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JUVENILE COHO SALMON LOCOMOTION AND MOSAIC MUSCLE ARE MODIFIED BY 3′,3′,5′-TRI-IODO-L-THYRONINE (T_3)

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Summary

Studies of maximum aerobic swimming performance in smolting juvenile salmonids indicate that these animals may be aerobically compromised during downstream migration. To test our hypothesis that hyperthyroid status contributes to decreased swimming performance through modification of muscle contractility in juvenile (112 mm mean total length) coho salmon (*Oncorhynchus kisutch*), we measured swimming performance and isolated muscle bundle contractility of fish implanted with 3′,3′,5′-tri-iodo-L-thyronine (T_3) pellets, of fish implanted with sham pellets and of fish with no pellet implantation (control group). After 3 weeks (*N*=12–13), critical swimming speeds (maximum aerobic swimming speed or U_{crit}) were measured. Muscle bundles (*N*=15–16) were dissected from the hypaxial musculature and stimulated to measure the force and velocity of an isometric twitch and tetani. T_3-treated fish demonstrated visible morphological changes associated with smoltification. Mean values of U_{crit} were significantly decreased and the prolonged contraction (tetani) and twitch rates of contraction, relaxation and maximum force were significantly increased by T_3 treatment compared with both the sham and control fish. Hematocrit, body mass and body length were not significantly affected by T_3 treatment. In conclusion, we suggest that the reported decrease in U_{crit} during salmonid smoltification may be mediated by endogenous T_3-induced contractile modification of mosaic muscle fibers.

Key words: tri-iodo-L-thyronine, skeletal muscle, fish, mosaic muscle, coho salmon, *Oncorhynchus kisutch*, swimming performance, critical swimming speed, smoltification.

Introduction

During its parr–smolt transformation (smoltification), a young anadromous salmon typically migrates downstream to the ocean. Many physiological and morphological changes accompany this transformation, which originates while the fish is still in fresh water (for a review, see Hoar, 1988). While swimming performance, measured as critical swimming velocity (U_{crit}, cm s^{-1}, or the maximum swimming speed that can be maintained for a period greater than 15 min), typically increases with increasing fish length (Davidson, 1949; Bainbridge, 1958), the swimming performance of smolting salmonids generally plateaus or decreases with increasing length (Folmar and Dickhoff, 1980; Flagg and Smith, 1982; Castleberry et al., 1991; but see Peake and McKinley, 1997). These results indicate that many young salmon are aerobically compromised (possibly because of a decrease in blood oxygen-carrying capacity, cardiac contractility, venous return or metabolic scope) during smoltification.

Plasma thyroxine (T_4) levels surge for approximately 1–2 months in coho salmon during smoltification (Grau et al., 1981; Dickhoff and Sullivan, 1987). During this time, T_4 has wide-ranging effects on metabolism, morphology and development (Dickhoff and Sullivan, 1987; McCormick and Saunders, 1987). Most measurements of thyroid hormone levels in smolting fish have determined the plasma concentrations of T_4, rather than the more active, once-deiodinated, 3′,3′,5′-tri-iodo-L-thyronine (T_3). Deiodination occurs mainly at tissue-specific sites in response to the enzyme activity of 5′ deiodinase, and T_3 is actively transported into the cell, where it binds to a tissue-specific nuclear receptor. Thyroid hormone has various effects on muscle development, growth and contractility in amphibians, birds and mammals (Everts, 1996; Muscat et al., 1995; Sachs et al., 1997), including promotion of slow to fast isoform transitions (e.g. Ca^{2+}, Na^+/K^+ and K^+ transporters; for a review, see Everts, 1996) and modification of myosin heavy and light chains in skeletal muscle (Li et al., 1996). Little is known about the role of T_3 in the muscle or locomotory development of fish. Martinez et al. (Martinez et al., 1995) found an accelerated transition from neonatal to adult myosin heavy chain isoforms in the locomotory skeletal muscle of Arctic charr (*Salvelinus alpinus*) fed a T_3-enriched diet. However, the contractile implications of this transition were not studied.

Thyroid hormone is one of the major hormones contributing to the onset of smolt characteristics (Hoar, 1988). Because

plasma levels are sensitive to environmental fluctuations such as exposure to novel flow, photoperiod, temperature and salinity (Dickhoff et al., 1982a; Specker and Schreck, 1984; McCormick et al., 2000), the physiological development of smolt characteristics may be mediated by environmental variability during residence in fresh water. Artificial elevation of thyroid hormone levels in salmonid parr results in altered behavior, including a reduction in aggressive behavior, negative rheotaxis, a preference for open areas, a preference for sea water and searching behavior (for reviews, see Iwata, 1995; Iwata, 1996). Thyroid hormone has also been implicated in the acceleration of body silvering and changes in the shape of the head and body during smoltification (Winans and Nishioka, 1987). Variation in rearing habitats resulting in variation in the frequency, duration and magnitude of the peak in thyroid hormone levels (Grau et al., 1982; Dittman et al., 1994) could explain the conflicting results of previous studies on the maximum aerobic swimming performance of smolting salmonids (Castleberry et al., 1991; Peake and McKinley, 1997).

To investigate the role of thyroid hormone in locomotory development, we tested the hypothesis that T_3 contributes to decreased swimming performance in juvenile coho salmon (*Oncorhynchus kisutch*) through modification of muscle contractility.

Materials and methods

Fish origin and implantation

Fish *Oncorhynchus kisutch* (Walbaum) were obtained in June 1999 from the Iron Gate Hatchery on the Klamath River and transported to the University of California, Davis, fish-holding facility in oxygenated, insulated plastic containers. Fish were maintained in 121 cm diameter, 400 l tanks at 19 °C, outdoors, under natural (October–November 1999 photoperiod; 38°31′N, 121°30′W) light levels, with continuous flows of well water (pH 8.0, hardness 307 mg l^{-1}, dissolved O_2 >90 % air saturation) for 5 months. Fish were sorted at random into three treatment groups (T_3, sham and control) and assigned to one of three round 400 l tanks. Well water flows into each tank were adjusted to 162±3 ml s^{-1} (N=3) to maintain dissolved O_2 at greater than 90 % air saturation.

Groups of five fish were anesthetized in $NaHCO_3^-$-buffered MS222, weighed in a beaker of water (without displacing water) and measured to the nearest millimeter for standard length (snout to caudal peduncle, SL), fork length (snout to caudal fork, FL), and total length (snout to caudal fin tip, TL). The T_3- and sham-treated fish received a 5 mm long ventral incision, starting directly ventral to the tip of the posteriorly extended pectoral fin. The incision extended through the muscle but did not penetrate the peritoneal fascia. A T_3 or sham pellet (Innovative Research of America's matrix-driven delivery, MDD, pellet system, Sarasota, Florida, USA) was inserted into the incision. Light pressure was applied to push the pellet through the fascia and into the peritoneal cavity close to the gut. The slit was then sealed with a drop of cyanoacrylate glue, and the fish was marked with fin clips. Following surgery (mean duration 1 min), fish were placed in an aerated recovery container (3‰ NaCl in well water) until they regained equilibrium and started swimming. They were then transferred to one of the holding tanks, which ultimately held 16 sham, 16 control and 16 T_3-implanted fish.

Fish from all treatment groups were maintained in tanks for 3 weeks and hand fed *ad libitum* rations of soft moist salmon pellets (Rangen; 4 mm diameter). During the first week post-surgery, three of the sham fish died, apparently as a result of surgery-related problems. After the 3 week dose period, during which most of the T_3 in the pellet was released into the bloodstream at a mean concentration of 15 ng ml^{-1} (G. A. Nevitt, personal communication), fish were chosen at random for the swimming or muscle contraction experiments. Following each experiment, sham and T_3-treated fish were dissected to establish the presence of an implanted pellet.

Swimming performance

We used a Brett-type swimming apparatus (Brett, 1964) to measure the critical swimming speed (U_{crit}) of the juvenile coho salmon (N=12) over the 2 week period following the 3 week T_3 dose period (end date 19 October 1999). Swimming performance experiments started at 07:00 h and continued until approximately 17:00 h (after which the fish, regardless of treatment group, generally refused to swim). Fish were individually netted from the holding tank, moved 20 m indoors in a plastic, insulated container and placed into the polyvinylchloride and acrylic swimming apparatus (150 mm diameter test section). Each fish was acclimated for 30 min at 0.02 m s^{-1}, after which the water velocity was increased in 0.1 m s^{-1} increments every 20 min until the fish fatigued (impinged at the back of the chamber and refused, despite encouraging decreases in flow velocity, to continue swimming further at the fatigue speed). The maximum aerobic swimming speed (U_{crit}, in cm s^{-1}) was calculated using the equation (Brett, 1964):

$$U_{crit} = V_f + [(T/t)V] ,$$

where V_f is the final velocity at which the fish swam for the entire 20 min period, T is the time swum (min) at the final velocity, t is 20 min and V is the velocity increment (0.1 m s^{-1}). The swimming chamber was thoroughly rinsed with fresh well water after each experiment to prevent the exchange of chemical alarm signals (Berejikian et al., 1999; Poulin et al., 1999). No solid blocking correction was necessary because the cross-sectional area of the fish was always less than 10 % of the cross-sectional area of the swimming chamber (Jones et al., 1974).

Following the swimming performance experiment, the fish was weighed in a tared beaker of water (without displacing water), maintained in an aerated, temperature-regulated plastic container for 30 min, netted and quickly killed by a sharp blow to the head and pithing. Blood was collected *via* heparinized hematocrit tube following caudal severence (Korcock et al., 1988). Plasma was collected, and hematocrit was measured after tube centrifugation. Plasma samples were frozen

immediately at –80 °C and assayed for T_3 using a homogeneous enzyme immunoassay similar to that of Schall et al. (Schall et al., 1978), except that T_3 antibodies and conjugates were used (the structure of T_3 is identical amongst vertebrates; R. Ewing, personal communication) and the detection system used alkaline phosphatase rather than peroxidase. The ventricle of the fish was removed and weighed. Gill filaments were removed from the first two gill arches, placed in a homogenizing medium (Sigma Chemical Company, St Louis, MO, USA) containing 0.3 mol l^{-1} sucrose, 0.05 mol l^{-1} imidazole, 0.02 mol l^{-1} Na_2EDTA and 0.01 mol l^{-1} 2-mercaptoethanol, frozen at –80 °C and assayed for Na^+/K^+-ATPase activity (Johnson et al., 1977). Assays of ATPase activity and T_3 plasma content were performed by Biotech Inc. (Corvallis, OR, USA).

Muscle contractility

Under oxygenated, iced saline solution (concentrations in mmol l^{-1}: 124.06 NaCl, 3.08 KCl, 2.74 $NaHCO_3$, 0.93 $MgSO_4 \cdot 7H_2O$, 3.49 Tes acid and 6.37 Tes salt), a muscle fiber bundle (188±12 fibers, mean ± S.E.M., N=3 fish, in cross section, muscle fiber count analysis performed on a Macintosh computer using the public-domain NIH Image program developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/) was carefully dissected from the hypaxial musculature of the pithed fish (ventral to the dorsal fin and immediately dorsal to pelvic fin musculature). The muscle bundle was cut from the thinnest area in the abdominal wall (locomotory and postural muscle) and included both epidermal and abdominal epithelial tissue from the lining of the abdominal cavity. Attempts to remove the epithelial/connective tissues resulted in loss of contractility. Bundle length, width and height were measured under a dissecting microscope using a calibrated ocular micrometer. Because the myosepta of these small fish were delicate, it was necessary to hook the muscle to a force transducer through the contractile tissue. Great care was taken to hook the muscle bundles consistently among treatments and replicates. Hooked bundles consisted of three cells and were 2.74±0.04 mm long, 0.96±0.13 mm wide and 0.97±0.20 mm high (means ± S.E.M., N=16). The hooks were connected vertically to the transducer (either a Statham UC4 or a Grass force displacement FT03) and a static bar. The muscle bundle was submerged in a temperature-controlled, oxygenated saline solution. Muscle bundles were acclimated at 19 °C in a slack position for 1 h before progressive stretching to the length at which the greatest force was produced (L_{max}, the plateau region on the length/tension curve). During stretching, peak tension (using 7.5 ms supramaximal stimuli *via* platinum electrodes) was measured after the muscle had relaxed to a steady baseline tension level. The muscle was rested for 10 min following length adjustment and then stimulated to twitch, rested again for 10 min and stimulated at 166 Hz to obtain a fused (tetanic) contraction. Muscle force, delay (time from peak stimulus to peak tension) and rates of contraction and relaxation (df/dt, where f is force, g s^{-1} and t is time) were measured using a MacLab data-acquisition system (AD Instruments). The typical measurements of 50 % time to peak force/50 % time to relaxation are not included here because total peak force was altered with treatment. Following contraction, muscle bundle stretched length, wet mass and (after 24 h in a drying oven at 60 °C) dry mass were measured.

Statistical analyses

Treatment groups were compared using analysis of variance (ANOVA) and Tukey–Kramer HSD *post-hoc* tests for normally distributed data and Wilcoxon/Kruskal–Wallis rank sums test for non-normally distributed data. Statistical significance was accepted at P<0.05. Tetanic *versus* twitch data were compared with using Student's t-tests. All statistical analysis was facilitated by JMP IN (student version 3.2, SAS) statistical software for the Macintosh. Values are presented as means ± S.E.M.

Results

Morphological and physiological measurements

Hematocrit data were arcsine-transformed to achieve a normal distribution. Fish mass, standard, fork and total lengths, heart mass/body mass and hematocrit did not differ significantly among treatment groups (ANOVA, P>0.05; Table 1). The T_3-treated fish had significantly greater (ANOVA/Tukey–Kramer HSD, P=0.02) plasma T_3 levels (10±1.3 ng ml^{-1}, N=29) than those of the control (5±1.3 ng ml^{-1}, N=27) or the sham (6±1.3 ng ml^{-1}, N=27) groups, but this difference was probably due to elevated T_3

Table 1. *Mean values of morphological and physiological variables for juvenile coho salmon pooled from both experiments and all three treatment groups*

	T_3 (N=38)	Sham (N=31)	Control (N=30)
Hematocrit	34.9±0.6	34.4±0.6	35.4±0.7
Fish mass (g)	15.5±0.7	16.7±0.8	14.3±0.8
Standard length (mm)	97.9±1.4	99.4±1.5	95.7±1.5
Fork length (mm)	105.3±1.4	106.7±1.6	102.3±1.6
Total length (mm)	111.7±1.5	113.5±1.7	109.1±1.7
Heart mass/body mass	0.0013±0.0001	0.0013±0.0001	0.0013±0.0001

Values are means ± S.E.M.
No significant differences were found among the treatments (ANOVA; $P \geqslant 0.05$).

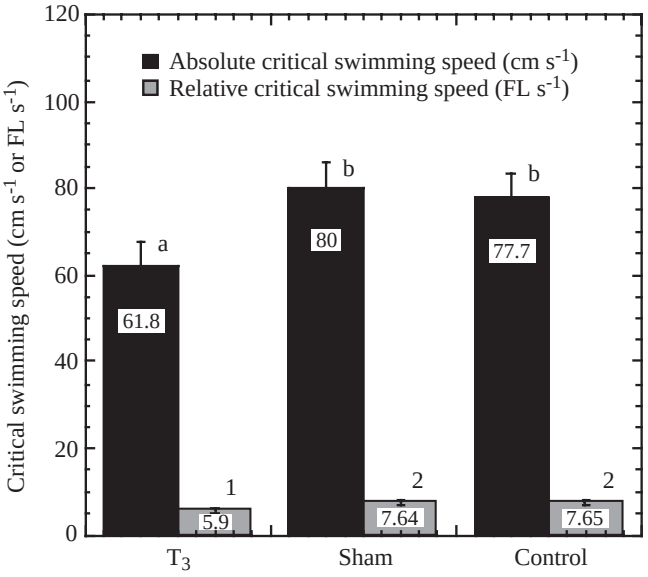

Fig. 1. Mean (including the numerical value in each column) absolute ($cm\,s^{-1}$) and relative ($FL\,s^{-1}$, where FL is fork length) critical swimming speeds (U_{crit}) of juvenile coho salmon from the three treatment groups. Values are means + S.E.M. (N=12). Significant differences (Tukey; P<0.05) are indicated by letters or numbers.

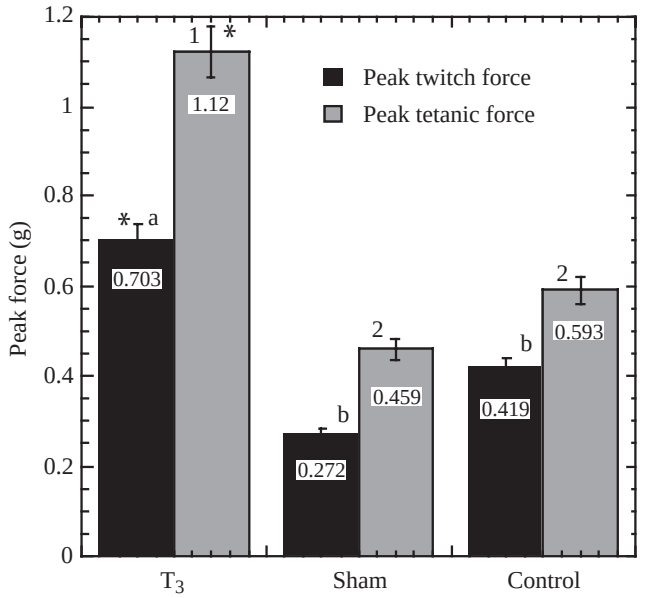

Fig. 2. Mean (including the numerical value in each column) peak force (g) of twitch and tetanic contractions measured in juvenile coho salmon muscle from the three treatment groups. Values are means + S.E.M. (N=15, control and T_3; 14, sham). Significant differences among treatment groups (Tukey; P<0.05) are indicated by letters or numbers. A significant difference between twitch and tetanic peak forces within a treatment group (t-test; P<0.05) is indicated by an asterisk.

levels on the first day of the experimental period, when the T_3 pellets were still actively releasing hormone. All T_3 levels declined to baseline levels of below $5\,ng\,ml^{-1}$ after the second day of experiments. All T_3-treated fish had visible smolt-like characteristics including a blunted rostrum and silvery scales. Gill Na^+/K^+-ATPase acitivities did not differ among treatment groups (ANOVA, P>0.05).

Critical swimming speed

Absolute U_{crit} ($cm\,s^{-1}$) and relative U_{crit} (U_{crit}/V, where V is swimming speed in $FL\,s^{-1}$) were significantly lower in the T_3-treated fish compared with the sham and control fish (ANOVA/Tukey–Kramer HSD, P<0.05, Fig. 1).

Muscle contraction kinetics

Mean peak twitch and tetanic contraction forces were significantly increased in the T_3-treated group compared with the sham and the control groups (ANOVA/Tukey–Kramer HSD, $P\leqslant0.001$; Fig. 2). Tetanic force was significantly increased over twitch force only in T_3-treated animals (Student's t-test, P<0.05). Delay (time from peak stimulus to peak tension) was not significantly different among treatment groups (ANOVA/Tukey–Kramer HSD, P>0.05, data not shown). In addition to the muscle force differences measured among groups, the rates of contraction and rates of relaxation were significantly higher in the T_3-treated group compared with the sham and control groups [Wilcoxon/Kruskal–Wallis (rank sums), P<0.005, Fig. 3]. The dry mass of T_3-treated fish muscle was significantly greater than the dry mass of sham fish muscle (ANOVA/Tukey–Kramer HSD, P=0.02) but not significantly different from the dry muscle mass of the control fish. Dry muscle mass was not significantly related to peak twitch force (r^2=0.005). Wet muscle mass, water content (wet mass minus dry mass), stretched length and cross-sectional area were not significantly different among treatment groups (ANOVA, P>0.05, data not shown).

Discussion

This is the first study to examine the role of T_3 in swimming performance and locomotory muscle contractility of juvenile salmonids. Our results demonstrate a significant decrease in swimming performance, peak muscle contractile forces and an increase in muscle contraction and relaxation rates in T_3-treated coho salmon parr compared with those in sham and control fish. The role of thyroid hormone in the smoltification of juvenile salmonids has been extensively studied (for a review, see Clarke and Hirano, 1995), but the decrease in swimming performance during smoltification is currently controversial. While many studies have shown a decrease in relative swimming performance (U_{crit} in $body\,lengths\,s^{-1}$) during smoltification (Folmar and Dickhoff, 1980; Flagg and Smith, 1982), Peake and McKinley (Peake and McKinley, 1997) recently attributed this decrease to the length differences among the fishes. Because the cost to transport a swimming organism over 1 body length increases with body mass (Videler and Nolet, 1990), longer fish would have lower relative values of U_{crit} than shorter fish (Brett, 1965). Because smolts are generally longer than parr, a decrease in relative U_{crit} could be

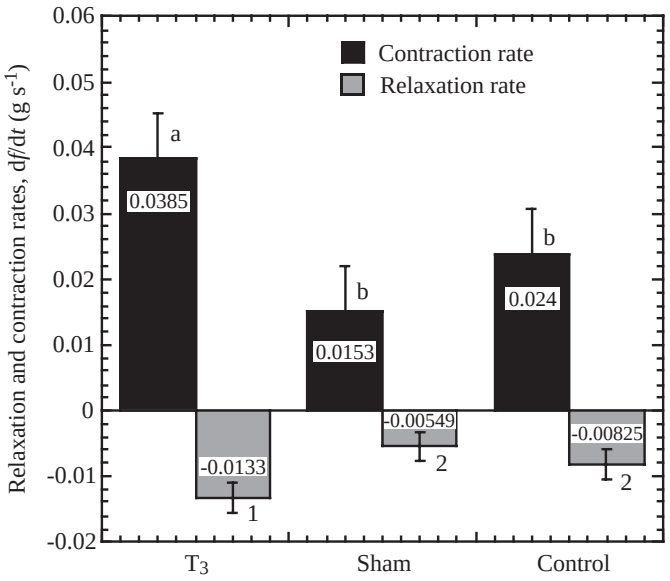

Fig. 3. Mean (including the numerical value in each column) contraction and relaxation rates (d*f*/d*t*, $g\,s^{-1}$) measured in juvenile coho salmon muscle from the three treatment gouups. Values are means + S.E.M. (*N*=15, control and T_3; 14, sham). Significant differences among treatment groups (Kruskal–Wallis; $P<0.05$) are indicated by letters or numbers.

attributed to their longer length. The results of Peake and McKinley (Peake and McKinley, 1997), presented in absolute terms ($cm\,s^{-1}$), demonstrate that critical swimming speed in wild Atlantic salmon (*Salmo salar*) does not decrease during smoltification. These results differ from those of Castleberry et al. (Castleberry et al., 1991) in which absolute ($cm\,s^{-1}$) swimming performance of chinook salmon (*O. tshawytscha*) smolts is reduced compared with that of parr. The differences in the results among previous studies can be attributed to species differences or to the individual environmental history of the population studied, potentially resulting in a different T_3 profile during development. The environmental history (exposure to changing environmental variables such as temperature and photoperiod throughout development) of a population could modify swimming performance because thyroid hormone, which can reduce swimming performance (Fig. 1), is sensitive to environmental influences such as temperature and flow (Youngson et al., 1989), diet (Zaugg et al., 1983), photoperiod (Nishioka et al., 1983), high rearing densities (Patino et al., 1986), moon phase (Grau et al., 1981), water quality and salinity (Dickhoff et al., 1982a; Dickhoff et al., 1982b; Specker and Schreck, 1984) and interactions between photoperiod and temperature (Solomon, 1978; McCormick et al., 2000). Evidence from hatchery studies indicates that plasma thyroid hormone levels can peak several times during development in response to environmental variation (Grau et al., 1982; Dittman et al., 1994). Thus, differences in environmental variation (e.g. changing photoperiod, salinity and temperature) resulting in unique patterns of T_3 exposure during freshwater residence may explain the conflicting results found among swimming performance studies, especially those that compare hatchery with wild fish. Our approach was to isolate the effects of T_3 on swimming performance and muscle contractility using fish of similar size.

While T_3 treatment influenced swimming performance and muscle contractility characteristics in coho salmon, it did not affect other variables such as hematocrit and gill Na^+/K^+-ATPase activity. Hematocrit levels were similar among treatment groups (Table 1). The stable hematocrit suggests that blood oxygen capacity limitations are not contributing to the decreased swimming performance of our T_3-treated fish. As in other studies (Saunders et al., 1985), our fish did not demonstrate increased Na^+/K^+-ATPase activity, indicating that T_3 did not increase the ability of our fish to osmoregulate successfully in sea water. This result supports a decoupling of the hormonal mechanisms responsible for downstream migration from the mechanisms responsible for seawater readiness in juvenile coho salmon if one of the essential components of downstream migration is a decreased ability of the fish to hold station in a fast current (implied by a decreased swimming performance). In previous studies, smolts were observed swimming passively downstream, while other studies noted active downstream movements (for a review, see Folmar and Dickhoff, 1980). Our data support the hypothesis that T_3 accelerates downstream migration through decreased swimming performance. We acknowledge that total T_3 levels may give an incomplete picture of thyroid status as a result of confounding factors such as T_3 receptor availability.

Heart mass/body mass ratios (Table 1) were also measured because T_3-treated mammals and birds demonstrated an increased heart volume compared with body mass (Kolar et al., 1992; Deaton et al., 1997). In addition, heart volume is increased in smolts compared with parr (Poupa et al., 1974). As in our T_3-treated coho salmon, heart mass/body mass ratio was not modified in T_3-treated lizards (Venditti et al., 1996). Although heart mass was not increased, it is possible that T_3-modified cardiac contractility (Kolar et al., 1992) contributed to the observed decrease in swimming performance.

In addition, we demonstrated that T_3 alters the contractile properties of the mosaic muscle fibers in coho salmon parr. The increased rates of contraction and relaxation indicate an upregulation of fast-type fiber contractile proteins, an alteration of contractile properties that would probably promote sprinting over sustained swimming. The fiber types that make up salmonid mosaic muscle have not been characterized completely. According to Johnston et al. (Johnston et al., 1975), the large fibers in the mosaic (rainbow trout, *O. mykiss*) muscle are of the fast glycolytic type, while the smaller fibers are of the slower, more oxidative type (FOG or fast oxidative glycolytic according to Webb, 1975). Alternatively, Higgins (Higgins, 1990) determined that the mosaic muscle of Atlantic salmon parr and smolt is composed of small and large white muscle fibers. Ennion et al. (Ennion et al., 1999) demonstrated that histochemical differences exist between the small (neonatal type) and large (adult isoform) white fibers of

carp (*Cyprinus carpio*) muscle. Although the contractile consequences of these differences in white fibers remain to be determined, it is obvious that histochemical analysis of mosaic muscle may not be the optimal method for fiber type characterization. We did not examine the histochemical properties of juvenile coho salmon muscle because the possibilities for T_3-related fiber-type modification cannot be addressed solely through determination of myofibrillar ATPase activity or through other histochemical/morphological methods. Studies on the effects of T_3 on mammalian muscle fibers indicate that T_3 can upregulate fast-type sarcoplasmic reticulum Ca^{2+}-ATPase (Thelen et al., 1994), regulate fiber type composition through activation of the *myoD* gene family (Downes et al., 1995), produce acquired myasthenia-gravis-type symptoms (Dewey et al., 1995), alter ratios of myosin heavy chain phenotypes (Devor and White, 1994), increase cyclic AMP levels (Ramachandran et al., 1996), alter electrophysiological properties (Venditti et al., 1996) and increase cation transport (for a review, see Everts, 1996). Our current studies focus on the effect of T_3 on ryanodine receptors because of the increase in contraction velocity in addition to force as indicated in our results. Furthermore, ryanodine receptors respond to T_3 treatment (Jiang et al., 2000) by increasing in abundance. Although further investigations of these mechanisms are beyond the scope of this paper, our results on juvenile salmonids imply a significant plasticity in juvenile salmonid mosiac muscle in response to elevated plasma T_3 content.

The T_3-treated fish also demonstrated increased peak contractile tetanic and twitch forces (Fig. 2) indicating a modification of the excitation–contraction coupling mechanism, an increased sensitivity to Ca^{2+} or an alternative modification of the molecular mechanism. Two possible muscle fiber modifications include the T_3-enhanced transition from neonatal to adult myosin heavy chains (Martinez et al., 1995) and the T_3-enhanced upregulation of fast myosin isoforms or sarcolemmal/sarcoplasmic ion exchangers, similar to that observed in other taxa (Everts, 1996; Li et al., 1996; Sachs et al., 1997). In addition, only our T_3-treated fish demonstrated a significantly greater tetanic than twitch force compared with the other treatment groups. This result may also be due to enhanced sensitivity to Ca^{2+} levels, modification of the excitation–contraction coupling mechanism or a reduced level of parvalbumin (a protein that acts as a time-delayed buffer to increased $[Ca^{2+}]$; Heizmann, 1984). A number of parvalbumin isoforms are expressed during development in salmonids, but the relationship between these isoforms and muscle contractility is not known (Huriaux et al., 1996). We are currently investigating possible molecular mechanisms behind the change in contractility using frozen muscle samples obtained from our treatment groups.

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