Tissue-specific thyroid hormone regulation of gene transcripts encoding iodothyronine deiodinases and thyroid hormone receptors in striped parrotfish (*Scarus iseri*)

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A B S T R A C T

In fish as in other vertebrates, the diverse functions of thyroid hormones are mediated at the peripheral tissue level through iodothyronine deiodinase (dio) enzymes and thyroid hormone receptor (tr) proteins. In this study, we examined thyroid hormone regulation of mRNAs encoding the three deiodinases *dio1*, *dio2* and *dio3* – as well as three thyroid hormone receptors *trαA*, *trαB* and *trβ* – in initial phase striped parrotfish (*Scarus iseri*). Parrotfish were treated with dissolved phase T_3 (20 nM) or methimazole (3 mM) for 3 days. Treatment with exogenous T_3 elevated circulating T_3, while the methimazole treatment depressed plasma T_4. Experimentally-induced hyperthyroidism increased the relative abundance of transcripts encoding *trαA* and *trβ* in the liver and brain, but did not affect *trαB* mRNA levels in either tissue. In both sexes, methimazole-treated fish exhibited elevated *dio2* transcripts in the liver and brain, suggesting enhanced outer-ring deiodination activity in these tissues. Accordingly, systemic hyperthyroidism elevated relative *dio3* transcript levels in these same tissues. In the gonad, however, patterns of transcript regulation were distinctly different with elevated T_3 increasing mRNAs encoding *dio2* in testicular and ovarian tissues and *dio3*, *trαA* and *trαB* in the testes only. Thyroid hormone status did not affect *dio1* transcript abundance in the liver, brain or gonads. Taken as a whole, these results demonstrate that thyroidal status influences relative transcript abundance for *dio2* and *dio3* in the liver, provide new evidence for similar patterns of *dio2* and *dio3* mRNA regulation in the brain, and make evident that fish exhibit *tr* subtype-specific transcript abundance changes to altered thyroid status.

1. Introduction

In teleost fish, the thyroid hormones (THs) thyroxine (T_4) and tri-iodothyronine (T_3) play key roles in regulating development and growth [24,36], reproduction [42,69], osmoregulation [45], and the behaviors and underlying physiology associated with rheotaxis and migration [13,37]. The ability for THs to regulate such diverse processes evolved in part through the mechanisms of TH action in peripheral tissues, where changes in TH metabolism occur via changes in hormone-converting iodothyronine deiodinase enzyme activity, and shifts in the type and density of TH receptors allow for differential effects at cell- and tissue-specific levels [67].

Teleost fish – similar to other vertebrates – express three types of iodothyronine deiodinase enzymes termed type I, type II, and type III (Dio1, Dio2, and Dio3, respectively), which function at the prereceptor level to regulate TH levels and, ultimately, TH action at the level of the target tissue (reviewed by [54]). Although Dio1, Dio2 and Dio3 are the protein products of three different genes, each enzyme catalyzes the removal of iodine atoms from either the outer-ring or inner-ring of THs to generate either more active or less active forms of hormone. The mechanism of hormone biotransformation, however, varies among the three deiodinase enzymes, leading each enzyme to have a different role in regulating TH bioactivity [29,30,54]. In fish, Dio2 acts as an outer-ring deiodinase ([ORD] or 5′-deiodinase) by removing iodine from the 5′ outer-ring site to convert T_4 to the more active form T_3, while Dio3 acts as an inner-ring deiodinase ([IRD] or 5-deiodinase) to remove iodine from the inner-ring of T_4 and T_3 and convert these hormones to inactive forms including reverse triiodothyronine (rT_3) and diiodothyronine (T_2). Dio1, in contrast, is thought to catalyze primarily outer-ring deiodination in fish, although – as in other vertebrates – the enzyme also has inner-ring deiodination properties, making its role in regulating TH bioactivity more difficult to define [54,67]. In fish, these three deiodinase enzymes show tissue and developmental stage specific patterns of expression suggesting that the distinct deiodinases contribute to the diverse – and sometimes tissue-specific – functions of THs [8,54,60]. At present, however, the importance of deiodinase enzymes as mediators of TH action in fish is still largely unknown,

and additional information on tissue-specific regulation of deiodinase expression is needed [54].

The effects of THs in a given cell or tissue also depend on signal transduction via the binding nuclear thyroid hormone receptors (TRs), which subsequently interact with thyroid response elements (TREs) in the promoter regions of genes to either enhance or inhibit gene transcription [79]. Teleost fishes have been found to express several distinct TR subtypes; although the number of types can vary depending on the species, these receptors can be broadly grouped in α- and β-type categories based on their structural characteristics [39,40,53] (reviewed by [51]). Although there is little data on functional differentiation among the teleost TR types and subtypes (but see [52]), several studies have found variation in the expression of TRs among tissues or stages of development [8,34,53].

Even though the functional roles of different deiodinase enzymes and TRs remain to be fully determined in teleost fishes – and may even vary between taxa – expression profiles for transcripts encoding *dio* and *tr* genes have recently begun to be used as indicators for endocrine disruption of the hypothalamic–pituitary–thyroid axis, and several studies have demonstrated that *dio* and *tr* relative gene transcript abundance in fishes can be impacted by exposure to pollutants [18,35,38,48,50,55,65,71]. For example, adult male and female fathead minnow (*Pimephales promelas*) given dietary exposures of the polybrominated diphenyl ether flame retardant 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) showed depressed circulating levels of T_4 and reduced transcript abundance for *trβ* – but increased *trα* – in the brain after 21 days of exposure [35]. While these and other studies suggest that *dio* and *tr* mRNA levels may be impacted by environmental pollutants, they also make clear that additional work is needed on the transcriptional dynamics of deiodinase enzymes, TRs, and other TH-regulated gene pathways in fish [18,34]. It is increasingly evident that many thyroid-disrupting chemicals appear to have multimodal or multitarget actions on the hypothalamic–pituitary–thyroid axis [6], and TH-regulated genes – including *dio*'s and *tr*'s – can show tissue-specific responses even when thyroid function or thyroid hormone status is disrupted systemically [64].

Therefore, in this study, we examined TH regulation of *dio* and *tr* gene expression in a teleost fish, the striped parrotfish (*Scarus iseri*). Striped parrotfish, similar to other parrotfish species, are perhaps most notable for their role grazing algae on coral reefs [23]. Parrotfish, however, are also unusual in that they are one of few teleost taxa that have a discrete thyroid gland organ [43,44]. Parrotfish have therefore been used previously as a model for examining thyrotropin regulation of TH production [19,70], and have the potential to provide new insights into mechanisms of HPT regulation in teleosts more broadly. Here, we isolated and sequenced cDNAs encoding *dio1*, *dio2* and *dio3* – as well as three distinct TH receptors *trαA*, *trαB* and *trβ* – from striped parrotfish and examined tissue-specific patterns of change in the relative abundance of these transcripts following systemic thyroid status modulation via administration of either the goitrogen methimazole or exogenous T_3.

2. Materials and methods

2.1. Animal collection and housing

Striped parrotfish (*S. iseri*) are a protogynous sex-changing species that can change from initial phase (male or female) to terminal phase (male only) phenotypes during life [31,57]. The focus of the current study, however, was only on initial phase (IP) striped parrotfish, which were collected using hand nets and a 60 cm diameter cast net from the fringing reef (12°04′01″N, 68°51′40″W) surrounding Curaçao, The Netherlands Antilles on 28 May 2009. Fish (N = 38; 17.80 ± 2.07 g, body mass; 102.5 ± 3.4 mm SL; mean ± SEM) were transported to the Caribbean Research & Management of Biodiversity (CARMABI) research station and maintained in flow-through seawater water tanks (128 L, 80 cm × 40 cm × 40 cm in size) at ambient ocean temperatures (27.4–29.1 °C; Hobo Light-Temperature Data Loggers, Onset Computer Corp., Bourne, MA, USA) for 10 days prior to beginning the experiment. Fish were housed at densities of 3 or 4 fish per tank, and tanks contained natural rock structures for cover. All fish were fed algae wafers (Hartz Mountain Corp., Secarcus, NJ, USA) twice daily. Seawater was pumped into the tanks at a rate of ∼1 L per min, so that the entire volume of each tank was exchanged approximately every 2 h. All parrotfish were collected and maintained in accordance with established guidelines of the Animal Care and Use Committee of the University of North Carolina, Wilmington (Protocol #A0809-020).

2.2. Methimazole and T_3 treatments

Each of the 12 holding tanks was assigned to one of three groups: control, methimazole treatment, or exogenous T_3 treatment, so that there were four tanks per treatment group (n = 12–13 fish per treatment). For each tank, flow-through seawater was turned off, and stock solutions of methimazole in NaOH, T_3 in NaOH, or NaOH vehicle alone (control) were added to each 128 L tank. Fish in the methimazole treatment were exposed to dissolved phase methimazole (Sigma, St. Louis, MO, USA) in the tank water at a concentration of 3 mM with 0.01 M NaOH. The exogenous T_3 treatment consisted of 20 nM T_3 (triiodo-L-thyronine, Sigma) with 0.01 M NaOH, while fish in control tanks were exposed to 0.01 M NaOH vehicle only. Every 12 h, water flow was turned on in each tank at a flow rate of >2 L per min for 1 h to cycle the tank, after which time water flow was again turned off and new stock solutions of methimazole, T_3 or NaOH vehicle alone were added to each tank to renew treatment concentrations.

After 3 days of treatment, parrotfish were euthanized (tricaine methanesulfonate, MS222) and body mass (g) and total length (mm) were recorded. Blood plasma was collected for measurement of plasma T_3 and T_4 by radioimmunoassay, and the liver, brain (without pituitary gland) and one gonad were dissected and immersed in RNAlater (Ambion, Austin, TX, USA) overnight at 4 °C before being stored at −20 °C. The other gonad was dissected and fixed in 4% paraformaldehyde overnight for later histological confirmation of sex. A small section of skeletal muscle tissue was also dissected from the tail of each fish. The muscle was stored in 100% ethanol for subsequent genetic confirmation of each fish's species identity.

2.3. T_3 and T_4 radioimmunoassays

Plasma total T_3 and total T_4 were measured by radioimmunoassay as described elsewhere [12,36]. Plasma (10 μl) was incubated for 2 h at 37 °C in sodium barbital buffer with either anti-L-T_3 antiserum (1:10,000; Accurate Chemical & Scientific Corp., Westbury, NY, USA) and I^{125}-labeled T_3 (Perkin-Elmer, Waltham, MA, USA), or anti-L-T_4 antiserum (1:4000; Accurate Chemical & Scientific Corp.) and I^{125}-labeled T_4 (Perkin-Elmer, Waltham, MA, USA). Ice cold sodium barbital buffer containing 20% polyethylene glycol was then added to each sample, and samples were centrifuged (1409*g*) for 20 min at 4 °C. The supernatant was removed to separate free and bound hormone, and the remaining pellet was assayed for radioactivity (Cobra II gamma counter, Packard, Downer's Grove, IL, USA). T_3 standards from 0.625 to 60 ng/ml – and T_4 standards from 1.25 to 60 ng/ml – defined the sensitivity of the assays. For each hormone, all samples were run in duplicate

in a single assay, and the intra-assay coefficient of variation (% CV) was 6.28% for the T_3 assay, and 7.68% for the T_4 assay.

2.4. Identification of partial cDNAs for deiodinase enzymes and TH receptors

2.4.1. RNA isolation and reverse transcription

Total RNA was extracted from the liver of an initial phase male striped parrotfish (8.46 g, 87.6 mm SL) using TriReagent (Molecular Research Center, Cinicinnati, OH, USA) with bromochloropropane as the phase separation reagent. Extracted RNA was DNase I treated (DNA-free Treatment kit, Ambion), quantified by spectrophotometry (260:280 = 2.02; NanoDrop 2000, ThermoScientific, Wilmington, DE, USA), and examined on a 0.8% agarose gel for RNA quality.

First strand cDNA was synthesized in a 20 μl reverse transcription reaction by incubating 5 μg of total RNA template from the liver (4.75 μl) with 1.0 μl annealing buffer, 1.0 μl of random primers, and 3.5 μl of RNase-free H_2O (Sigma, St. Louis, MO) at 65 °C for 5 min. Subsequently, 10 μl of 5× buffer and 2 μl of Superscript III Reverse Transcriptase Enzyme Supermix (Invitrogen, Carlsbad, CA, USA) were added, and the mixture was incubated under a thermal profile of 25 °C for 10 min followed by 50 °C for 50 min and 85 °C for 5 min (PT-100 thermal cycler, MJ Research).

2.4.2. Isolation and sequencing of partial cDNA sequences

PCR was performed using degenerate primers designed from consensus regions of sequences for *dio* and *tr* cDNAs isolated from other teleost fish (On-line Supplemental materials, Table 1). The deiodinase primers for *dio1*, *dio2* and *dio3* were designed to the following teleosts cDNA sequences: *dio1*: shortjaw mudsucker (*Gillichthys seta*, GenBank Accession No. FJ208960), Japanese flounder (*Paralichthys olivaceus*, AB362421), and Nile tilapia (*Oreochromis niloticus*, Y11109); *dio2*: Japanese flounder (*P. olivaceus*, AB362422), Japanese medaka (*Oryzias latipes*, NM_001136521), turbot (*Psetta maxima*, AF467779), and mummichog (*Fundulus heteroclitus*, FHU70869), and *dio3*: Atlantic halibut (*Hippoglossus hippoglossus*, DQ856303), Nile tilapia (*O. niloticus*, Y11111), puffer (*Takifugu rubripes*, NM_001136146), and Senegalese sole (*Solea senegalensis*, AM902722).

For the *trαA* receptor degenerate primers were designed based off cDNA sequences from bastard halibut (*P. olivaceus*, GenBank Accession No. PAITHRAA1), ice goby (*Leucopsarion petersii*, AB204858), pufferfish (*T. rubripes*, AF302243), and turbot (*P. maxima*, AF302253). Primers for *trαB* were designed from Nile tilapia (*O. niloticus*, AF302249) and bastard halibut (*P. olivaceus*, D16462). Degenerate primers for *trβ* were designed from bastard halibut (*P. olivaceus*, Q91279), gilthead seabream (*Sparus aurata*, AAO86517), fire clownfish (*Amphiprion melanopus*, ACH43022), orange-spotted grouper (*Epinephelus coioides*, ABP62962), Pacific bluefin tuna (*Thunnus orientalis*, BAG12083), and black porgy (*Acanthopagrus schlegelii*, ABQ96862).

In addition, a partial cDNA sequence for elongation factor-1α (*ef-1α*) was isolated for use as a control transcript. For *ef-1α*, degenerate nested primers (On-line Supplemental materials, Table 1) were designed to consensus regions of cDNAs from *O. latipes* (NM_001104662), *Pagrus major* (AY190693), *Carassius auratus* (AB056104) and *Seriola quinqueradiata* (AB032900). These primers amplified an 835-bp partial cDNA of *ef-1α* from striped parrotfish (GenBank Accession No. HM120251).

2.4.3. Phylogeny construction

Deduced amino acid sequences for all identified parrotfish *dio* and *tr* cDNAs were aligned to *dio* and *tr* transcripts or genes from other vertebrates (for GenBank Accession Nos., see On-line Supplemental materials, Table 2). Amino acid sequences were aligned using Clustal X [33], and phylogenetic analyses were conducted using MEGA v.5 [72], using the Neighbor-Joining method and a *p*-distance model for tree construction [59]. All positions containing alignment gaps were eliminated only in pairwise sequence comparisons (pairwise deletion of gaps). Confidence values for clusters of associated taxa were obtained by bootstrap tests (1000 replicates).

2.5. SYBR green real-time quantitative RT-PCR assays

Total RNA was extracted from liver, brain and gonadal tissues using TRI-Reagent (Molecular Research Center) with bromochloropropane. The resulting RNA was DNase I treated (DNA-free kit, Ambion) and quantified by spectrophotometry (Nanodrop 2000). Total RNA was then reverse transcribed in 10 μl reactions by incubating 0.5 μg of total RNA template (4.75 μl) with 2.0 μl of 5× buffer, 1.0 of 0.1 M dithiothreitol (Invitrogen), 0.25 μl of RNaseOUT (40 U/μl) (Invitrogen), 1.0 μl of random hexamer (500 ng/ml) (Promega, Madison, WI, USA), 0.5 μl dNTPs (stock of 10 mM each of dCTP, dGTP, dTTP and dATP; Promega), and 0.5 μl of Superscript III Reverse Transcriptase (200 U/ml) (Invitrogen), under a thermal profile of 25 °C for 10 min followed by 50 °C for 50 min and 85 °C for 5 min (MyCycler thermal cycler, Bio-Rad).

Primers for SYBR green quantitative real-time PCR assays were designed (Primer Quest, Integrated DNA Technologies, and Primer Express 2.0, Applied Biosystems, Inc.) to protein coding regions of the partial cDNAs for deiodinase enzymes (*dio1*, *dio2*, *dio3*) and thyroid hormone receptors (*trαA*, *trαB*, *trβ*). Primers were also designed for *ef-1α* from striped parrotfish for use as a control gene. All primers were synthesized by Integrated DNA Technologies (Coralville, IA, USA) (Table 1). Specificity of these SYBR green primer sets was confirmed by sequencing PCR products, as well as by melt-curve analysis during the quantitative PCR runs.

Quantitative real-time PCRs were conducted in 18 μl reactions. Each reaction contained 4.56 μl nuclease-free water (Sigma, St. Louis, MO, USA), 9.0 μl iQ SYBR green Supermix (Bio-Rad, Hercules, CA, USA), 0.72 μl each of forward and reverse primers (5 μM), and 3.0 μl of reverse-transcribed cDNA template. The PCR thermal profile for each reaction was 50 °C for 2 min, 95 °C for 10 min, 42 cycles of 95 °C for 15 s and 59 °C for 1 min, and all assays were run on a Bio-Rad iCycler with a MyiQ™ Single Color PCR Detection System (Bio-Rad, Hercules, CA, USA). Melt curve analysis was also performed to confirm amplification of a single product and the absence of primer–dimers. For each gene, a standard curve was made from a pool of RNA from samples representing all treatments and sexes. Each standard was serially diluted and assayed in triplicate. DNA contamination was assessed for each gene by analyzing RNA samples that were not reverse-transcribed, and each qPCR run included two samples without cDNA template to further control for contamination. Transcript abundance for *ef-1α* was quantified as the normalizing gene. For each gene, correlation coefficients (r^2) for the standard curve ranged from 0.982 to 0.992. PCR efficiencies for each gene were calculated using the equation: efficiency = $[10^{(-1/\text{slope})} - 1]$, and are provided in Table 1. For each gene, relative mRNA levels were subsequently calculated using the standard curve and normalized to *ef-1α* mRNA expression. Finally, expression of each gene of interest was expressed as a relative level by dividing the resulting values by the mean values of males in the control treatment group.

2.6. Histological confirmation of gonadal sex identity

Gonads that were fixed in 4% paraformaldehyde were subsequently transferred to 70% ethanol, dehydrated through a graded series of ethanol, embedded in paraffin, and sectioned longitudinally (10 μm). Three sections were collected from each fish's

Table 1
Primers for SYBR green quantitative real-time PCR assays.

Transcript	Primer	Sequence (5′–3′)	Amplicon size (bp)	% PCR efficiency (avg.)
dio1	Forward	CAACAGACCGCTGGTGCTGAATTT	92	99.87
	Reverse	TGAAGTCCCTGACGAGTTGCTTGA		
dio2	Forward	TTGGATTTCGAGTCATCGGATCGC	312	95.00
	Reverse	ATTGTTGTCCATGCTGTCTGCCAC		
dio3	Forward	TGTGTGTCTCCGACTCCAACAAGA	280	99.33
	Reverse	AAGTCCGCAATGTCTTGGTACTGG		
trαA	Forward	AAGCGCAAGTTCTTGCCAGATGAC	275	102.03
	Reverse	TCGCCGCTTAGTGTCAATGTCTCA		
trαB	Forward	TATGGCAGCTCGCAGTGACATGA	249	103.10
	Reverse	CTCCCAGTGGAAACAGAAACGCAAA		
trβ	Forward	AAAGCCACGGGCTACCACTA	121	101.67
	Reverse	TGACGCATTTCCCCTCGTA		
ef1α	Forward	CATCGACATTGCTCTGTGGAA	121	100.23
	Reverse	GGCGCAGTCAGCCTGAGA		

gonad; the first section was collected approximately halfway through the tissue with two additional sections collected at subsequent 100–200 μm intervals. All sections were stained with hematoxylin and eosin, and examined qualitatively under a light microscope to confirm gonadal sexual identity.

2.7. Sequencing of partial cytochrome B gene from genomic DNA

Striped parrotfish are morphological similar to their sister taxon, the princess parrotfish (*Scarus taeniopterus*), and commonly change body coloration upon capture making definitive species identification difficult based on morphology alone. As such, species identity of all striped parrotfish collected was confirmed by sequencing the cytochrome *B* (*cytB*) gene. Nested sets of gene-specific primers for a 381-bp region of *cytB* were designed to consensus regions of the genes described previously from striped parrotfish (*Scarus iserti*, GenBank Accession No. EU601382) and princess parrotfish (*S. taeniopterus*, EU601394) [66]. The outer nested primer set was (forward) 5′-CCTAGTTGACCTCCCTGC-3′ and (reverse) 5′-GTTGCACCTCAGAAGGA-3′, and the inner nested primer set was (forward) 5′-TCAAACATCTCTGTCTGATGAA-3′ and (reverse) 5′-TAGCCTACGAATGCTGTTAT-3′.

Genomic DNA was isolated from skeletal muscle tissue samples using the DNeasy Cell and Tissue Kit (Qiagen), and the resulting genomic DNA was amplified in 50 μl PCRs containing 2 μl of DNA template (62–168 ng), 25 μl GoTaq Colorless Master Mix (Promega), 21 μl nuclease-free H_2O, and 1 μl each of forward and reverse primer (10 mM) using a thermal profile of 42 cycle of 94 °C for 10 min, 42 cycles of 95 °C for 1 min, 48 °C for 1 min, and 72 °C for 2 min, followed by a final extension of 72 °C for 10 min. The resulting PCR products were purified and sequenced. All fish used in the experiment were confirmed genetically to be striped parrotfish.

2.8. Statistical analyses

Hormone data failed to conform to the assumptions of normality, so was square-root transformed prior to analysis. Transformed data was then analyzed by two-factor ANOVA models using 'treatment' and 'sex' as factors, as well as the interaction between these factors, followed by Tukey HSD tests for multiple comparisons among the treatment groups for each sex separately. Transcript abundance data was likewise analyzed using two-factor ANOVA models with 'treatment' and 'sex' as factors. When a significant effect of 'treatment' was found, Tukey HSD tests were calculated for each sex separately. When transcript abundance data failed to conform to the assumptions of parametric statistics, data were square root transformed prior to analysis.

3. Results

3.1. Identification of partial cDNAs encoding deiodinase enzymes and TH receptors

Degenerate primer PCR amplified partial cDNAs encoding *dio1* (169-bp nucleotides, GenBank Accession No. HM120152), *dio2* (363-bp nucleotides, HM120253) and *dio3* (480-bp nucleotides, HM120254) from striped parrotfish liver. BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and phylogenetic analyses of the deduced amino acid sequences of these partial cDNAs confirmed their identities as encoding iodothyronine deiodinases (Fig. 1A).

Similarly, degenerate primer PCR using degenerate primers designed to consensus regions of teleost *trα* transcripts identified two distinct cDNAs from striped parrotfish. BLAST and phylogenetic analyses revealed that both cDNAs encoded putative partial coding regions of *trα* subtypes (Fig. 1B). One partial cDNA of 540-bp nucleotides encoded for a partial coding region of 179 amino acid residues for a *trαA* gene (GenBank Accession No. HM120255), while a second cDNA of 630-bp nucleotides in length encoded for a second receptor *trαB* (HM120256). Although these *trαA* and *trαB* cDNAs showed 83.0% identify in their nucleotide sequences and 91.6% identity in their deduced protein sequences, the pattern of nucleotide differentiation suggests that the two mRNAs represent two distinct *trα* genes, rather than splice variants of a single gene (On-line Supplemental materials, Fig. 1). In addition to these two *trα* cDNAs, degenerate primer PCR also amplified a *trβ* partial cDNA of 1080-bp nucleotides from the parrotfish liver, which encoded 359 amino acid residues (GenBank Accession No. HM120257).

3.2. Plasma T_4 and T_3 levels

Plasma total T_4 levels decreased in fish treated with methimazole (males: mean = 18.4 ng/ml, range = 10.0–30.6 ng/ml; females: mean = 18.8 ng/ml, range = 9.5–28.7 ng/ml) from levels in control fish (males: mean = 33.9 ng/ml, range = 23.3–39.8 ng/ml; females: mean = 31.5 ng/ml, range = 21.6–52.0 ng/ml) ($F_{2,22}$ = 4.3037, p = 0.026) (Fig. 2A). Pairwise statistical comparisons revealed that the extent of this methimazole-induced decrease was greater in males than in females. There were no differences in plasma total T_4 levels between males and females.

Plasma total T_3 levels increased in both male and female IP striped parrotfish exposed to exogenous T_3 compared to levels in fish in the control group ($F_{2,27}$ = 13.403, $p < 0.0001$) (Fig. 2B). Although mean T_3 levels increased by 106% in T_3-exposed females over levels in control females, and 85% in T_3-exposed males relative to control males, these increases were in the physiological range normally encountered by parrotfish, since some parrotfish in the

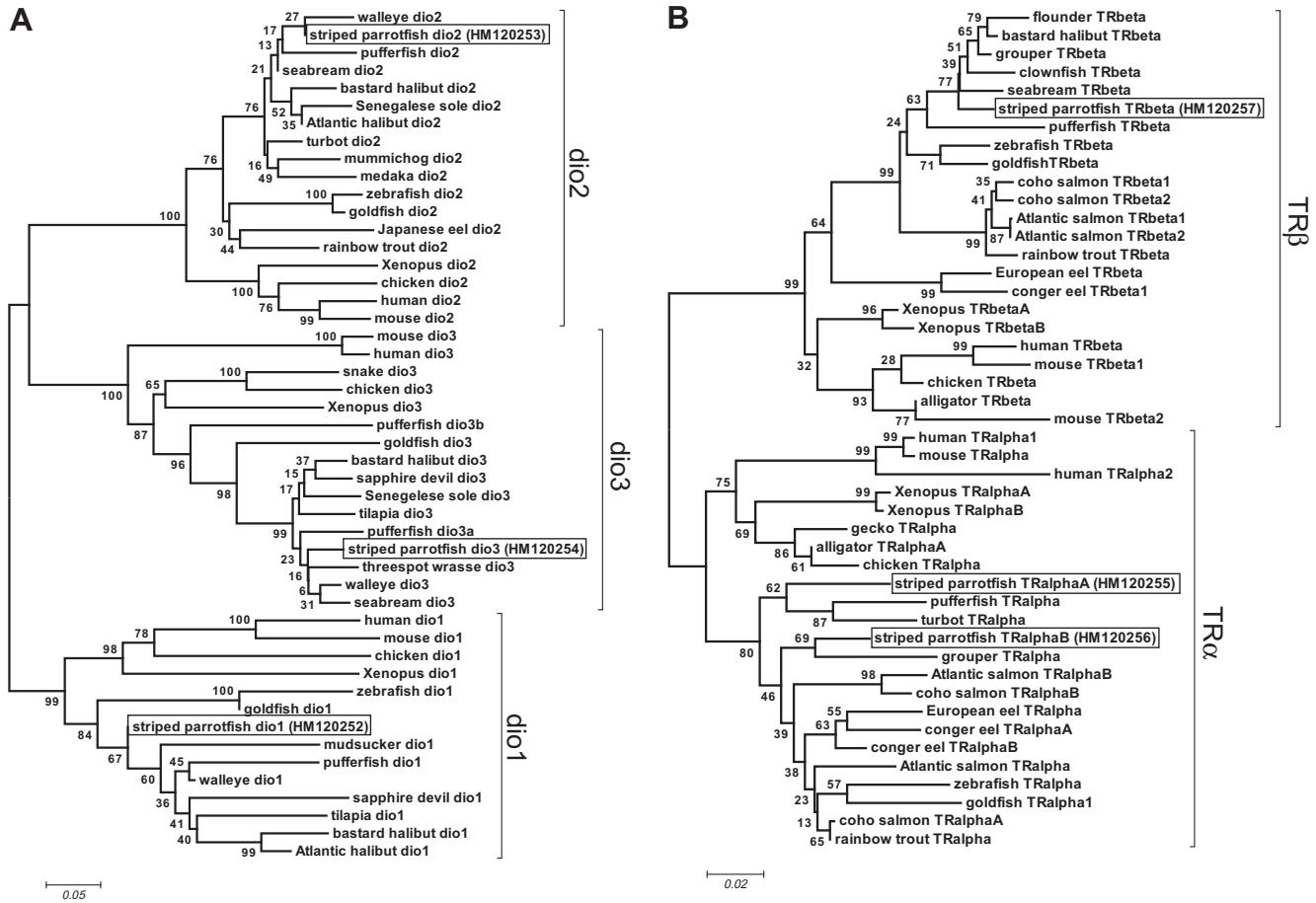

Fig. 1. Phylogenetic trees of deiodinase enzymes (A) and thyroid hormone receptors (B) based on alignment of deduced amino acid sequences from fish and other vertebrates. Partial cDNAs identified from striped parrotfish are shown in boxes with associated GenBank accession numbers. Clades for type 1 (dio1), type 2 (dio2) and type 3 (dio3) deiodinase enzymes (A), and for thyroid hormone receptor types α (TRα) and β (TRβ) (B), are demarked by brackets. Trees were assembled with the Neighbor-Joining method with pairwise deletion of gaps, and bootstrap values are indicated at each node. GenBank accession nos. for all taxa are provided in Online Supplemental Materials, Table 2.

control groups (males: mean = 23.8 ng/ml, range = 8.1–51.0 ng/ml; females: mean = 26.0 ng/ml, range = 9.4–44.6 ng/ml) had T_3 levels approaching mean values observed in T_3-exposed individuals of the same sex (males: mean = 44.0 ng/ml, range = 23.6–61.6 ng/ml; females: mean = 51.3 ng/ml, range = 41.1–57.9 ng/ml). There were no differences in plasma total T_3 levels between males and females.

3.3. Regulation of deiodinase and thyroid hormone receptor mRNAs in liver

In the liver, transcripts encoding *dio2* were significantly elevated in parrotfish treated with methimazole ($F_{2,32} = 10.984$, $p = 0.0002$), although *post hoc* pairwise statistical comparisons revealed that this increase was greater in males than in females (Fig. 3B). Liver *dio2* transcript abundance was unaffected by treatment with exogenous T_3. Transcript levels of *dio2* were also similar between sexes. Transcript abundance for *dio3* showed a significant increase in the liver of T_3-treated parrotfish of both sexes ($F_{2,32} = 18.425$, $p < 0.0001$) (Fig. 3C). Levels of *dio3* mRNAs also varied in the liver between the sexes, with males exhibiting a higher abundance of *dio3* transcript than females ($F_{1,32} = 7.258$, $p = 0.011$). There was no change in liver *dio3* abundance in methimazole-treated fish. Liver *dio1* mRNA levels were unaffected by either methimazole or T_3, but were higher in males than in females ($F_{2,32} = 5.266$, $p = 0.028$) (Fig. 3A).

Relative transcript abundance for *trαA* was elevated in the liver of T_3-treated fish ($F_{2,32} = 3.517$, $p = 0.042$), although this increase was not significant in pairwise comparisons (Tukey HSD tests) (Fig. 3D). Levels of *trαA* mRNAs in the liver were similar between males and females. Transcripts encoding *trβ* also increased in the liver of T_3-treated fish of both sexes ($F_{2,32} = 5.753$, $p = 0.0073$) (Fig. 3F), but were unaltered by methimazole. Male and female parrotfish had similar levels of *trβ* mRNAs in the liver. Transcript abundance for *trαB* did not vary with treatment or sex (Fig. 3E).

3.4. TH regulates brain expression of dio2, dio3, trαA, and trβ mRNAs

In the brain, *dio2* transcript abundance increased following methimazole exposure and showed a trend of being reduced by T_3 elevation, although the magnitude of this effect differed between the sexes (treatment $*$ sex interaction, $F_{2,32} = 3.909$, $p = 0.030$) (Fig. 4B). Overall, the pattern of change observed was suggestive of brain *dio2* gene transcription being induced by reductions in circulating THs, and inhibited with TH elevation (treatment effect, $F_{2,32} = 12.097$, $p = 0.0001$). Brain *dio2* mRNA levels also differed between the sexes, with females having a greater abundance of *dio2* transcripts than males (sex effect, $F_{1,32} = 17.395$, $p = 0.0002$). Brain *dio3* relative mRNA levels also differed in the parrotfish brain following experimental manipulation of circulating TH levels, with male and female parrotfish showing elevated *dio3* transcript abundance following treatment with T_3 ($F_{2,32} = 17.763$, $p < 0.0001$) (Fig. 4B). Transcript abundance for *dio1* in the brain did not vary among treatments, although *dio1*

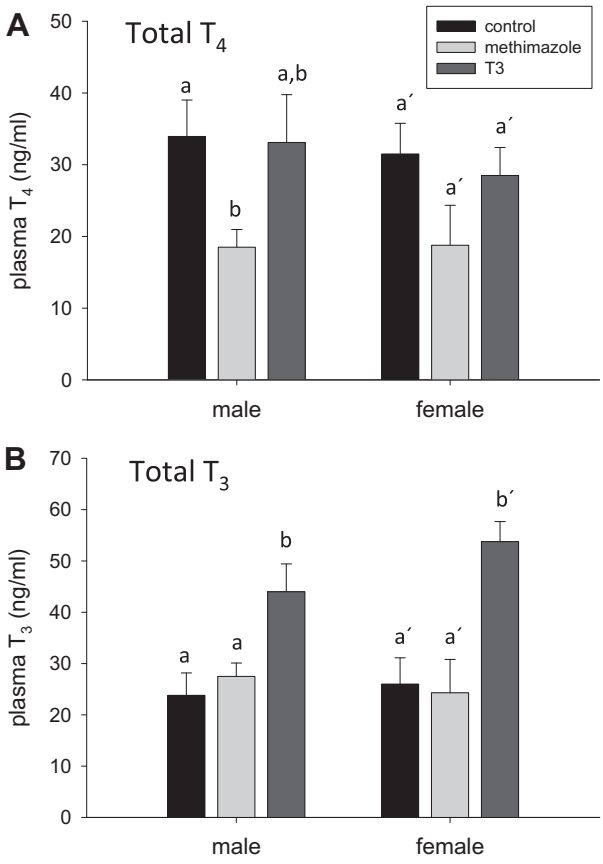

Fig. 2. Exposure to dissolved phase methimazole and T_3 altered plasma levels of total T_4 (A) and total T_3 (B). Methimazole depressed total T_4 in males, while exogenous T_3 significantly increased total T_3 plasma levels in both sexes. Letters indicate significant differences within each sex separately (Tukey HSD tests). Data are shown as mean ± SEM.

mRNAs were higher in the brains of males than in females ($F_{1,32} = 6.769$, $p = 0.014$) (Fig. 4A).

Relative transcript abundance for *trαA* was elevated 67% in the brain of T_3-exposed males, and 77% in the brains of T_3-exposed females, relative to control fish of the same sex ($F_{2,32} = 9.014$, $p = 0.0008$) (Fig. 4D). While mRNA levels for *trαB* did not differ with treatment or sex (Fig. 4E), *trβ* mRNAs were autoinduced in the brain of both sexes by T_3 ($F_{2,32} = 12.685$, $p < 0.0001$) (Fig. 4F). Females in the T_3 treatment showed an 82% increase in the relative abundance of *trβ* transcripts in the brain, while males showed an increase of 56%. While exogenous T_3 induced clear increases in *trαA* and *trβ* receptor mRNAs, treatment with methimazole did not alter mRNA levels for any TH receptor.

3.5. Deiodinase and thyroid hormone receptor mRNA regulation in the gonad

Comparisons of relative transcript abundance for *dio1* in the gonads revealed no effect of methimazole or exogenous T_3 in either sex (Fig. 5A). Females did, however, show higher levels of *dio1* transcript in the ovaries compared to the testes of male fish ($F_{1,31} = 9.657$, $p = 0.004$). Males given exogenous T_3, however, had testes *dio2* transcript levels that were elevated 135% over control fish ($F_{2,31} = 7.430$, $p = 0.0023$) (Fig. 5B). Females treated with T_3 showed an even greater magnitude (459%) of *dio2* mRNA increase in the ovary, but the high variation seen in *dio2* mRNA levels among T_3-treated females led this increase to be non-significant in *post hoc* pairwise statistical comparisons. While the effects of T_3 on *dio2* mRNA levels were similar between the sexes, there was a greater abundance of *dio2* transcript in the testis than in ovary ($F_{1,31} = 12.855$, $p = 0.0011$). Gonadal levels of mRNAs encoding *dio3* varied among treatments ($F_{2,31} = 3.579$, $p = 0.040$), with increased *dio3* mRNAs in the testes of T_3-treated male parrotfish. Similar to *dio2* transcripts, *dio3* mRNA levels differed significantly between the sexes with a higher abundance of *dio3* transcript in the testis than ovary ($F_{1,31} = 5.293$, $p = 0.0283$) (Fig. 5C).

Transcripts encoding *trαA* were present at relative levels 9 times greater in the testes than ovary ($F_{1,30} = 34.629$, $p < 0.0001$) regardless of treatment (Fig. 5D). In both sexes, exogenous T_3 induced a moderate increase in *trαA* mRNA levels ($F_{2,32} = 3.199$, $p = 0.055$), although this increase was only significant in the testis in pairwise statistical tests. Transcripts for *trαB* were also expressed at a level 10 times greater in testes than in ovarian tissue (sex effect: $F_{1,31} = 30.759$, $p < 0.0001$), and also showed increased expression in response to T_3 ($F_{2,31} = 3.755$, $p = 0.035$) (Fig. 5E). Transcripts for *trβ* were also greater in the testes than in the ovary ($F_{1,30} = 18.834$, $p = 0.0001$) (Fig. 5F), but were unaltered in either sex by T_3 or methimazole ($F_{2,30} = 2.318$, $p = 0.11$).

4. Discussion

Here, we examined the influence of circulating TH status on relative levels of mRNAs encoding deiodinase enzymes (*dio1*, *dio2* and *dio3*) and thyroid hormone receptors (*trαA*, *trαB* and *trβ*) in the liver, brain and gonads of male and female initial phase striped parrotfish. Parrotfish treated with dissolved phase T_3 for 3 days showed elevated plasma total T_3 levels, while fish treated with methimazole exhibited depressed plasma total T_4. These alterations in TH status led to tissue- and transcript-specific changes in the relative abundance of mRNAs encoding deiodinase enzymes and TRs. Although *dio1* transcripts in all tissues examined were unaffected by changes in thyroid hormone levels, the methimazole-induced reduction in T_4 resulted in increased *dio2* mRNA abundance in liver and brain, but not in testes or ovary. Transcripts for *dio3*, in contrast, were higher in relative abundance in the liver, brain and testes – but not in the ovary – of parrotfish experimentally made hyperthyroid with exogenous T_3. The relative abundance of *tr* mRNAs was likewise regulated in several tissues, although again the pattern of change depended on the tissue and transcript. Elevated T_3 induced an increased abundance of transcripts encoding *trαA* and *trβ* in both liver and brain, as well as *trαA* in the testes. Our results also indicate that transcripts for *trαB* increased in the testes – but not ovary – under both hypo- and hyperthyroidism, even though *trαB* mRNA levels were not affected in liver or brain by either condition.

4.1. Tissue-specific patterns of dio mRNA abundance changes with TH status

Taken as a whole, our results with parrotfish provide a comprehensive examination of TH-mediated *dio* transcript abundance changes in a teleost fish that, when compared to the results of previous studies (Table 2), indicate that teleost fishes exhibit tissue-specific patterns of *dio* transcript abundance change in response to alterations in thyroid status. While not demonstrated previously in fishes, there is precedent for similar tissue-specific patterns of *dio* transcript regulation in other vertebrates including mammals and amphibians (e.g., [29,67,79]). In mammals, for instance, it is well established that THs regulate both transcriptional and post-transcriptional mechanisms to influence Dio enzyme activity [4,67]. While elevated TH levels are generally considered to increase Dio1 and Dio3 activity and decrease Dio2 activity in mammalian tissues, these regulation patterns vary among tissue

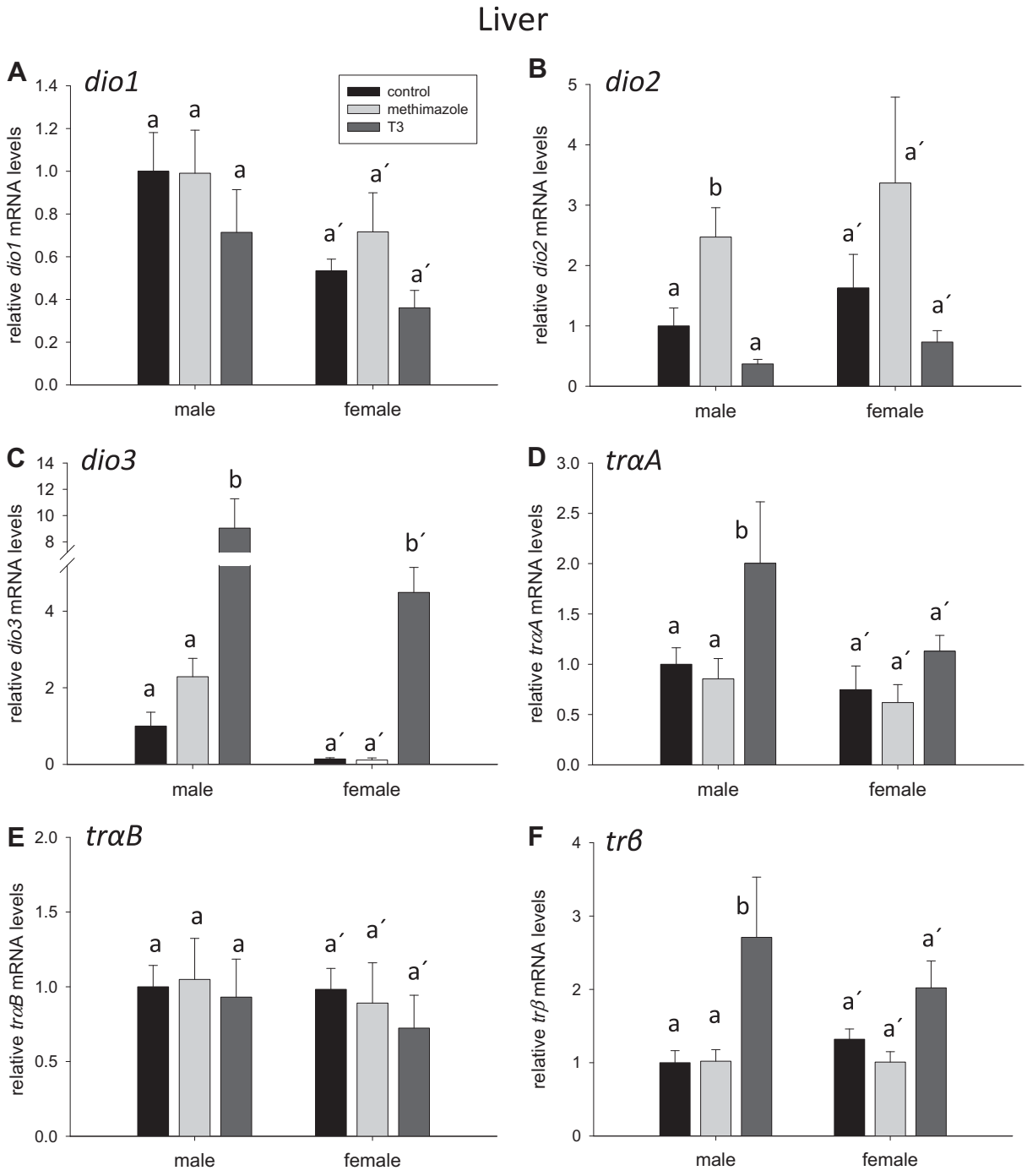

Fig. 3. Hypothyroidism induced by exposure to dissolved phase methimazole induced an elevation in hepatic *dio2* mRNA levels in male parrotfish (B), while hyperthyroidism induced increases in *dio3* (C), *trαA* (D) and *trβ* (F) transcript abundance in the liver. Transcripts encoding *dio1* and *trαB* were unaffected by changes in thyroid hormone status (A and E). Letters indicate significant differences within each sex separately (Tukey HSD tests). Results are expressed as normalized transcript abundance with respect to *ef-1α* mRNA levels from the same sample, with the mean value for the male control group for each gene normalized to a value of 1.0. All data are shown as mean ± SEM.

types. For example, in rats *dio2* mRNA levels decrease in the cerebral cortex under hyperthyroid status [7], even though *dio2* mRNAs are upregulated by T_3 in cultured brown fat adipocytes [41]. These and other studies therefore suggest that specific patterns of *dio* gene regulation vary cell- and tissue-specific patterns, likely in accordance with the distinct tissue-specific functions for TH action during a given developmental period [67].

The functional roles of the three Dio enzymes therefore may lend some insight into why the patterns of *dio* mRNA regulation vary among tissues. In teleost fishes as in other vertebrates, Dio2 is a T_4-outer-ring deiodinase (T_4-ORD) that converts T_4 to the more bioactive T_3 [54]. In parrotfish, hepatic *dio2* mRNA levels increased under conditions of depressed systemic T_4, a result consistent with that observed previously in the liver of other fishes [74] (Table 2). Accordingly, other studies have found that hyperthyroidism induced by T_3 administration reduces *dio2* mRNA levels in teleost liver [5,17] (Table 2), even though we did not observe a similar effect in striped parrotfish. Taken together, these findings support the idea that changes in *dio2* transcript abundance – and, subsequently, Dio2 activity – in liver help regulate peripheral T_3 status [15,54].

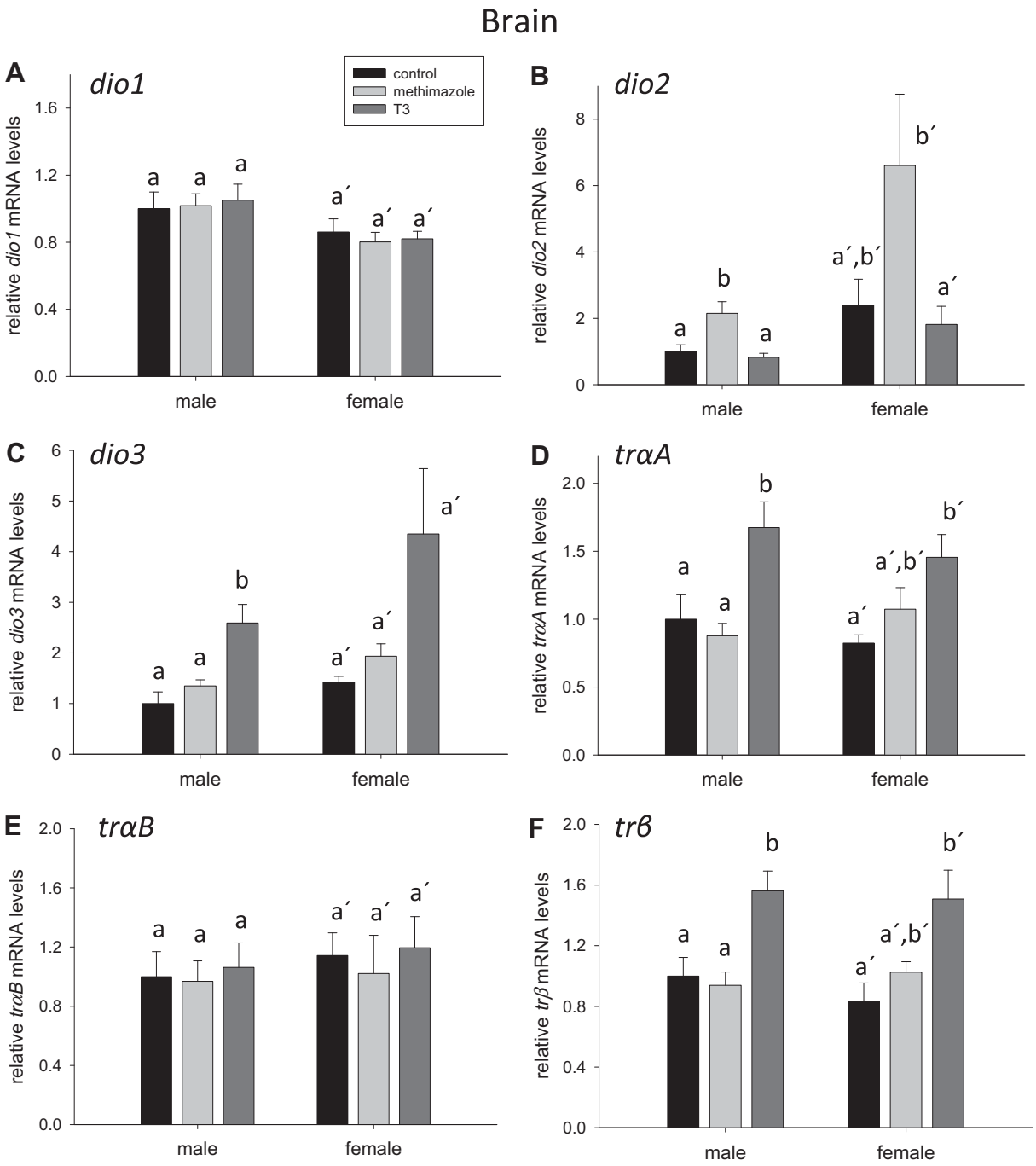

Fig. 4. Regulation of *dio* and *tr* mRNA levels in the brain by changes in plasma thyroid status. Methimazole-induced depression in T_4 induced increased *dio2* mRNA levels in both male and female initial phase parrotfish (B). Exogenous T_3 increased relative gene transcripts for *dio3* (C), *trαA* (D) and *trβ* (F) in the brain. Transcripts encoding *dio1* (A) and *trαB* (D) in the brain were unaltered by thyroid hormone status. Letters indicate significant differences among treatments within each sex separately (Tukey HSD tests). Data are plotted as mean ± SEM.

In addition to these effects on liver *dio2*, however, we also detected *dio2* transcripts in the brain tissues of all parrotfish examined, and even observed increased *dio2* relative abundance under conditions of depressed T_4 in methimazole-treated fish (see Fig. 4B). While some early investigations in teleosts were unable to detect *dio2* transcripts in the brain using Northern blot or RT-PCR techniques [5,60], our findings using the more sensitive technique of quantitative real-time RT-PCR provide evidence that *dio2* transcripts can be present in the teleost brain and that relative *dio2* mRNA abundance can respond to changes in circulating TH status in patterns similar to those seen in liver. While this finding may at first appear to contradict those of previous studies, levels of *dio2* mRNA were 10- to 30-fold lower (3–5 C_t values) in parrotfish brain than in liver, suggesting that *dio2* transcripts may be present in the brain of many teleosts at levels too low for detection with less sensitive methods. Recently, studies in other fish species have also localized *dio2* mRNAs to the fish brain, which provides further support for this idea [21,48,55,63,68]. Further, several studies have found T_4-ORD activity in the teleost fish brain, although generally at low levels [14,16,46,49,56], and previous studies with rainbow trout and Atlantic salmon (*Salmo salar*) even provide evidence that Dio2 activity in the brain of these species may be TH regulated

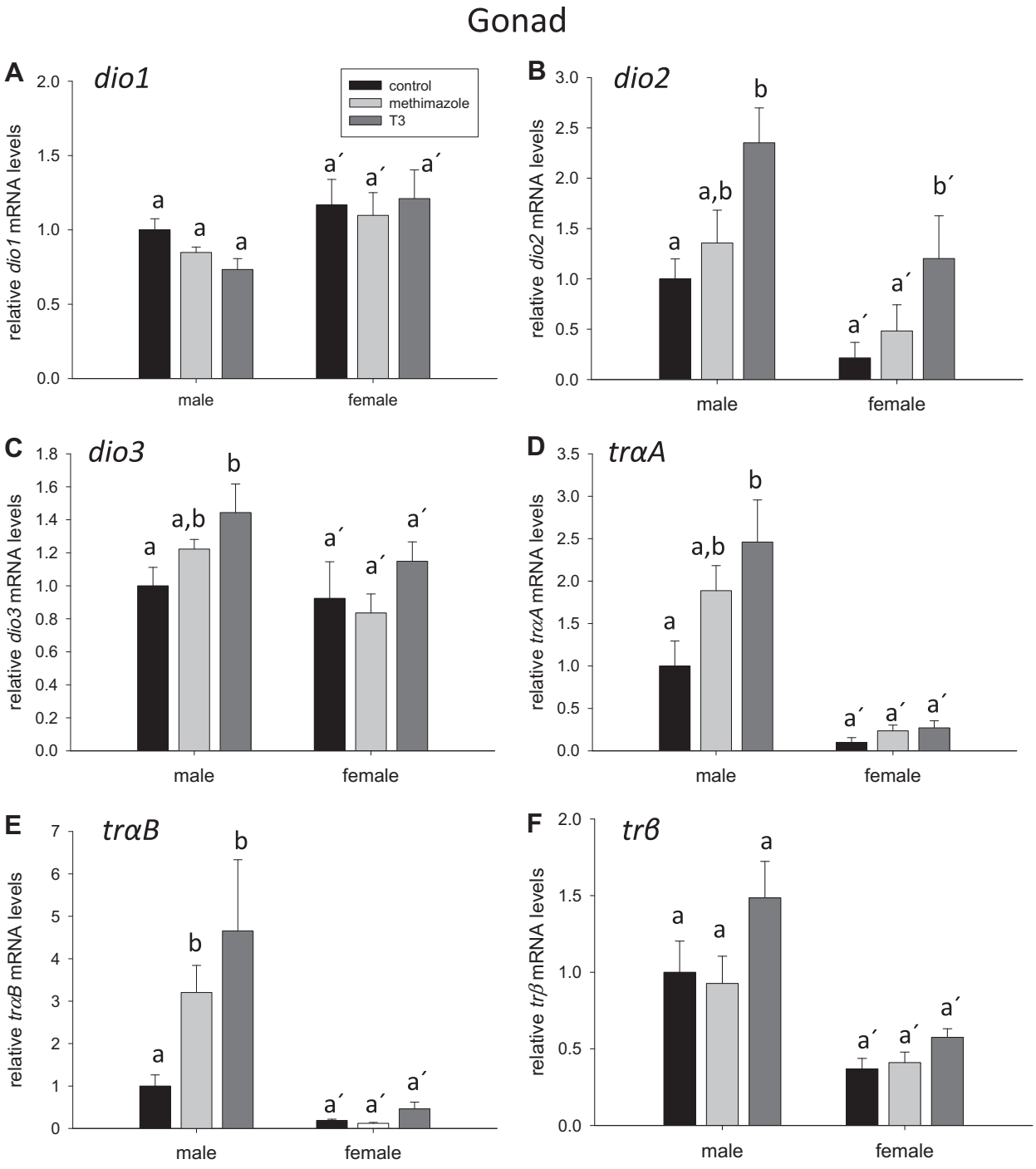

Fig. 5. Gonadal gene transcript abundance for *dio2* (B), *dio3* (C), *trαA* (D) and *trαB* (E) were significantly elevated in the testes of male parrotfish experimentally made hyperthyroid by exogenous T_3, while *dio1* (A) and *trβ* (F) were unaffected. Transcripts encoding *trαB* (E) also increased in the testes of males made hypothyroid by methimazole exposure. In females, only ovarian *dio2* mRNA levels were affected by altered thyroid hormone status (B). Transcripts encoding *dio2*, *dio3*, *trαA*, *trαB* and *trβ* were higher in abundance in the testes than ovary, while *dio1* mRNA levels were greater in ovarian tissue than testicular tissue. Letters indicate significant differences among treatments within each sex separately (Tukey HSD tests). All data are plotted as mean ± SEM.

[14,49]. Similar findings have been documented in other vertebrates; in rats, for instance, hypothyroidism elevates – and exogenous T_3 decreases – *dio2* mRNA levels and Dio2 activity in the brain [7,73], and thyroid hormone-induced changes in Dio2 expression have been shown to occur in part through TH-induced changes in *dio2* transcriptional regulation [27]. Clearly more work is needed to explicate the functional significance of *dio2* mRNAs in the brain of fish, and future studies should address whether *dio2* transcript abundance in the brain may vary in accordance with life history stage or under environmental conditions that modulate plasma or brain TH levels.

In addition to our findings with *dio2*, our results also indicate that TH status influences relative levels of *dio3* transcript in the liver and brain of parrotfish. These thyroid hormone-induced changes in relative *dio3* abundance are similar to those observed in other fishes, where hyperthyroidism increases – and hypothyroidism decreases – *dio3* mRNA abundance and Dio3 activity in liver and brain [5,8,48,52] (Table 2). While the specific mechanism

Table 2
Summary of evidence for thyroid hormone effects on deiodinase (*dio*) and thyroid hormone receptor (*tr*) mRNA abundance in teleost fishes.

Transcript	Tissue/cell	Context	Hormone manipulation	Relative mRNA abundance response	Species	Ref.
dio1	liver	*in vivo*	↑ T_4	n.c. after 12 h; ↓ after 24 h	*Fundulus heteroclitus* (adult)	[17]
			↑ T_3, or ↑ T_2	↓ after 12 h; ↓ after 24 h		
	liver	*in vivo*	↓ T_3 (via MMI administration)	↑ after 90 days	*Oreochromis niloticus* (juv.)	[74]
	liver	*in vivo*	↓ T_3 (via MMI administration)	↑ after 90 days	*Sarotherodon melanotheron* (juv.)	[74]
	whole body	*in vivo*	↑ T_3	↓ after 7 days; n.c. after 18 and 31 days	*Sparus aurata* (larval)	[8]
	whole body	*in vivo*	↑ T_3 (embryos exposed beginning at 2 hpf)	n.c. from 8 to 75 hpf	*Danio rerio* (embryo/larval)	[78]
dio2	liver	*in vivo*	↑ T_4, ↑ T_3, or ↑ T_2	↓ after 12 h; ↓ after 24 h	*Fundulus heteroclitus* (adult)	[17]
	liver	*in vivo*	↓ T_3 (via MMI administration)	↑ after 90 days	*Oreochromis niloticus* (juv.)	[74]
	liver	*in vivo*	↓ T_3 (via MMI administration)	↑ after 90 days	*Sarotherodon melanotheron* (juv.)	[74]
	liver	*in vivo*	PTU administration (n.c. in plasma T_4 or T_3)	↑ after 21 days	*Sparus aurata* (juv.)	[48]
	liver	*in vivo*	↑ T_3	↓ after 7 days	*Oncorhynchus mykiss*	[5]
			↑ T_4	n.c. after 7 days		
	brain	*in vivo*	PTU administration (n.c. in plasma T_4 or T_3)	↓ after 21 days	*Sparus aurata* (juv.)	[48]
	whole body	*in vivo*	↑ T_3	↓ after 7 days; n.c. after 18 and 31 days	*Sparus aurata* (larval)	[8]
	whole body	*in vivo*	↑ T_3 (embryos exposed beginning at 2 hpf)	↓ at 75 hpf; n.c. from 8-48 hpf	*Danio rerio* (embryo/larval)	[78]
dio3	liver	*in vivo*	↑ T_3	↑ after 7 days	*Oncorhynchus mykiss*	[5]
			↑ T_4	n.c. after 7 days		
	liver	*in vivo*	↑ T_3	↑ after 12 and 24 h	*Carassius auratus* (adult)	[52]
	hepatocytes	*in vitro*	↑ T_3	↑ after 5 h	*Carassius auratus*	[52]
	brain	*in vivo*	PTU administration (n.c. in plasma T_4 or T_3)	↓ after 21 days	*Sparus aurata* (juv.)	[48]
	whole body	*in vivo*	↑ T_3	↓ after 18 days; n.c. after 7 and 31 days	*Sparus aurata* (larval)	[8]
trα	liver	*in vivo*	↑ T_3	↑ after 10 days (both male and female)	*Pimephales promelas* (adult)	[34]
			↓ T_4 (via MMI administration)	n.c. after 10 days (both male and female)		
	hepatocytes	*in vitro*	↑ T_3, ↑ T_4	↑ after 19 h	*Conger myriaster*	[25]
	brain	*in vivo*	↑ T_3	↑ after 10 days (both male and female)	*Pimephales promelas* (adult)	[34]
			↓ T_4 (via MMI administration)	n.c. after 10 days (both male and female)		
	gonad	*in vivo*	↑ T_3	n.c. after 10 days (both testis and ovary)	*Pimephales promelas* (adult)	[34]
			↓ T_4 (via MMI administration)	n.c. after 10 days (both testis and ovary)		
	gonad	*in vivo*	↑ T_3	*trα1*: n.c. after 12 h (both testis and ovary); ↓ after 24 & 36 h (testis); ↓ after 24 h but n.c. after 36 h (ovary) *trα2*: n.c. after 12, 24 & 36 h (testis and ovary) *trα-t*: ↑ after 12, 24 & 36 h (testis) ↑ after 24 & 36 h (ovary)	*Carassius auratus* (adult)	[81]
		in vitro	↑ T_3	*trα1*: ↓ after 8 h (ovary); n.c. after 8 h (testis); n.c. after 4 & 24 h (both testis and ovary) *trα2*: n.c. after 12, 24 & 36 h (testis and ovary) *trα-t*: ↑ after 4, 8 & 24 h (testis) ↑ after 8 h (ovary); n.c. after 4 & 24 h (ovary)		
	whole body	*in vivo*	thiourea administration	n.c. after 8 days, ↑ after 13 days	*Solea senegalensis* (larval)	[40]
			↑ T_4	n.c. after 8 and 13 days		
	whole body	*in vivo*	↑ T_3	↓ after 31 days; n.c. after 7 and 18 days	*Sparus aurata* (larval)	[8]
	whole body	*in vivo*	↑ T_3 (embryos exposed beginning at 2 hpf)	↑ at 48 hpf; n.c. from 8-36 hpf and at 75 hpf	*Danio rerio* (embryo/larval)	[78]
	whole body	*in vivo*	↑ T_3 (beginning at 0 hpf)	↓ at 24 hpf, ↑ at 72 and 144 hpf	*Danio rerio* (embryo/larval)	[39]
	HeLa cells	*in vitro*	↑ T_3	↑ after 24 h	*Danio rerio*	[39]
trβ	liver	*in vivo*	↑ T_3	↑ after 10 days (both male and female)	*Pimephales promelas* (adult)	[34]
			↓ T_4 (via MMI administration)	n.c. after 10 days (both male and female)		
	hepatocytes	*in vitro*	↑ T_3, ↑ T_4	↑ after 19 h	*Conger myriaster*	[25]
	hepatocytes	*in vitro*	↑ T_3	↑ after 19 h	*Anguilla japonica*	[26]
	brain	*in vivo*	↑ T_3	↑ after 10 days (both male and female)	*Pimephales promelas* (adult)	[34]
			↓ T_4 (via MMI administration)	n.c. after 10 days (both male and female)		
	gonad	*in vivo*	↑ T_3	↑ after 10 days (both testis and ovary)	*Pimephales promelas* (adult)	[34]
			↓ T_4 (via MMI administration)	n.c. after 10 days (both male and female)		
	gonad	*in vivo*	↑ T_3	↓ after 12, 24 & 36 h (testis); n.c. after 12, 24 & 36 h (ovary)	*Carassius auratus* (adult)	[81]
		in vitro	↑ T_3	↓ after 8 h (ovary); n.c. after 4 & 24 h (ovary); n.c. after 4, 8 & 24 h (testis)		
	whole body	*in vivo*	thiourea administration	↓ after 11 & 15 days	*Solea senegalensis* (larval)	[40]
			↑ T_4	↑ after 8 days; n.c. after 13 days		
	whole body	*in vivo*	↑ T_3	↓ after 18 days; n.c. after 7 & 31 days	*Sparus aurata* (larval)	[8]
	whole body	*in vivo*	↑ T_3 (embryos exposed beginning at 2 hpf)	↑ at 75 hpf; n.c. from 8-48 hpf	*Danio rerio* (embryo/larval)	[78]
	whole body	*in vivo*	↑ T_3 (beginning at 0 hpf)	↓ at 24 hpf, ↑ at 72 and 144 hpf	*Danio rerio* (embryo/larval)	[39]
	HeLa cells	*in vitro*	↑ T_3	↑ after 24 h	*Danio rerio*	[39]

Abbreviations: hpf, hours post-fertilization; juv, juvenile; MMI, methimazole; n.c., no change; PTU, propilthiouracil; T_2, 3,5-diiodothyronine.

by which THs regulate Dio3 expression in fish remains to be determined fully, recent findings suggest that both TRα and TRβ can mediate T_3-induction of *dio3* transcription [52]. In goldfish (*Carassius auratus*), siRNA inhibition of both *trα-1* and *trβ* mRNAs diminished T_3 induction of *dio3* gene transcription in cultured hepatocytes [52] (reviewed by [51]). Interestingly, siRNA inhibition of a third goldfish receptor transcript (*trα-t*), which is truncated by a premature stop codon and lacks the ligand-binding domain, was found to increase steady-state *dio3* mRNA levels and enhance T_3 induction of *dio3* transcription, providing evidence for TR-subtype specific functions in teleost deiodinase regulation [52]. Beyond these recent findings, however, the overall response patterns of

dio3 mRNA abundance and Dio3 activity appear to correspond to Dio3's proposed function in teleosts as an inner-ring deiodinase (IRD) that protects tissues from excesses of active TH [54,61] (Table 2), although there is also some evidence that the specific TH-induced dynamics of Dio3 expression can again vary with cell and tissue type. In tilapia, for instance, treatment with gonadotropin (porcine FSH) elevated circulating T_4 and increased brain T_3-IRD activity, even though exogenous T_3 failed to have a similar effect; a methimazole-induced depression in circulating T_4 and T_3, however, was observed to reduce brain T_3-IRD activity in these same fish [47]. In other studies with tilapia, however, T_3-IRD activity in brain was not affected by either T_4 or T_3, even though T_3-IRD activity increased in gills and liver with T_3 elevation [5,75].

Beyond these effects in liver and brain, TH status also affected the relative abundance of *dio2* and *dio3* mRNAs in the parrotfish gonads (see Fig. 5B and C). There is little data available on *dio* transcripts or Dio activity in the teleost gonad, although *dio2* and *dio3* transcripts have previously been localized to gonadal tissues of fish [21,60], and *dio2* mRNA levels in the gonad of rainbow trout were found at levels nearly has high as those seen in liver [60]. In trout, transcripts encoding *dio2* appear to reach highest levels in the testis during early spermatogenesis stages [60], suggesting a role for Dio2 in the regulation of sperm production. Consistent with this idea, there is evidence that THs regulate testicular development and spermatogenesis in teleosts [11]. For instance, in Nile tilapia, hypothyroidism experienced during early life results in a larger mass testis with more Sertoli cells and increased spermatozoa after maturation [42], while 21-day thiourea exposures of pre-spawning male catfish (*Clarias gariepinus*) showed narrowed seminiferous tubules and fewer spermatozoa [69]. In rats, deiodinase activity has been demonstrated within the testes [3], and recently Dio2 expression – and *dio2* mRNAs – were found to be highly expressed in testicular germ cells [77]. Transcripts for *dio2* have also been shown to be upregulated in the rodent testis under hypothyroid status [76]. Beyond what is presented here with parrotfish, however, comparable data are currently unavailable for teleost fishes, and future studies that localize and quantify deiodinase expression in the gonads are needed.

Lastly, we found no evidence for TH regulation of *dio1* mRNA levels in any parrotfish tissues examined. Previous studies in larval and adult fishes have found mixed results, with some studies concluding that *dio1* transcript abundance was unaffected by changes in systemic TH levels [78], while other studies observed TH-dependent *dio1* mRNA regulation [8,17,74] (Table 2). As such, the picture of TH-mediated *dio1* transcriptional regulation in teleost fishes is less clear than that for *dio2* and *dio3* [54]. In mammals, THs upregulate *dio1* gene transcription [4,29,80], at least in part via TRβ receptor action on TREs in *dio1*'s promoter region [1,22,28], although the degree of *dio1*'s T_3-dependent regulation varies among tissues [1]. As found here in parrotfish (Figs. 3F and 4F) and elsewhere, THs have been shown to autoinduce *trβ* transcription in teleosts [34,39,78] (Table 2). Exogenous T_3 treatment might therefore have altered expression levels of TRβ in ways that confound clear patterns of T_3 influence on *dio1* mRNA levels. Alternatively, even though the teleost *dio1* gene is homologous to the mammalian *dio1* gene (see Fig. 1), there is considerable evidence that the biochemical properties of Dio1 vary between fish and mammals, which may indicate that mechanisms for teleost *dio1* regulation may also be divergent from that of mammalian *dio1* [54,62].

4.2. Evidence for thyroid hormone receptor subtype-specific mRNA regulation

In addition to the observed changes in *dio* transcript abundance, we also found that thyroid hormone status influenced relative *tr* mRNA levels in patterns indicative of tissue-specific roles for TR receptors. In brief, TRs function to induce or repress the transcription of genes by acting as ligand-dependent transcription factors that bind in dimer pairs to thyroid response element (TRE)-binding domains in the promoter region of genes. In mammals and amphibians, *tr* genes themselves contain TREs so that transcription of these genes is autoinduced by T_3 [79], and although there has been comparatively little work on TREs in fish, TRs appear to function through similar a mechanism [39,51]. Fish generally appear to have at least one α and one β receptor type, which are encoded by at least two genes [40,51], although additional α and β receptor mRNA subtypes have been identified in several species. These receptors subtypes result from either alternative splicing or from gene duplication events, which are now recognized as common within bony fishes (e.g., [32]). In parrotfish, we identified three distinct *tr* cDNAs, termed *trαA*, *trαB* and *trβ* based on their phylogenetic relationships (see Fig. 1). Although these cDNAs are only partial sequences, the extent of nucleotide and deduced amino acid sequence divergence between the *trαA* and *trαB* cDNAs from parrotfish suggest that these transcripts may be encoded by two distinct *trα* genes (On-line Supplemental materials, Fig. 1).

Here, the relative abundance of transcripts encoding *trαA* and *trβ* – but not *trαB* – were elevated by T_3 in the brain and liver of both sexes of parrotfish, suggestive of T_3 autoinduction of *trαA* and *trβ* – but not *trαB* – in these tissues. This result is similar to that found in other fishes, where alterations in thyroid status have been found to alter *trα* and *trβ* transcript levels (Table 2). For instance, in the fathead minnow (*Pimephales promelas*), oral exposure to exogenous T_3 resulted in elevated *trα* and *trβ* transcript levels in the brain and liver, as well as *trβ* transcripts in the testes, but not ovary [34]. These receptor-subtype specific – and tissue-specific – patterns of *tr* transcript abundance change are analogous to the complex patterns of *tr* regulation observed in other vertebrates, where *tr* mRNAs are either induced or repressed depending on the receptor type (or subtype) and tissue [10,20,58]. In fish, it is also clear that TR subtypes are TH-regulated in developmental stage specific patterns [8,39,40,78].

While we found evidence for male-female differences in *dio* mRNA levels in several parrotfish tissues, the only sex differences in *tr* transcript abundance that we observed occurred in the gonads where *trαA*, *trαB* and *trβ* mRNA levels were all greater in testicular tissue than ovarian tissue (see Fig. 5D–F). There is little data available on TR expression in the teleost gonads, although transcripts encoding *trα* have previously been found to be greater in the testis than ovary of sexually mature fathead minnows [34]. Transcripts for *trβ*, however, were at greater levels in ovarian tissues than in testicular tissues of several other fishes [2,34,53], a pattern opposite to that observed here with parrotfish. Part of the explanation for the opposite sex pattern of *trβ* expression observed in parrotfish may lie in this study's examination of initial phase male parrotfish only. Striped parrotfish exhibit both initial phase and terminal phase male phenotypes, which differ from each other in coloration, behavior and spawning frequency. In stoplight parrotfish (*Sparisoma viride*), initial phase males have been shown to exhibit lower 11-KT and higher E_2 levels than terminal phase fish [9], implying that testicular function may also differ between initial and terminal phase male parrotfish. Whether such differences explain why gonadal *trβ* mRNA levels in these initial phase striped parrotfish showed a pattern opposite to that seen in these testes other fishes is unclear, but would be an interesting avenue for future use of these sex changing parrotfishes as a model for understanding TH-mediated reproductive function in teleost fishes.

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