

Thyroxine Induces Transitions in Red Muscle Kinetics and Steady Swimming Kinematics in Rainbow Trout (*Oncorhynchus mykiss*)

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ABSTRACT During normal development, rainbow trout undergo a shift in red muscle contraction kinetics and swimming kinematics. Young trout parr have faster muscle kinetics and faster tailbeat frequency during swimming than older, larger juvenile trout. In this study, the thyroid hormone thyroxine (T_4) was used to induce these changes in trout parr. This allowed a comparison of swimming kinematics, through the use of video analysis and electromyography, and red muscle contractile properties, through the use of in vitro muscle preparations, between natural parr and same-sized induced juveniles. The red muscle of natural parr has faster contractile properties than induced juveniles, including faster twitch time and a faster maximum shortening velocity (V_{max}). Further, natural parr swim with faster tailbeat frequencies than induced juveniles. The results suggest that the natural shift in red muscle contraction kinetics observed during parr-smolt transformation in trout directly affects swimming behavior in these fish. Also, thyroid hormones appear to induce a shift towards *slower* isoforms of the muscle protein myosin heavy chain (MHC), a result distinct from work on rats where thyroid hormones induce shifts towards faster forms of MHC. *J. Exp. Zool.* 290:115–124, 2001. © 2001 Wiley-Liss, Inc.

Many fish show developmental changes in swimming behavior. These changes can be associated with body form, such as the morphological transformation observed in flounder at metamorphosis, or they can be associated with ontogenetic shifts in niche, such as in trout. During development, trout and other salmonids pass through several life history stages that vary in terms of swimming behavior (Groot and Margolis, '91). First feeding trout, or alevins, are poor swimmers that rarely leave the bottom of their resident streams. Fish in the first juvenile stage, called parr, swim continuously. These fish rise off of the stream bottom and hold position in the moving water. Older juveniles, termed smolts in anadromous salmonids, typically mark the onset of downstream migration. The parr-smolt transformation (PST) is associated with a suite of morphological, physiological and behavioral changes (Hoar, '88).

In nonanadromous salmonids, such as rainbow trout, the older juveniles can be termed "pseudosmolts" because they are not physiologically adapted for marine life (Dickhoff and Sullivan, '87). In this paper, we employ the simpler term "older juvenile" for rainbow trout that have undergone a developmental transition from the typi-

cal parr characteristics to typical smolt features, such as a more slender body, a silvery in appearance, and a shift in visual sensitivity.

In rainbow trout, *Oncorhynchus mykiss*, there is a developmental shift in the kinematics of steady swimming and in the kinetics and molecular composition of the aerobic or red muscle (Coughlin et al., 2001). Steady, aerobic swimming by parr occurs at a higher tailbeat frequency and with a lower relative tailbeat amplitude compared to older juveniles. Further, parr red muscle has faster activation and relaxation times and faster maximum shortening velocity (V_{max}) compared to older juveniles (Coughlin et al., 2001). Faster red muscle kinetics are found in the fish that swim steadily with a higher tailbeat frequency. Lastly, SDS-PAGE analysis of rainbow trout red muscle composition reveals a shift in the myosin heavy chain composition (Coughlin et al., 2001) as has been previously observed in Atlantic salmon

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(Martinez et al., '93). This shift involves a developmental reduction in the number of identifiable MHC bands on an electrophoretic protein gel. Since steady (continuous) swimming is powered by only the red or aerobic muscle fibers (Johnston et al., '77; Coughlin and Rome, '99), a shift in red muscle kinetics and composition would predictably affect steady swimming kinematics.

The correlation between changes in MHC composition and a shift in red muscle function is confounded by fish size. Since the developmental changes described above occur over a period of growth from 30 to 60 g in rainbow trout (Hawryshyn et al., '89), the effects of the change in muscle kinetics cannot be easily separated from the effects of growth on the swimming of trout parr and older juveniles. Developmental changes in swimming behavior could be partly a function of size. Larger fish might naturally swim with a slower tailbeat frequency due to the inertial effects of greater body mass. To separate the effects of changing contraction kinetics and of growth on swimming, thyroid hormone treatment was used to induce developmental changes in trout parr. The red muscle contraction kinetics and steady swimming performance of the parr and same-sized hormone manipulated fish or induced juveniles could then be compared.

The thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) are associated with normal development in salmonids. For instance, in anadromous salmonids a surge in plasma T_4 and T_3 concentrations occurs during smoltification (Folmar and Dickhoff, '80; Dickhoff and Sullivan, '87; Hoar, '88; Youngson, '89; Dittman and Quinn, '96). Although the exact role of thyroid hormones in smoltification continues to be an area of research, it is clear that thyroid hormones can be used to artificially induce several of the changes associated with natural development in trout (Hoar, '88; Prunet et al., '89; Morin et al., '95). For example, salmonids treated with either T_4 or T_3 show the typical smolt transformations in morphology and vision (Browman and Hawryshyn, '92, '94). However, these fish do not generally show the physiological changes associated with adaptation for seawater (Dickhoff and Sullivan, '87). In the nonanadromous rainbow trout, the exogenous application of T_4 has been successfully used to generate induced juveniles the same size as control parr (Browman and Hawryshyn, '92, '94). These experimental rainbow trout juveniles show changes in visual system, a silvering in appearance and the changes in body shape (Beaudet et

al., '91). A similar effect can be generated through the dietary administration of T_3 (Dickhoff and Sullivan, '87).

In other animal systems, the role of thyroid hormones in regulating muscle composition and function has been established. In flounder, thyroid hormones regulate developmental changes in myotomal muscle at metamorphosis, such as shifts in troponin and myosin light chain expression, but not myosin heavy chain (Yamano et al., '91, '94). In rats, thyroid hormone can regulate skeletal muscle Na^+ and K^+ ATPase isoforms (Azuma et al., '93) and myosin isoform transitions (Gambke et al., '83; Butler-Browne et al., '84). Treatment of rats with thyroid hormones can alter the expression of Ca^{2+} ATPase pumps and myosin heavy chain isoforms, typically changing fiber type from slower to faster forms of MHC (e.g., Van der Linden et al., '96; Yu et al., '99).

To investigate the relationship of changes in contraction kinetics to swimming performance, thyroxine was used to generate induced juvenile rainbow trout. Through the regulation of diet, growth of the induced juveniles was restricted. Therefore, the red muscle kinetics, swimming kinematics, and MHC composition of same sized natural parr and induced juveniles could be examined. The hypothesis being tested is that the changes in swimming performance observed in natural older juveniles are associated with the shift in red muscle kinetics and muscle composition. The prediction is that induced juveniles, like natural older juveniles, will swim with slower tailbeat frequencies and display slower red muscle kinetics.

MATERIALS AND METHODS

Experimental animals

Rainbow trout, *Oncorhynchus mykiss*, parr were obtained from the Huntsdale Fish Culture Station, Carlisle, PA, of the Fish and Boat Commission of the Commonwealth of Pennsylvania. Fish were maintained on a diet of prepared fish feed (Ziegler Trout Grower) in a re-circulating aquarium system at 10°C. At the onset of experimental treatment, the parr used in this research had obvious parr marks and a mean (\pm SD) total length of 12.9 ± 1.9 cm ($n = 57$). They were approximately 6–8 months post hatching. All handling of experimental animals was reviewed by the Widener University Institutional Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals of the National Research Council.

Animals were randomly assigned to either the

normal (control) group or to the hormone treatment (experimental) group. The first group of experimental fish received a 6-week exogenous treatment with thyroxine (Sigma L-thyroxine, $300 \mu\text{g l}^{-1}$). Sample size for this "moderate thyroxine" treatment was $n = 15$ for both experimental and control fish groups. This dosage was based on published values (Browman and Hawryshyn, '92). A second group of experimental fish received an increased hormone dose, nine weeks at $600 \mu\text{g l}^{-1}$ exogenous thyroxine. The higher dose was used to see if the differences in muscle kinetics and swimming kinematics observed under the moderate treatment would be enhanced by more intense hormone treatment. Sample sizes for the "high thyroxine" treatment were $n = 13$ for the experimental fish group and $n = 14$ for the control fish group. During treatment, all fish (experimental and control) were maintained in 8-l tanks, with two fish per tank kept separate by a partition. The tanks were aerated and kept at 10°C in an environmental chamber. Water was changed three times weekly and diet was restricted to three feedings weekly of ~ 0.5 g of trout grower pelleted food (Ziegler Brothers). At the end of treatment, the experimental fish were visibly different from natural parr. The induced juveniles were silvery and had generally lost their parr marks (Fig. 1),

as well as being more slender (Table 1). However, the induced juveniles and natural parr were the same length and had the same mass (Table 1).

Experimental procedures

Muscle mechanics

For muscle mechanics experiments, trout were sacrificed by spinal transection and pithing. Live preparations were dissected from the red muscle, which runs in longitudinal bands down the midline of each side of the fish. After removing the scales, 0.5 mm wide strips of red muscle were extracted from just above and below the lateral line of the fish. Muscle preparations were dissected from two longitudinal positions: anterior, 0.25–0.55 BL and posterior, 0.55–0.85 BL. Both positions were used in the moderate thyroxine treatment experiment, only the posterior position was studied in the high thyroxine treatment experiment. Subsequent dissection was carried out at 4°C with the use of a stereomicroscope.

For dissection and mechanics experiments, a physiological saline solution was used (Altringham and Johnston '90), as previously described (Coughlin, 2000). Live bundles were the length of one myomere (~ 2.5 – 4 mm) with an approximate cross-sectional area of 0.25 mm^2 . The bundles were then tied into a muscle mechanics system



Fig. 1. Induced smolt (top) and natural parr from moderate thyroxine treatment. After six weeks of exogenous thyroxine (T_4 , $300 \mu\text{g l}^{-1}$), the induced juveniles had a silvery appearance, while the control fish retained their parr marks.

There were no significant differences in length or mass of hormone treated versus control fish (Table 1). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

TABLE 1. Sizes of natural parr (control fish) and induced juveniles (experimental fish)

Thyroxine treatment		Pre-treatment		Post-treatment	
		Natural parr	Induced juveniles	Natural parr	Induced juveniles
Moderate thyroxine 300 $\mu\text{g l}^{-1}$ for 6 weeks	Total length (cm)	14.3 \pm 1.1 (n = 15)	14.4 \pm 1.4 (n = 15)	14.5 \pm 1.2	14.7 \pm 1.5
	Mass (g)	23.0 \pm 4.1 (n = 10)	23.8 \pm 5.1 (n = 10)	25.3 \pm 4.1	25.8 \pm 5.7
	Depth/SL	0.225 \pm 0.004 (n = 10)	0.221 \pm 0.008 (n = 10)	0.225 \pm 0.004	0.214 \pm 0.004 ^a
High thyroxine 600 mg l^{-1} for 9 weeks	Total length (cm)	11.2 \pm 0.8 (n = 13)	11.1 \pm 1.0 (n = 12)	12.2 \pm 0.7	11.7 \pm 1.1
	Mass (g)	16.0 \pm 2.7 (n = 13)	14.9 \pm 4.6 (n = 12)	20.8 \pm 3.6	18.3 \pm 5.5

^aDifference between natural parr and induced juveniles ($P < 0.05$).

For both moderate and high thyroxine experiments, there were no differences between the mass or total length of control and experimental fish. This was true for both pre-treatment and post-treatment observations. For the moderate thyroxine experiment, measurements of body shape were made. Following treatment, the moderate thyroxine-induced juveniles had a more slender body shape, with a significant reduction in the ratio of maximum fish depth to standard depth.

with a servosystem (Cambridge Technology 300S) and a load cell (Entran, 2–20 g). Temperature in the apparatus was maintained at 10°C for all experiments. Experimental control and data collection were carried out using a PC, Keithley-Metrabyte DAS-1601 input/output board and custom software.

For each bundle, the optimal length for maximal tetanic isometric stress was first found. Stimulation conditions were typically 150–250 ms pulse trains of 2–3 ms pulses at a frequency of 125 Hz. Stimulus duration, pulse frequency, amplitude, and duration were optimized to maximize isometric stress. Isometric twitch and tetanus contractions were recorded at 10°C, and the force traces analyzed for activation and relaxation times. For tetanic contractions, time of activation (TA) was defined as the time from 10–90% of peak isometric stress, and time of relaxation (TR) was the time from 90–10% of isometric stress. Twitch time (TW 90) was the time from stimulation to 90% recovery (10% of peak isometric stress) in twitch contractions.

For POST preparations at the high thyroxine treatment (n = 6 for experimental and n = 6 for control fish), the maximum shortening velocity of the muscle, V_{max} , was estimated through the force-clamp technique. Shortening velocity was measured at loads between 0.05 and 0.8 of peak tetanic isometric stress. After the force-velocity curve was plotted, the data were corrected for passive tension, and V_{max} was found by fitting the Hill equation hyperbola to the force - velocity data using a custom curve-fitting program. The fit of the hyperbolic curves at low force levels was improved by not constraining the curves to pass

through an intercept of 1 (Rome and Sosnicki, '90), and A^* and P_o^* were therefore used to represent variables of the Hill equation. V_{max} was expressed in muscle lengths per sec (ML s^{-1}). Peak steady-state power production (W_{max}) and the optimal shortening velocity for peak power ($V_{\text{opt}} / V_{\text{max}}$) were found from the curve (Coughlin et al., 2001).

Determination of the live muscle fiber area of muscle bundles was carried out at the end of each experiment based on previous histological work described by Coughlin (2000). Muscle bundles have a roughly rectangular cross-section. Height and width of the muscle fibers of each bundle could be accurately determined with the ocular micrometer. Live fiber area was calculated as total muscle fiber cross-section *0.48. The constant was determined from the previous determination that (1) 70% of the total muscle bundle area represented live fibers and (2) 69% of live muscle area was actual muscle fibers (the rest being connective tissue) (Coughlin, 2000).

Swimming kinematics

To examine the effect of development on swimming capabilities, fish were swum in a re-circulating flow chamber. The temperature-controlled flume was maintained at 10°C and had a 0.40 m long, 0.15 m internal diameter test section of clear acrylic pipe. A DC motor permitted speeds of 0.10 to 1 m s^{-1} . Two types of swimming experiments were carried out. In the first, experimental and control fish from the moderate thyroxine treatment were swum across a range of length-specific swimming speeds of 1 to 6 body lengths per sec (BL s^{-1}), both before and after hormone treatment. Swimming was videotaped from above (Panasonic

WV-BP510 video camera and ProLine AG1970 VCR), and kinematics were analyzed with 16.67 ms resolution. Maximum steady swimming speed was determined as the highest swimming speed at which the fish would swim steadily for 30 sec (i.e., no burst and coast swimming). This definition was arbitrary, but allows for an internal comparison of these two groups of fish. Tailbeat frequency and tailbeat amplitude were determined from video analysis. Tailbeat frequency was calculated from the total time of five consecutive tailbeats in steadily swimming fish. Tailbeat amplitude was measured as the mean distance traveled in each direction from the resting or straight body position for five tailbeats and was expressed in units of total length. For instance, a value of 0.10 means that during swimming, the tail moved in each direction a distance equal to 10% of total length.

In the second round of swimming experiments, electromyograms were recorded from two longitudinal positions, anterior (0.35 L) and posterior (0.75 L) for parr and induced juveniles swimming at speeds from 1 to 5 BL s⁻¹. This experiment was carried out for both the moderate and high thyroxine experiments. The EMG signals were recorded using Grass EMG amplifiers (P511, filtering bandwidth of 10 to 1000 Hz with a 60 Hz notch filter) and a PC. EMG signals were analyzed for duty cycle of EMG activity, defined as the duration of

EMG bursts expressed as a proportion of one tailbeat cycle. In addition, the frequency of muscle activity could be determined via the EMG signals. This provided an alternate measure of tailbeat frequency for comparison with the data from video analysis described above.

RESULTS

Muscle mechanics

In general, the red muscle of the induced juveniles was significantly slower than that of natural parr (Fig. 2). Under the moderate thyroxine treatment, induced juveniles had significantly longer activation times in the anterior muscle than control fish (Fig. 2A) and longer relaxation times in the anterior and posterior muscle during tetanic contractions (Fig. 2B). Under both moderate and high treatments, posterior muscle from the hormone treated fish had significantly longer twitch times (Fig. 2C). One exception to this trend is the shorter activation time of red muscle from the high thyroxine induced juveniles relative to control (Fig. 2A). While high thyroxine treatment led to results similar to those for the moderate treatment, there was no evidence of an enhanced effect with higher T₄ concentration.

The posterior red muscle of induced juveniles had a slower V_{max} than the natural parr under the high thyroxine treatment (*t*-test, *P* = 0.047).

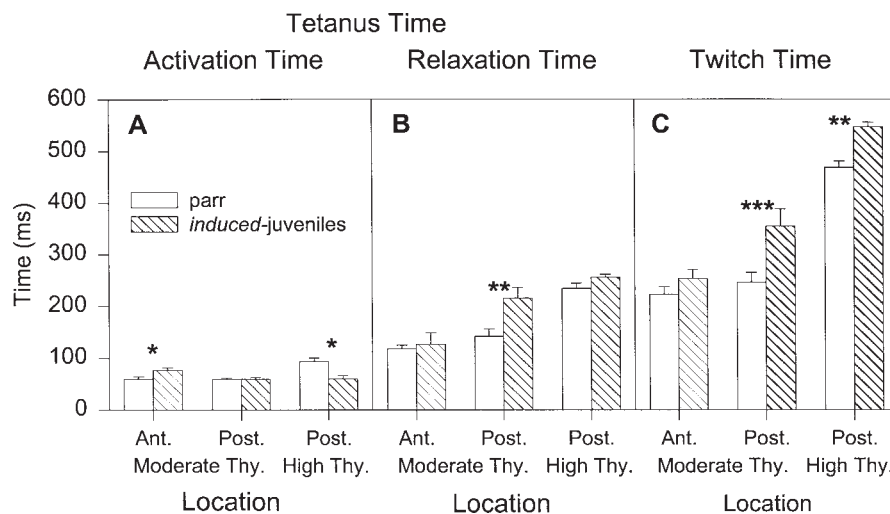


Fig. 2. Red muscle kinetics from natural parr and induced juveniles. For tetanic contractions, activation time (A) is the time from 10 to 90% of peak isometric stress and relaxation time (B) is the time from 90 to 10% of peak isometric stress. Twitch time (C) is the time from twitch stimulation to 90% recovery from peak twitch isometric stress. Measurements were made for muscle bundles from anterior and posterior

positions (n = 10 for each position in both natural parr and induced juveniles) for the moderate thyroxine experiment and for the posterior position (n = 6 for both natural parr and induced juveniles) for the high thyroxine experiment. Asterisk indicates a significant difference between natural parr and induced juveniles at a given position and treatment level (*t*-test; * *P* < 0.05; ** *P* < 0.025; *** *P* < 0.01).

The mean (\pm SE) V_{\max} for natural parr was $3.08 \pm 0.35 \text{ ML s}^{-1}$ and for induced juveniles was $2.33 \pm 0.15 \text{ ML s}^{-1}$. There was no difference in the ratio of A^* / P_o^* between the two groups of fish (t -test, $P = 0.84$), indicating that the force - velocity curves for muscle from each group had the same shape. The mean value of A^* / P_o^* for all fish was 0.35 ± 0.06 . Also, there was no difference between the optimal shortening velocity for maximum power output-between the induced juveniles and natural parr (t -test, $P = 0.69$). The mean value of $V_{\text{opt}} / V_{\max}$ for all fish was 0.34 ± 0.02 . However, there was a significant difference in the maximum steady-state power output of muscle (t -test, $P = 0.04$). The natural parr produced significantly more steady state power than the induced juveniles; mean values for W_{\max} were $61.1 \pm 2.0 \text{ W kg}^{-1}$ for natural parr and $42.4 \pm 7.0 \text{ W kg}^{-1}$ for induced juveniles.

Swimming experiments

Before hormone treatment, there were no differences in the swimming kinematics of control and experimental fish (data not shown). After treatment, induced juveniles swam with lower tailbeat frequencies than natural parr across a range of length specific swimming speeds. This difference was significant (Fig. 3). In addition, induced juveniles swim with a significantly greater tailbeat amplitude (expressed as fraction of total length). Analysis of their maximum steady swimming speeds indicates that parr can use their aerobic musculature to swim significantly faster than induced juveniles. Furthermore, at the maximum steady swimming speed, the tailbeat frequency of parr is significantly faster than of induced juveniles (Table 2).

From the measurements of EMG activity during swimming, conflicting results were observed between the moderate vs. high thyroxine experiments. Under moderate thyroxine treatment, the hormone had a significant effect on the duty cycle of EMG activity (multi-way ANOVA, $P = 0.025$ for the effect of hormone treatment on duty cycle). The EMG activity of hormone treated fish was significantly longer than for natural parr (Figs. 4 and 5). This effect was not observed in the high thyroxine treatment experiment (multi-way ANOVA, $P = 0.93$ for the effect of hormone treatment on duty cycle). For both moderate and high thyroxine treatment, tailbeat frequency was generally greater in the natural parr than in the induced juveniles (Fig. 6), although this effect was not significant for either treatment group (multiway ANOVA, $P = 0.114$

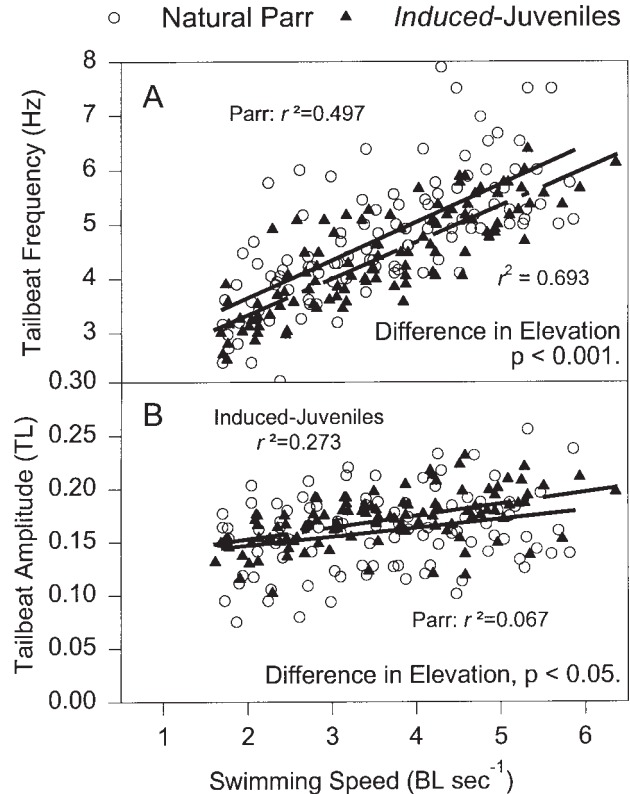


Fig. 3. Tailbeat frequency (A) and tailbeat amplitude (B) of swimming rainbow trout. Tailbeat amplitude is the mean distance traveled by the tip of the tail in each direction from the resting or straight body position and is expressed in units of total length (TL). Natural parr swam with significantly greater tailbeat frequencies, while induced juveniles swam with significantly greater tailbeat amplitudes across a range of length specific swimming speeds. All regressions were significant (Regression ANOVA, $P = 0.02$ for tailbeat amplitude of natural parr; $P < 0.001$ for all others). The natural parr regression lines are solid, while the induced smolt regression lines are broken. This experiment was carried out with fish from the moderate thyroxine treatment. For both groups of fish, ten fish swam at eight speeds each.

for high thyroxine treatment, $P = 0.161$ for moderate thyroxine treatment).

DISCUSSION

Natural and induced development in rainbow trout red muscle

Thyroid hormone treatment affects the red muscle kinetics and the steady swimming kinematics of rainbow trout parr. Compared to natural parr (control fish), the experimental induced juveniles show slower red muscle kinetics, with longer twitch times and slower shortening velocities (V_{\max}). The induced juveniles also swim with lower tailbeat frequencies than natural parr. The slower kinetics of the experimental fish are correlated with lower tailbeat

TABLE 2. Swimming performance of natural parr and induced juveniles

Thyroxine treatment	Pre-treatment		Post-treatment	
	Natural parr	Induced juveniles	Natural parr	Induced juveniles
Maximum steady swimming speed (BL s ⁻¹)	2.77 ± 0.11	2.90 ± 0.08	3.12 ± 0.10	2.84 ± 0.09 ^a
Tailbeat frequency at maximum speed (Hz)	4.44 ± 0.16	4.61 ± 0.18	4.18 ± 0.19	3.25 ± 0.19 ^a

^aDifference between natural parr and induced juveniles ($P < 0.05$).

Following moderate thyroxine treatment, natural parr had a maximum steady swimming speed and swam with a higher tailbeat frequency than the induced juveniles. There were no differences between the two groups pre-treatment. Sample size was $n = 10$ for parr and for induced juveniles.

frequency during swimming. Previous research on natural development found the same general results reported here for hormone treatment. During normal development, the larger, older juveniles have slower muscle kinetics than parr, and this is reflected in slower swimming speeds and lower tailbeat frequencies (Coughlin et al., 2001).

An important result of the current work is that differences in red muscle kinetics and steady swimming kinematics were observed between normal and hormone treated fish that were the same size. In the previous study shifts in swimming kinematics could be attributed in part to growth. A larger fish may simply oscillate its tail more slowly as a consequence of larger tail mass, for instance. However, the present study shows that changes in the kinetics of the myotomal red muscle have specific effects on swimming kinematics. By controlling for animal size, the connection of kinetics to kinematics is confirmed.

Thyroid hormone, development, and muscle activity during swimming

The examination of EMG activity between natural parr and induced juveniles raises more ques-

tions than it answers. While the moderate thyroxine treatment led to a significant shift in EMG activity, with longer EMG duty cycles in the hormone treated fish, there was no change in EMG duty cycle in the high thyroxine treatment. If no change had been observed in either group, then the results would suggest that the nervous control of the swimming musculature is constrained and relatively unresponsive to external influence. However, the difference in EMG activity between natural parr and induced juveniles in the moderate thyroxine experiment points to plasticity of nervous control. Further research is needed to address this issue, examining thyroxine dose-response curves and possible seasonal variations in trout responses to T₄.

Duty cycle of EMG activity in rainbow trout appears to increase during development. For instance, for anterior red muscle from rainbow trout, the duty cycle increases from 0.15–0.25 for 10–15-cm natural parr (Fig. 5A) to 0.4 for 20-cm natural juveniles (Coughlin, 2000) to >0.5 for larger adult rainbow trout (Hammond et al., '98). Interestingly, the duty cycle of anterior red muscle of the induced juveniles from the moder-

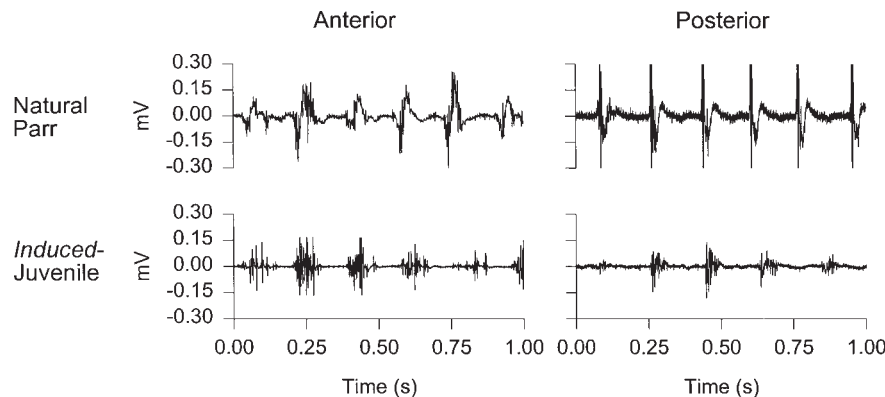


Fig. 4. Sample electromyograms from red muscle of a natural parr and an induced smolt swimming at 3.5 BL s⁻¹. Fish are from the moderate thyroxine experiment. The length

of EMG bursts of muscle activity at each position was typically longer in induced juveniles.

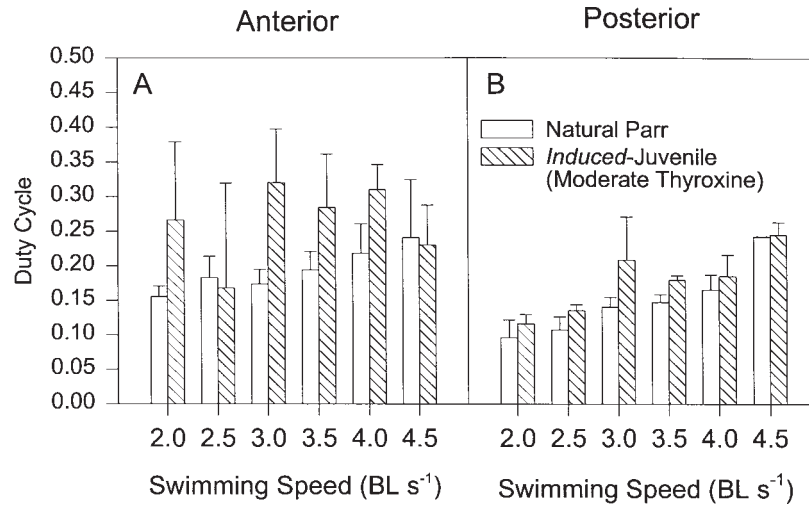


Fig. 5. Duty cycle of EMG activity for natural parr and induced juveniles from the moderate thyroxine experiment. At most speeds, the duty cycle of red muscle activity, which is EMG burst duration expressed as a proportion of the

tailbeat period, was longer in induced juveniles than in natural parr. The effect is more obvious in the anterior (A) than posterior (B). Combining all data, induced juveniles have a significantly longer duty cycle (multiway ANOVA, $P = 0.025$).

ate thyroxine experiment ranged from 0.25 to 0.35 (Fig. 5A), close to the values observed in natural juveniles.

The meaning of the increase in duty cycle of EMG activity with development is not obvious. Larger trout have slower red muscle kinetics (Hammond et al., '98; Coughlin, 2000). Coupling longer duty cycles with slower kinetics in the larger fish points to a shift in red muscle function during swimming. Kinetically slower muscle typically produces less mass-specific oscillatory

power for a given set of in vivo muscle activity conditions (Rome et al., '93, 2000; Coughlin, 2000). Furthermore, longer duty cycles can lead to reduced power output by decreasing the time available for relaxation, as has been observed in many fish species that have longer duty cycles in the anterior myotome compared to the posterior (Rome et al., '93, 2000; Jayne and Lauder, '95; Swank and Rome, 2000; Coughlin 2000). Developmental changes in duty cycle *and* kinetics suggest variations in how power is produced by the

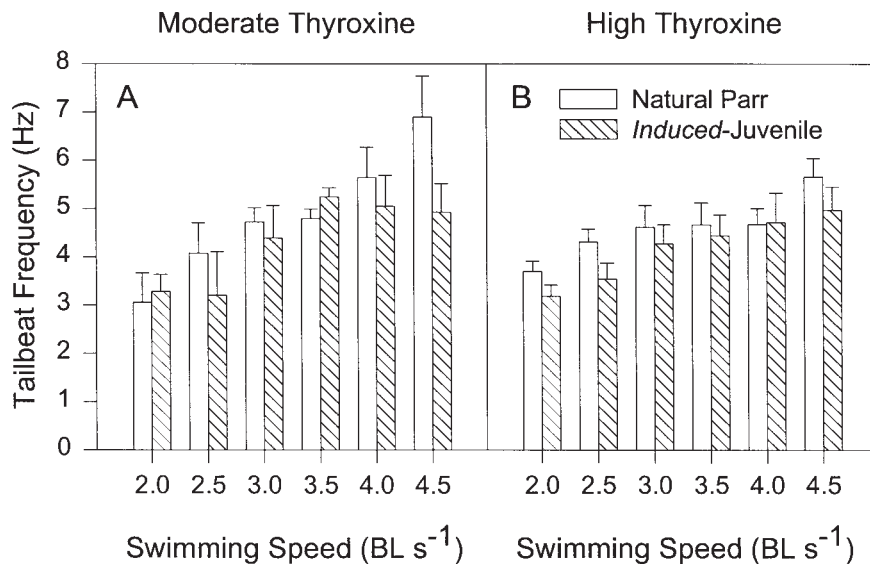


Fig. 6. Tailbeat frequency for natural parr and induced juveniles as determined from the electromyography experiments with both moderate (A) and high thyroxine (B) treated

fish. At most speeds for both thyroxine levels, frequency was higher in the natural parr than in induced juveniles, although overall the differences were not significant.

red muscle during steady swimming. This concept requires continued study.

Thyroid hormone and muscle fiber type

Thyroid hormones are a "potent" regulator of muscle proteins in rats (Yu et al., '99). Thyroid hormones affect isoform expression of various myofibrillar proteins in a variety of ways, including direct regulation of gene expression, as well as at post-transcriptional and post-translational levels (Caiozzo and Haddad, '96). However, the specific mechanisms of thyroid hormone regulation of MHC expression in various muscle types are not known and remain a subject of speculation (Caiozzo et al., '98, Adams et al., '99; Yu et al., '99). Importantly, there are other influences on MHC composition, such as nervous stimulation and muscle loading (e.g., Caiozzo et al., 1998). Nevertheless, the effect of thyroid hormones on protein expression during development in rats has been well studied. In early development, thyroid hormones are important in the regulation of a shift from embryonic and neonatal to adult forms of myosin heavy chain (Gambke et al., '83; Adams et al., '99).

The transition in MHC isoforms in developing rats is typically from slower to faster forms. For instance, in the rat plantaris muscle, T_3 is important in the transition from slow embryonic and neonatal isoforms of MHC directly to fast twitch types IIb and IIx, and thyroid deficiency represses the expression of at least type IIb (Adams et al., '99; Jakubiec-Puka et al., '99). In the rat soleus muscle, the thyroid hormone T_3 is involved in the shift from slow embryonic and neonatal forms to the adult type I (slow) and type IIa (fast) forms of MHC (Adams et al., '99). Treatment of adult rats with T_3 induces an up regulation of types IIa and IIx and a down-regulation of the type I MHC expression in the soleus muscle (Caiozzo et al., '91, '98; van der Linden et al., '96; Jakubiec-Puka et al., '99; Yu et al., '99).

The influence of thyroid hormone on slow twitch muscles in rats, such as the soleus, contrasts sharply with the current work on slow twitch or red muscle in rainbow trout. Whereas thyroid hormone induces a shift to faster MHC isoforms in rats, it apparently induces a shift to slower forms in trout. In rats, the thyroid hormone induced change in MHC expression is accompanied by transitions in maximum shortening velocity, with faster velocities in muscle from hormone (T_3) treated (hyperthyroid) animals (Caiozzo et al., '91; Yu et al., '98). Since muscle shortening velocity is

closely associated with MHC isoform (Moss et al., '95; Schiaffino and Reggiani, '96), a shift in V_{max} is at least indirect evidence of a thyroid hormone-induced change in MHC expression in rainbow trout. The hormone (T_4) treated induced juveniles had a slower muscle shortening velocity, as has been previously observed in natural older juveniles. Further, in natural juveniles the shift in V_{max} has been correlated with a change in MHC protein composition (Coughlin et al., 2001).

We set out to show that developmental transitions in red muscle contractile properties results in changes in behavior. The use of thyroid hormones to induce a shift in muscle kinetics showed that previously observed differences in swimming between rainbow trout parr and older juveniles are not simply due to size, but result from shifts in muscle contraction kinetics. Like natural juveniles, the induced juveniles of this study had slower rates of activation and relaxation and a slower V_{max} than natural parr. Furthermore, the effect of a shift in kinetics is clear: slower red muscle contractile properties resulted in slower tailbeat frequency during swimming. As research continues on the mechanism of thyroid hormone regulation of MHC expression in mammals, rainbow trout provide an intriguing model animal due to the distinctive shift in MHC expression they display as a result of thyroid hormone treatment.

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