola* species.

Species	Duration (days)	Mean Swim Speed (BL s-1)	Temp (°C)	Initial Mass (g)	Initial FL (cm)	Initial TL (cm)	Final Mass (g)	Diff. in Mass from Controls (%)	Diff. in Growth from Controls (%)	Control SGR (%M day-1)	SGR (%M day^{-1})	Diff. in SGR from Controls (%)	Control FCR	Exercise FCR	Diff. in FCR from Controls (%)	Source
S. dorsalis (Cohort 1)	14	6.7 (U_{opt})	22.5 ± 0.5	4.35 ± 1.27	6.39 ± 0.68	7.05 ± 0.71	25.97 ± 5.82	9.4	11.5	11.3	11.9	5.3	-	-	-	Current Study
S. dorsalis (Cohort 1)	21	6.1 (U_{opt})	22.5 ± 0.5	4.35 ± 1.27	6.39 ± 0.68	7.05 ± 0.71	39.52 ± 9.73	**13.4**	15.3	9.9	10.5	6.0	-	-	-	Current Study
S. dorsalis (Cohort 1)	28	5.7 (U_{opt})	22.5 ± 0.5	4.35 ± 1.27	6.39 ± 0.68	7.05 ± 0.71	**69.81 ± 16.32**	**34.6**	37.8	8.9	9.9	12.0	-	-	-	Current Study
S. dorsalis (Cohort 2)	14	4.4 (U_{opt})	21.8 ± 0.8	29.14 ± 8.56	12.65 ± 1.19	13.6 ± 1.30	73.24 ± 17.03	7.1	13.2	6.2	**6.8**	9.3	0.86	0.88	2.3	Current Study
S. dorsalis (Cohort 2)	21	4.3 (U_{opt})	21.8 ± 0.8	29.14 ± 8.56	12.65 ± 1.19	13.6 ± 1.30	96.72 ± 20.61	7.0	9.6	5.4	5.7	6.0	0.85	0.91	7.1	Current Study
S. dorsalis (Cohort 2)	28	4.2 (U_{opt})	21.8 ± 0.8	29.14 ± 8.56	12.65 ± 1.19	13.6 ± 1.30	126.50 ± 22.70	10.0	**13.6**	5.0	**5.4**	8.0	0.86	0.92	7.0	Current Study
S. dorsalis (Cohort 3)	14	4.2 (U_{opt})	22.0 ± 0.7	39.60 ± 5.37	13.94 ± 0.65	15.04 ± 0.68	87.78 ± 11.40	2.3	5.5	5.4	5.7	5.0	0.79	0.85	7.6	Current Study
S. dorsalis (Cohort 3)	21	4.1 (U_{opt})	22.0 ± 0.7	39.60 ± 5.37	13.94 ± 0.65	15.04 ± 0.68	113.70 ± 13.95	3.6	6.0	4.9	5.1	4.2	0.84	0.89	6.0	Current Study
S. dorsalis (Cohort 3)	28	3.9 (U_{opt})	22.0 ± 0.7	39.60 ± 5.37	13.94 ± 0.65	15.04 ± 0.68	147.11 ± 18.51	5.4	9.8	4.5	4.7	4.0	0.85	0.90	5.9	Current Study
S. dorsalis (Cohort 4)	14	6.6 (U_{opt})	22.0 ± 0.7	5.39 ± 0.68	6.93 ± 0.31	7.71 ± 0.35	**26.35 ± 3.01**	**11.3**	**14.2**	10.6	**11.4**	7.8	0.67	0.73	9.0	Current Study
S. dorsalis (Cohort 4)	21	6.3 (U_{opt})	22.0 ± 0.7	5.39 ± 0.68	6.93 ± 0.31	7.71 ± 0.35	**41.11 ± 5.26**	**11.3**	**12.7**	9.2	**9.6**	5.2	0.72	0.78	8.3	Current Study
S. dorsalis (Cohort 4)	28	5.8 (U_{opt})	22.0 ± 0.7	5.39 ± 0.68	6.93 ± 0.31	7.71 ± 0.35	**61.36 ± 7.74**	**11.9**	**12.1**	8.2	**8.7**	5.7	0.74	0.79	6.8	Current Study
S. dorsalis (Cohort 5)	14	6.6 (U_{opt})	22.0 ± 0.2	4.47 ± 0.49	6.88 ± 0.26	7.50 ± 0.28	**24.29 ± 2.23**	**11.2**	**20.3**	11.3	**12.1**	7.5	0.69	0.67	-2.9	Current Study
S. dorsalis (Cohort 5)	21	6.2 (U_{opt})	22.0 ± 0.2	4.47 ± 0.49	6.88 ± 0.26	7.50 ± 0.28	**43.12 ± 3.40**	**16.6**	**18.5**	10.1	**10.7**	6.2	0.69	0.72	4.3	Current Study
S. dorsalis (Cohort 5)	28	5.8 (U_{opt})	22.0 ± 0.2	4.47 ± 0.49	6.88 ± 0.26	7.50 ± 0.28	**63.77 ± 4.97**	**19.5**	**16.2**	8.8	**9.5**	7.9	0.74	0.75	1.4	Current Study
S. quinqueradiata (HF)	28	1.00	22.0-24.6	4.30 ± 0.02	NR	7.3 ± 0.0^{+}	30.55 ± 1.72	34	41	6.0^{++}	7.0^{++}	15.9	1.41*	1.59*	-12.8	Yogata and Oku (2000)
S. quinqueradiata (HF)	28	2.25	22.0-24.6	4.37 ± 0.02	NR	7.3 ± 0.0^{+}	30.50 ± 1.09	34	40	6.0^{++}	6.9^{++}	15.1	1.41*	1.62*	-14.9	Yogata and Oku (2000)
S. quinqueradiata (LF)	28	1.00	22.0-24.6	4.31 ± 0.05	NR	7.3 ± 0.0^{+}	29.94 ± 0.66	19	24	6.2^{++}	6.9^{++}	11.1	1.47*	1.61*	-9.5	Yogata and Oku (2000)
S. lalandi	18	2.46 (U_{opt})	23.6 ± 0.1	504 ± 27	NR	34.6 ± 0.6	735 ± 23	11	46	1.5	2.1	40.0	1.77*	1.21*	31.6	Palstra et al. (2015)
S. lalandi	42	0.75	21.1 ± 0.03	1591 ± 6.6	47.60 ± 0.08	NR	NR	NR	NR	0.8	0.9	10.0	2.05	1.89	-7.8	Brown et al. (2011)
S. lalandi	42	1.5	21.1 ± 0.03	1591 ± 6.6	47.60 ± 0.08	NR	NR	NR	NR	0.8	0.9	7.5	2.05	1.93	-5.9	Brown et al. (2011)
S. lalandi	42	2.25	21.1 ± 0.03	1591 ± 6.6	47.60 ± 0.08	NR	NR	NR	NR	0.8	0.8	2.5	2.05	1.98	-3.4	Brown et al. (2011)
S. lalandi	49	0.75	14.9 ± 0.1	179 ± 4.6	23.66 ± 0.20	NR	NR	NR	NR	0.4	0.4	-4.8	2.39	2.18	-8.8	Brown et al. (2011)

FL, fork length; SGR, specific growth rate; TL, total length; U_{opt}, optimal swimming speed

* Both studies calculated the ratio of biomass gain/total food intake (which is the inverse of the calculation used in this study (dry feed intake/total weight gained)). Therefore the "Diff. in FCR from Controls" for these fish was calculated to reflect this difference in the underlying calculation.

+ reported as body length

++ Calculated using published data and the equation: $SGR = 100 * ((\ln(W_2) - \ln W_1)) / T)$

NR= not reported

Values are are mean ± standard deviation except for Brown et al. (2011) and Yogata and Oku (2000) which are mean ± SE.

Significant differnces of exercised groups from controls indicated in **bold** (NOTE: significance of SGR and Diff. in Growth from Controls could not be evaluated for Cohort 1 since fish were not individually tagged).

NOTE: For the current study, U_{opt} depends on the length of the fish. Each cohort started the trials at its own size-specific U_{opt}, which was then adjusted as fish grew throughout the duration of the exercise trial. The mean swimming speeds listed above are thus the mean U_{opt} that each group from each cohort experienced over the course of their swim trials.

Figure 3. Mean percent differences in mass (± standard error) of *S. dorsalis* exercised for different durations (2, 3, 4 weeks) in comparison to non-exercised controls over the growout period. Percent differences are the averaged means from all five cohorts.

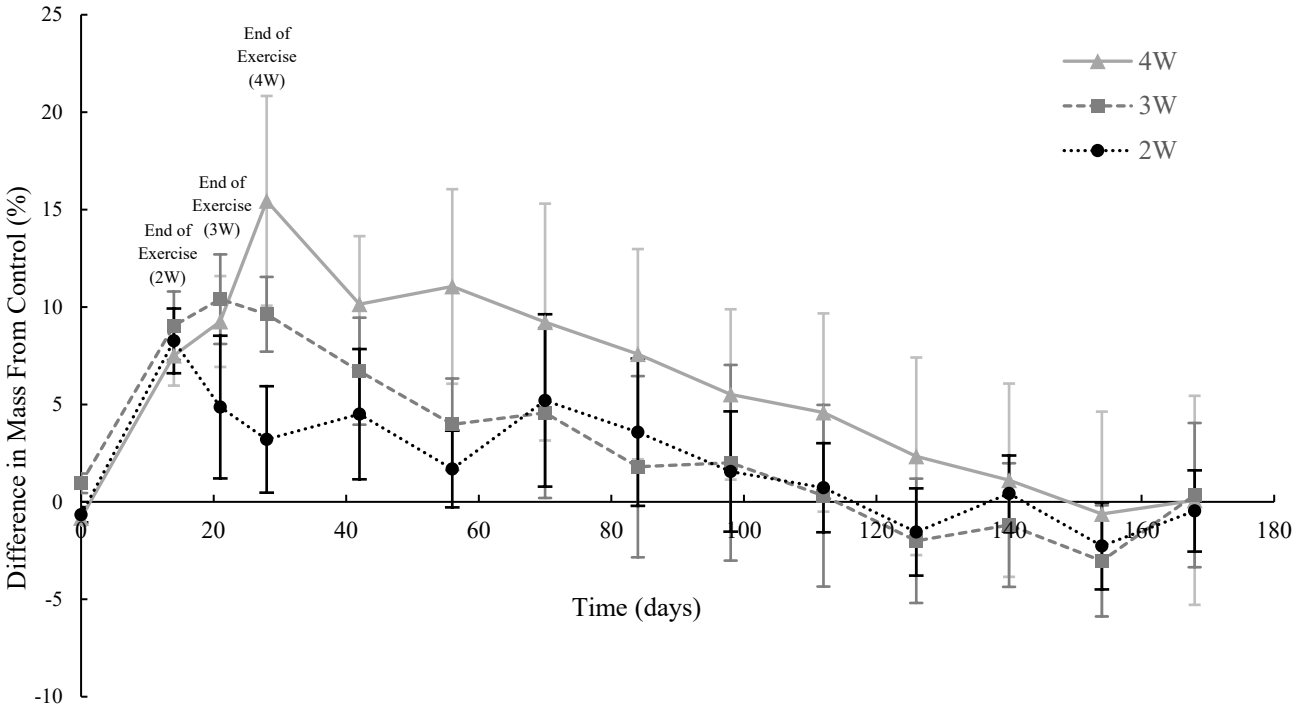

Figure 4. Percent difference in mean white muscle fiber area of *S. dorsalis* exercised for different durations (2,3,4 weeks) in comparison to a non-exercised control group for Cohort 4 (A) and Cohort 1 (B). Note: Samples were collected opportunistically based on fish and staff ability resulting in fewer sampling points for Cohort 4.

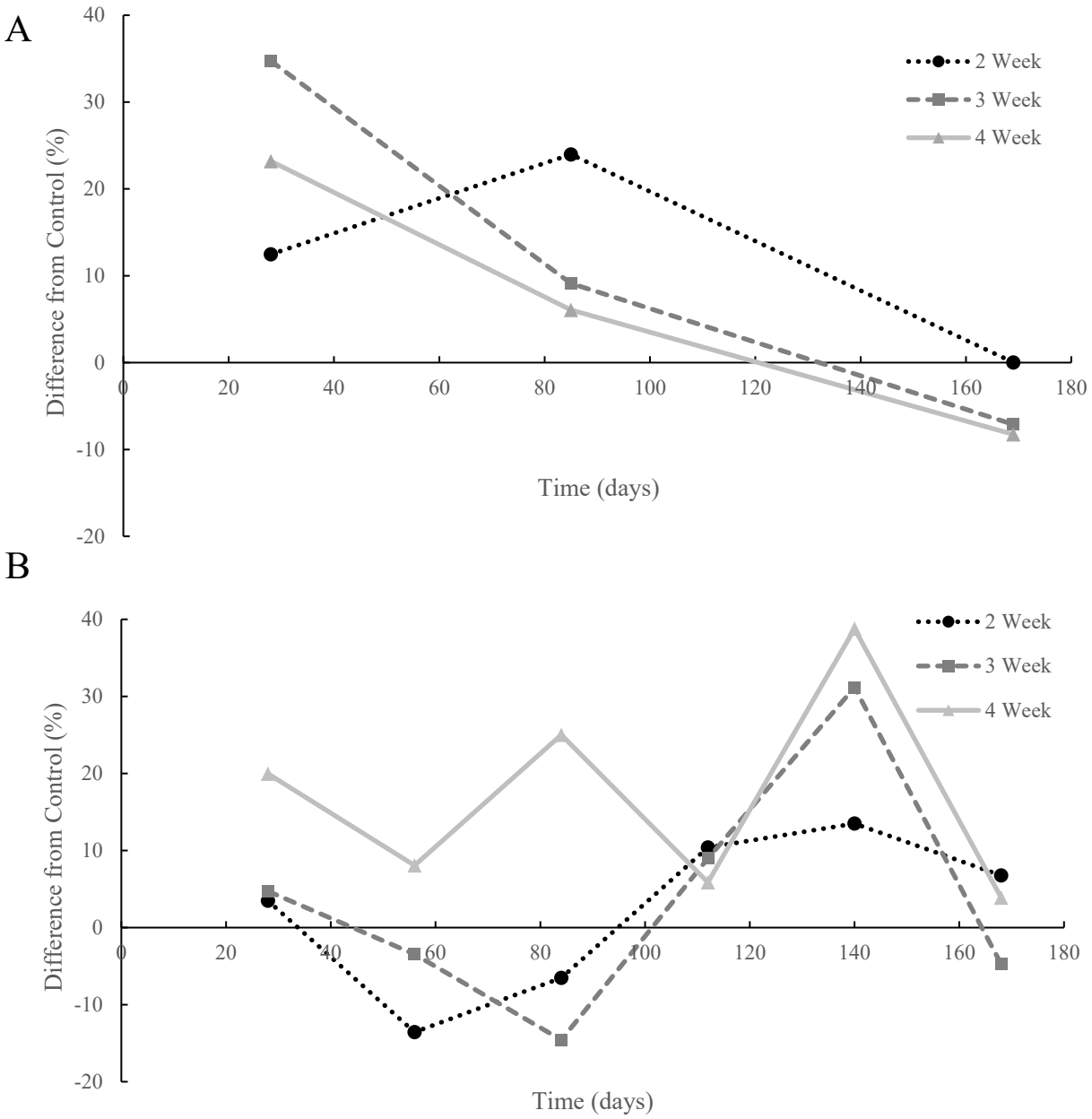

Figure 5. Relationship between feed conversion ratio and body mass for exercised *S. dorsalis* in comparison to non-exercised controls during the exercise period (A) and during growout post-exercise (B). (A) includes data from Cohorts 2-5 (feed consumption was not measured during exercise for Cohort 1), while (B) includes data for all five cohorts.

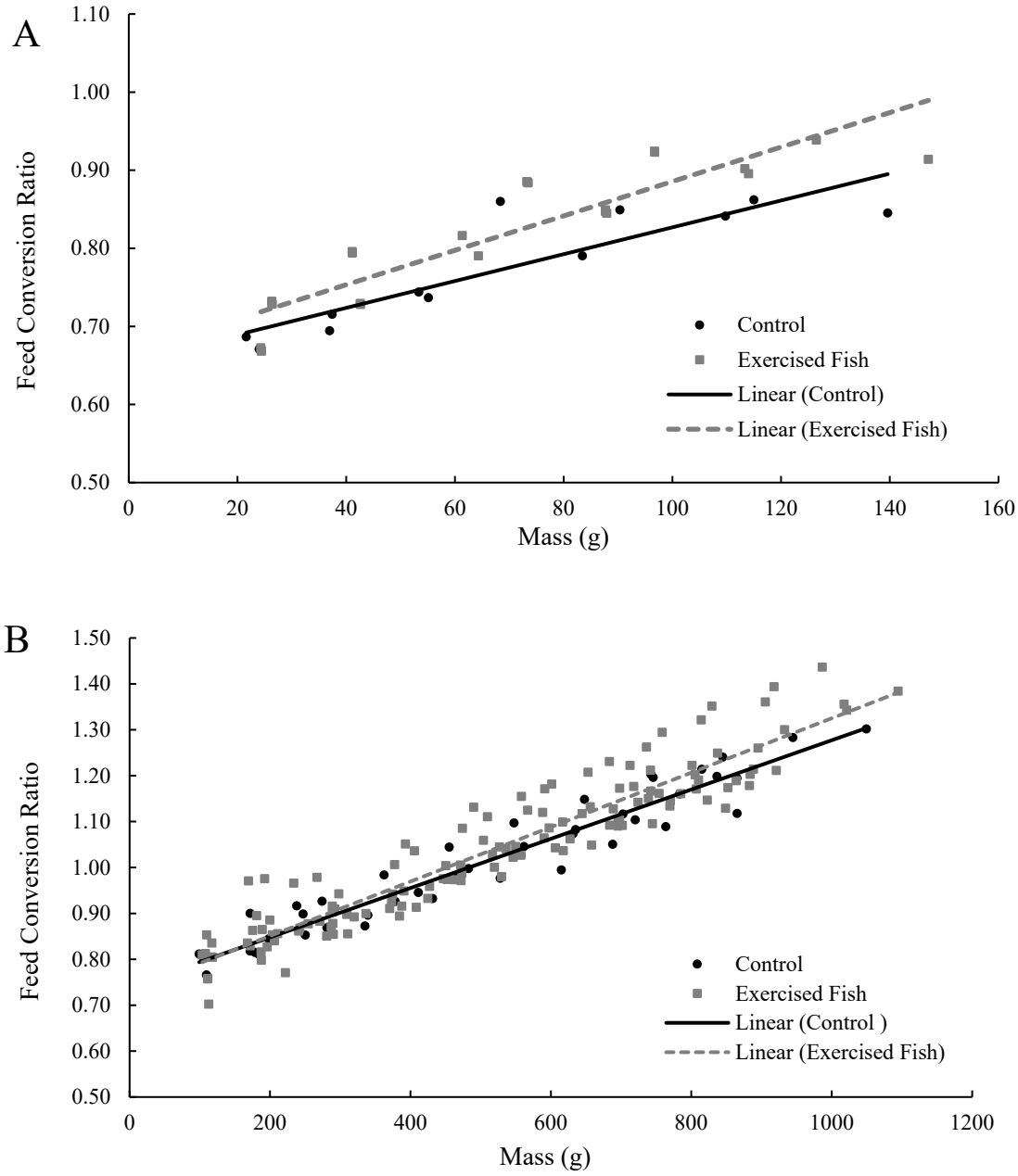

Table 3. Estimates of standard metabolic rate (mean ± standard deviation) for *S. dorsalis* subjected to continuous exercise (2W, 3W, 4W) compared to non-exercised controls at three different sizes during post exercise growout.

	SMR Size A ($mgO_2\ kg^{-1}\ min^{-1}$)	**SMR Size B (mgO2 kg-1 min-1)**	**SMR Size C (mgO2 kg-1 min-1)**
Control	5.59 ± 0.30	3.22 ± 0.38	2.00 ± 0.18
2W	5.05 ± 0.83	3.70 ± 0.40	**3.36 ± 0.40**
3W	4.96 ± 0.61	3.74 ± 0.29	2.02 ± 0.17
4W	4.78 ± 0.64	3.02 ± 0.24	2.13 ± 0.21

Data are from eight individuals randomly selected from each experimental group at each size. For direct comparison between groups, SMR data for individual fish were adjusted to a temperature of 22.0 °C using Q_{10}=2.0, and scaled to 55 g at Size A, 205 g at Size B, and 410 g at Size C using $mass^{0.80}$. Significant difference indicated in **bold**.