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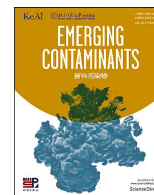

The antithyroid drug methimazole as an emerging aquatic contaminant: Physiological and reproductive disruption in female goldfish (*Carassius auratus*) and partial mitigation by thyroxine

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A B S T R A C T

Thyroxine (T4), a key thyroid hormone, plays a crucial role in regulating growth, metabolism, and reproduction in fish, whereas methimazole (MMI), a thyroid peroxidase inhibitor, can disrupt these processes by inducing hypothyroidism. This study investigates thyroid-modulating compounds' physiological and reproductive effects on the growth and development of mature female goldfish (*Carassius auratus*). A total of 240 adult female goldfish were divided into four treatment groups: control (coconut oil), T4, MMI, and T4+MMI (combined). The fish were acclimatized for four weeks before receiving injections of the respective compounds. Growth performance, blood biochemistry, thyroid hormone (THs) levels, oocyte development, and liver histology were evaluated over a 28-day experimental period. Results indicated significant differences in growth indices, with the T4 group showing the highest weight gain, specific growth rates, and lowest feed conversion ratio ($P < 0.05$), while the MMI group exhibited the lowest growth parameters. Blood glucose, triglyceride, and total protein levels were significantly elevated in the T4-treated group, whereas cholesterol was reduced ($P < 0.05$). Plasma T3 and T4, were highest in the T4 group and lowest in the MMI group. Histological analysis revealed advanced oocyte maturation in the T4 group, with a higher proportion of oocytes at the O4 stage, while the MMI group showed delayed development, with most oocytes remaining at the O2 stage. The T4+MMI group exhibited intermediate effects, with some improvement in oocyte development relative to the MMI group. Hepatic histopathology demonstrated normal liver structure in the control and T4 groups, while severe hepatic alterations, including necrosis and vacuolation, were observed in the MMI group. The T4+MMI group displayed intermediate liver damage. These findings demonstrate that the pharmaceutical methimazole, an emerging aquatic contaminant, acts as a potent endocrine disruptor, inducing hypothyroidism that severely impairs growth, metabolic homeostasis, and reproductive maturation in fish. The partial mitigation by thyroxine suggests potential complex interactions in environments contaminated with multiple bioactive compounds. This study underscores the significant ecological risk of antithyroid drugs in aquatic ecosystems and contributes critical data for environmental risk assessment.

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1. Introduction

Thyroid hormones (THs), including triiodothyronine (T3) and thyroxine (T4), are critical regulators of growth, metabolism, and reproduction in aquatic organisms, particularly fish [1–3]. The hypothalamic-pituitary-thyroid axis controls TH synthesis and release [4–6]. Acting through THs receptors (Thyroid hormone

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receptor alpha and thyroid hormone receptor beta), THs influence gene transcription and play essential roles in developmental processes such as neural maturation, sensory organ formation, and the morphogenesis of key structures like cranial bones, fins, and the swim bladder [7,8]. They also regulate crucial life-cycle transitions, including metamorphosis and osmoregulation, enabling fish to adapt to varying environmental conditions [9–12]. Their effects are mediated at the tissue level by deiodinase enzymes, which convert T4 into the biologically active T3, ensuring tissue-specific responses [13–15]. TH disruption can lead to developmental abnormalities affecting survival and reproduction [16–21]. Consequently, THs system disruption is a significant concern in aquatic ecosystems due to its impact on both individual health and population stability [22–24].

Disruption of THs by environmental chemicals can impair growth, metabolism, and reproduction [7,25,26]. This disruption is frequently induced by exposure to thyroid-disrupting chemicals, which are increasingly detected as emerging contaminants in aquatic environments. Similar effects have been observed in fish exposed to thyroid-disrupting chemicals such as methimazole (MMI), organochlorine pesticides, polychlorinated biphenyls, and estrogenic hormones [27–31]. They can cause developmental delays, reduced growth, and impaired reproductive performance, particularly during early life stages. THs are also essential regulators of reproductive processes, and dysregulation can negatively affect gonadal development and fertility in fish [25,32–36].

Of particular concern is the antithyroid drug MMI. Although primarily used clinically and in veterinary medicine as a thyroid-peroxidase inhibitor, MMI may enter aquatic environments via pharmaceutical wastewater discharge, aquaculture effluent, and improper disposal of unused medications [37–40]. It has been reported as an emerging contaminant in surface waters and sediments [41], and its chemical stability suggests potential resistance to conventional wastewater treatment [37–40]. Dedicated environmental monitoring for MMI is nevertheless sparse. Compilations and regional surveys indicate that, where measured, MMI concentrations are generally trace typically in the low nanograms per liter (ng/L) range in receiving waters (often <10–50 ng/L) and higher in raw sewage or concentrated hospital effluent (reported up to low-μg/L in extreme cases) [41–43]. Sediment detections are rare and usually close to analytical limits. Geographic coverage is uneven (most data come from Europe, East Asia and North America), leaving large gaps in many regions. Although measured environmental concentrations are generally low, these observations combined with MMI's bioactivity and persistence justify mechanistic ecotoxicological evaluation of its effects on non-target aquatic organisms and motivate the present laboratory study. MMI's persistence and chemical stability suggest it may resist conventional wastewater treatment [38,39]. Extensive research has explored THs and antithyroid drugs in fish physiology, particularly regarding growth, metabolism, and reproduction. T4 administration enhances somatic growth and development, whereas MMI inhibits THs synthesis and delays development [7,30,44–50]. The combined effects of T4 and MMI in fish remain poorly understood, despite potential co-exposure in natural environments. Evidence of T4-MMI interactions on growth, metabolism, and reproduction is inconsistent [27,51–54] highlighting the need for further study.

Although thyroid regulation influences gonadal development and fertility, histopathological outcomes of combined T4 and MMI exposure on reproductive tissues, particularly ovarian architecture and oocyte maturation remain poorly characterized. Most prior studies have relied on systemic endpoints (e.g., hormone titers or growth indices), with fewer efforts focused on tissue-level histology or staging of oocyte development in response to thyroid modulation. Despite extensive literature on thyroid-axis disruption using hormone titers and growth indices, there is a clear paucity of studies that (i) examine tissue-level histopathology of reproductive organs (ovarian architecture and oocyte staging), (ii) evaluate combined T4–MMI interactions, and (iii) incorporate molecular markers of thyroid and reproductive pathways. The present study addresses these gaps by combining systemic endpoints (growth, plasma THs), biochemical markers) with detailed ovarian histology and liver pathology in mature female goldfish, and by testing whether exogenous T4 can mitigate MMI-induced hypothyroid effects.

The goldfish, a cyprinid species, is extensively used as a vertebrate model in ecotoxicology, reproductive biology, and endocrinology research due to its well-characterized physiology and sensitivity to endocrine disruption [55–59]. Its relevance for studying the impacts of aquatic contaminants is well-established [60,61]. With a synchronized, photothermally regulated reproductive cycle and physiological similarity to other cyprinids like common carp [60,61], goldfish exhibit broad environmental tolerance (8–28 °C), an adaptable omnivorous diet, rapid juvenile growth, high fecundity, and resilience to fluctuating conditions [62–65]. Ease of captive breeding and high stocking density compatibility further supports their use in laboratory studies of endocrine disruption, growth, neurochemical signaling, and applied aquaculture research [65–68].

This study addresses the gap in understanding how exposure to the pharmaceutical contaminant MMI affects growth, THs, biochemical profiles, and gonadal histology in goldfish, and whether exogenous T4 can mitigate these effects. By integrating systemic and histological endpoints, we aim to clarify T4's protective potential and its influence on reproductive maturation, providing mechanistic insight into THs regulation in teleosts and informing ecological risk assessment of pharmaceutical contaminants.

2. Materials and methods

2.1. Ethical information

All experimental procedures involving animals were conducted in strict accordance with institutional and international ethical standards for animal research. The study adhered to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines and complied fully with Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes. Ethical approval for this research was granted by the Ethics Committee of the University of Guilan under approval number IR.GUILAN.REC.1400.049. All animals were housed and cared for under standardized conditions, with continuous monitoring to ensure well-being throughout the study. Veterinary best practices were followed, and efforts were made at every stage to minimize discomfort, stress, or pain. No signs of pain, distress, or abnormal behavior were observed during the experimental procedures. Anesthesia and handling protocols were selected based on current veterinary guidelines to ensure humane treatment.

2.2. Experimental animals and husbandry

A total of 240 adult female goldfish, with an average initial weight of 29.0 ± 0.2 g and length of 10.4 ± 0.1 cm, were purchased from a commercial ornamental fish hatchery located in Sangar, Guilan, Iran. The experiment was conducted at the Faculty of Natural Resources, University of Guilan (Sowmeh Sara, Guilan, Iran). Upon arrival, fish were randomly distributed into 16 glass aquaria (dimensions: 70 × 45 × 40 cm; 100 L capacity each), with 15 fish per

tank, to maintain appropriate stocking density and reduce social stress. Fish were acclimatized for four weeks under controlled water quality, photoperiod, and feeding conditions to stabilize physiological and behavioral parameters prior to the initiation of experimental treatments. The water temperature was maintained at 26 °C with a 300 W heater (Mahiran, Tehran, Iran), while 30 % (30 L) of the water was replaced daily with dechlorinated water at the same temperature. Aeration was provided via air stones to maintain dissolved oxygen levels at 90 % saturation, and pH was held at 8.3 ± 0.4. Water quality parameters, including temperature, dissolved oxygen, and pH, were measured twice daily using a mercury thermometer, an oxygen meter (YK-2001DO, Lutron, Taipei, Taiwan, China), and a pH meter (WTW 537, Xylem, Munich, Germany), respectively. The photoperiod was maintained at 12 h light (08:00–20:00) and 12 h dark using 20-W white fluorescent lamps (Pars Shahab, Tehran, Iran) positioned 30 cm above the water surface, with an intensity of 700 lux measured via a lux meter (LI-COR Li-1776, Lincoln, NE, USA). Fish were fed thrice daily (09:00, 14:00, and 19:00) at a rate of 2.5 % of their body weight per day using commercial ornamental fish pellets (Daneh Talaie, Boroujerd, Iran) containing 38.0 ± 0.3 % protein, 13.0 ± 0.5 % fat, 11.0 ± 0.2 % ash, 3.0 ± 0.1 % crude fiber, 1.0 ± 0.1 % phosphorus, and 8.0 ± 0.2 % moisture (pellet size: 4.5 ± 0.1 mm). This feeding rate was selected based on standard protocols for cyprinids that balance growth performance and feed efficiency. Feeding frequency was divided evenly across meals to ensure consistent nutrient availability and reduce feed waste. Although twice-daily feeding is commonly reported in aquaculture studies, we adopted a thrice-daily feeding schedule based on our previous experience, which showed better feed distribution, reduced competition, and minimized feed wastage [57]. This approach ensured that all fish had equal access to food, especially in experimental settings where social hierarchies and behavioral differences can affect feeding success. Moreover, spreading the ration across three meals helped maintain consistent nutrient availability and metabolic stability, which is particularly important in endocrine and reproductive studies where fluctuations in nutrient intake may confound physiological and hormonal responses. This practice is further supported by findings from goldfish [69] and other cyprinids such as the red garra (*Garra rufa*) [70], where thrice-daily feeding resulted in improved growth performance and feed utilization.

2.3. Experimental treatments and injection

Two thyroid-modulating compounds, T4 (Merck, Darmstadt, Germany; CAS no. 51-48-9) and MMI (Sigma-Aldrich, St. Louis, MO, USA; CAS no. 60-56-0), were used. Both compounds were dissolved in 1 mL of 96 % ethanol (Razi, Khuzestan, Iran). After the ethanol had completely evaporated, the residues were mixed with coconut oil (Giah Kala, Tehran, Iran) to ensure a gradual release upon injection. Coconut oil, rich in medium-chain triglycerides and widely used for the delivery of lipophilic drugs in pharmacological studies, was selected as the using 20 W white fluorescent (IP) vehicle [71]. Coconut oil–derived medium-chain triglycerides (MCTs) can be assimilated by teleost fish (e.g., rainbow trout (*Oncorhynchus mykiss*)) [72], supporting its suitability as a metabolic carrier in fish experiments. Before injection, fish were visually inspected for any signs of microbial infection or disease to ensure only healthy individuals were used. IP injections were performed using a sterile 1-mL syringe and a 26-gauge needle at the midline of the ventral surface, just anterior to the pelvic fins, to ensure precise dosage delivery with minimal stress. After injection, the injection site was treated with a topical anti-infective agent (10 % povidone-iodine solution, Tolid Darou, Tehran, Iran), to prevent possible infections and promote healing [71]. Control group fish received an identical volume of coconut oil without active substances to maintain procedural uniformity. The experiment consisted of four treatment groups with two replicates per group (30 fish per replicate): (1) control group (1 mL coconut oil per fish), (2) T4 group (10 mg/kg body weight of T4 in 1 mL coconut oil), (3) MMI group (20 mg/kg body weight of MMI in 1 mL coconut oil), and (4) T4+MMI group (co-injection of 10 mg/kg T4 and 20 mg/kg MMI, each in 1 mL coconut oil). All fish received a single intraperitoneal injection at the beginning of the experiment. Dose selection followed prior toxicological and endocrine-manipulation protocols [3,25,73,74] and was intended to produce a robust, experimentally tractable hypothyroid (MMI) or hyperthyroid (T4) phenotype via a single intraperitoneal pulse; these pharmacological doses facilitate mechanistic inference but do not model chronic environmental exposure.

2.4. Sampling and growth parameter analysis

The experimental period lasted 28 days. On the final day, fish were fasted for 24 h and anesthetized with 200 ppm clove powder extract (SHAFA Company, Sanandaj, Iran) before length and weight measurements [75]. Fish weights were measured to the nearest 0.1 g and lengths to the nearest mm. Growth performance indices were calculated using the following standard formulas:

Weight gain (WG, g) = Final weight − Initial weight.

Specific growth rate for the weight (SGR_W, % day^{-1}) = 100 × [Ln (Final weight) – Ln (Initial weight)]/Days.

Body weight increase (BWI, %) = 100 × (WG/Initial weight)

Condition factor (CF) = 100 × (weight/total $length^3$)

Feed conversion ratio (FCR) = Feed intake/WG.

FCR values were calculated at the tank level based on two replicates per treatment group, as feed intake was recorded per aquarium. This approach ensured consistency in feed delivery and allowed for treatment-level comparison of feed efficiency metrics.

Survival rate (SR, %) = 100 × (Number of survivors/Total fish stocked)

Blood samples were collected from 10 fish per group by caudal venipuncture. Plasma was separated via centrifugation at 1600g for 8 min (Hettich, Kirchlengem, Germany) and stored at −20 °C for biochemical and hormonal analyses.

2.5. Blood biochemical and hormonal analysis

Plasma biochemical parameters (cholesterol, triglycerides, albumin, and total protein) were measured using commercial assay kits validated for use in fish [3,76] (Pars Azmun, Karaj, Iran). Glucose (product no. product number: 1500017, detection range 5~400 mg/dL) concentration was determined using a color-based assay method. Albumin (product no. 1500001, limit: 0.2 g/dL) and total protein (product no. 1500028, range: 1–120 g/dL) were determined via bromocresol green binding and biuret methods at 540 nm. Cholesterol (product no. 1500010, range: 5–500 mg/dL) and triglycerides (product no. 1500012, range: 5–700 mg/dL) were analyzed via CHOD-PAP and GPO-PAP methods, respectively.

Plasma T3 and T4 levels were assessed using competitive enzyme immunoassay kits (Lake Forest, CA, USA; T3: product no. 125–300, sensitivity 0.04 ng/mL; T4: product no. 225–300, sensitivity 0.128 μg/dL) following the manufacturer's protocol. Absorbance was recorded at 450 nm (Epoch 2 Microplate Spectrophotometer, Vermont, USA) [3,75]. Intra-assay coefficients of variation were 6.4 % for T3 and 5.9 % for T4, while inter-assay coefficients were 7.2 % and 6.4 %, respectively (n = 20).

2.6. Histological analysis

Histological analysis was conducted on the ovary and liver tissue from 10 fish per treatment group. Tissues were fixed in Bouin's solution (HT10132-1L, Sigma-Aldrich, USA) overnight and then kept in 70 % ethanol for further analysis. at the next step, tissues were dehydrated using an increasing concentration of ethanol (70, 96, 100 %) and embedded in paraffin. Sections (6 μm thick) were mounted on slides and exposed to decreasing concentrations of ethanol (100, 96, 70 %). Finally, the prepared samples were stained with hematoxylin and eosin and examined under a light microscope (Olympus BX51, Tokyo, Japan). Oocyte development was classified according to standard histological stages, and gonadal cell types were identified. Staging criteria followed the methodologies described by Li et al. [77] and Al-Khalaifah et al. [78], with modifications for goldfish. Liver histological analysis followed the methodology described by Raghu et al. [79] and Clouston et al. [80], ensuring a standardized evaluation by a certified pathologist. Quantitative analysis of tissue structure was performed using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). The assessment focused on hepatic architecture, cellular integrity, necrotic lesions, cytoplasmic vacuolation, and other histopathological alterations indicative of metabolic or toxicological effects.

2.7. Statistical analysis

The normality of data was tested using the Kolmogorov-Smirnov test, and homogeneity of variances was assessed with Levene's test. One-way ANOVA was performed to evaluate differences among groups, followed by Tukey's post hoc test for pairwise comparisons. All statistical analyses were conducted using SPSS (version 27.0.1, Chicago, IL, USA). Results are expressed as mean ± standard error, with statistical significance set at $P < 0.05$.

3. Results

3.1. Growth indices

The growth performance of female goldfish varied significantly among the different groups. Final weight showed notable variation ($P < 0.05$, Table 1) in which fish treated with T4 exhibited the highest value (55.8 ± 1.2 g) and following final biomass (558.4 ± 11.6 g) was significantly outperforming all other groups ($P < 0.05$), while the MMI group showed the lowest final weight (40.6 ± 0.1 g) and final biomass (406.1 ± 0.7 g) ($P < 0.05$, Table 1). The WG and SGR were significantly higher in the T4 group (19.7 ± 0.9 g, 1.5 ± 0.0 %/day) compared to all other groups ($P < 0.05$), while the MMI group exhibited the lowest WG (5.0 ± 0.6 g) and SGR (0.5 ± 0.0 %/day). Similarly, BWI was greatest in the T4 group (54.3 ± 2.0 %), followed by the T4+MMI group (34.6 ± 1.8 %), with the lowest values in the MMI group (14.1 ± 1.8 %) ($P < 0.05$, Table 1). The FCR was most efficient in the T4 group (1.4 ± 0.1), while the MMI group displayed the least efficient FCR (3.0 ± 0.4) ($P < 0.05$, Table 1). Feed intake per fish was highest in the T4 group (2.7 ± 0.2 g) and lowest in the MMI group (1.5 ± 0.0 g) ($P < 0.05$). CF was significantly higher in the T4 group (1.7 ± 0.1), while the MMI group had the lowest CF (1.2 ± 0.1) ($P < 0.05$, Table 1). All groups maintained a 100 % survival rate throughout the experiment, with no significant differences observed ($P > 0.05$). These results suggest that T4 treatment significantly enhances the growth of goldfish, while MMI treatment negatively impacts growth indices ($P < 0.05$, Table 1).

3.2. Biochemical and hormonal parameters

The glucose levels varied significantly across treatments ($P < 0.05$, Table 2). The highest glucose concentration was observed in the T4 group (40.2 ± 0.9 mg/dL), which was significantly greater than the T4+MMI group (21.5 ± 0.9 mg/dL), the control group (12.3 ± 2.3 mg/dL), and the MMI group (4.6 ± 0.8 mg/dL). These results indicate that T4 treatment markedly increased glucose levels compared to other groups. Albumin levels were significantly elevated in the T4 group (13.1 ± 0.9 g/dL, $P < 0.05$, Table 2) compared to all other treatments. Control (6.8 ± 0.1 g/dL), MMI (6.6 ± 0.2 g/dL), and T4+MMI (7.1 ± 0.1 g/dL)

Table 2
Effects of thyroid-modulating compounds on the biochemical parameters of female goldfish (*Carassius auratus*) after a 28-day experimental period. Control: coconut oil; T4: 10 mg/kg thyroxine + coconut oil, MMI: 20 mg/kg methimazole + coconut oil, and T4+MMI: 10 mg/kg thyroxine and 20 mg/kg methimazole + coconut oil. Data are presented as mean ± SE (n = 10).

Parameters	Control	T4	MMI	T4+MMI
Glucose (mg/dL)	12.3 ± 2.3^{c}	40.2 ± 0.9^{a}	4.6 ± 0.8^{d}	21.5 ± 0.9^{b}
Albumin (g/dL)	6.8 ± 0.1^{b}	13.1 ± 0.9^{a}	6.6 ± 0.2^{b}	7.1 ± 0.1^{b}
Total Protein (g/dL)	22.4 ± 0.4^{b}	36.0 ± 2.7^{a}	21.8 ± 0.1^{b}	24.2 ± 0.7^{b}
Cholesterol (mg/dL)	90.0 ± 9.0^{a}	34.3 ± 0.1^{c}	38.5 ± 3.8^{c}	66.4 ± 3.7^{b}
Triglyceride (mg/dL)	37.1 ± 9.1^{b}	67.1 ± 3.4^{a}	38.9 ± 4.1^{b}	29.8 ± 3.8^{b}

Statistical significance was determined using one-way ANOVA at $P < 0.05$. Different letters indicate significant differences among treatment groups for each parameter.

Table 1
Effects of thyroid-modulating compounds on the growth performance of female goldfish (*Carassius auratus*) after a 28-day experimental period. Control: coconut oil; T4: 10 mg/kg thyroxine + coconut oil, MMI: 20 mg/kg methimazole + coconut oil, and T4+MMI: 10 mg/kg thyroxine and 20 mg/kg methimazole + coconut oil. Data are presented as mean ± SE (n = 30).

Parameters	Control	T4	MMI	T4+MMI
Initial length (cm)	13.1 ± 0.3	12.8 ± 0.2	13.2 ± 0.2	13.3 ± 0.1
Initial weight (g)	36.7 ± 0.8	36.2 ± 0.3	35.6 ± 0.6	36.5 ± 0.6
Final length (cm)	15.4 ± 0.4	15.0 ± 0.3	14.9 ± 0.3	15.8 ± 0.2
Final weight (g)	45.7 ± 1.4^{bc}	55.8 ± 1.2^{a}	40.6 ± 0.1^{c}	49.2 ± 1.5^{b}
Final biomass (g)	457.2 ± 13.9^{bc}	558.4 ± 11.6^{a}	406.1 ± 0.7^{c}	491.9 ± 15.0^{b}
Feed consumed (g)	21.3 ± 0.7^{a}	26.7 ± 1.7^{a}	14.7 ± 0.3^{b}	23.7 ± 1.9^{a}
WG (g)	9.0 ± 0.6^{c}	19.7 ± 0.9^{a}	5.0 ± 0.6^{d}	12.7 ± 0.9^{b}
SGR (%/days)	0.8 ± 0.0^{c}	1.5 ± 0.0^{a}	0.5 ± 0.0^{d}	1.1 ± 0.0^{b}
BWI (%)	24.5 ± 1.0^{c}	54.3 ± 2.0^{a}	14.1 ± 1.8^{d}	34.6 ± 1.8^{b}
CF	1.3 ± 0.1^{a}	1.7 ± 0.1^{a}	1.2 ± 0.1^{b}	1.3 ± 0.0^{b}
Feed intake/Fish (g)	2.1 ± 0.1^{bc}	2.7 ± 0.2^{a}	1.5 ± 0.0^{c}	2.4 ± 0.2^{b}
FCR	2.4 ± 0.1^{ab}	1.4 ± 0.1^{c}	3.0 ± 0.4^{a}	1.9 ± 0.1^{bc}
Survival rate (%)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

WG: weight gain, FCR: feed conversion ratio, SGR: specific growth rate, BWI: body weight increase, CF: condition factor. Statistical significance was determined using one-way ANOVA at $P < 0.05$. Different letters indicate significant differences among groups for each parameter.

groups showed no significant differences among themselves, suggesting that T4 treatment had a unique effect on albumin levels. Total protein levels were significantly elevated in the T4 group (36.0 ± 2.7 g/dL, $P < 0.05$, Table 2) compared to all other treatments. The control (22.4 ± 0.4 g/dL), MMI (21.8 ± 0.1 g/dL), and T4+MMI (24.2 ± 0.7 g/dL) groups displayed no significant, indicating that T4 treatment uniquely increased total protein levels. Cholesterol levels were significantly reduced in the T4 group (34.3 ± 0.1 mg/dL) and the MMI group (38.5 ± 3.8 mg/dL) compared to the control group (90.0 ± 9.0 mg/dL, $P < 0.05$). The T4+MMI group (66.4 ± 3.7 mg/dL) exhibited intermediate cholesterol levels that were lower than the control but higher than the T4 and MMI groups ($P < 0.05$). Triglyceride concentrations were significantly higher in the T4 group (67.1 ± 3.4 mg/dL, $P < 0.05$, Table 2) compared to the control (37.1 ± 9.1 mg/dL) and T4+MMI (29.8 ± 3.8 mg/dL) groups.

The plasma T4 levels were highest in the T4-treated group (52.2 ± 0.0 ng/mL), significantly surpassing those of the other groups (Fig. 1A; $P < 0.05$). The T4+MMI group displayed intermediate T4 levels (44.0 ± 1.5 ng/mL), which were higher than the control group (38.5 ± 0.4 ng/mL) but lower than the T4 group. In contrast, the MMI group showed the lowest T4 levels (26.5 ± 1.1 ng/mL), significantly differing from all other treatments. Similarly, T3 levels were significantly elevated in the T4 group (72.2 ± 0.1 ng/mL) compared to the control, T4+MMI, and MMI groups ($P < 0.05$). The T4+MMI treatment resulted in intermediate T3 levels (Fig. 1B; 50.5 ± 0.7 ng/mL), which were higher than the control group (44.0 ± 0.5 ng/mL) but lower than the T4 group ($P < 0.05$). The MMI group had the lowest T3 levels (30.9 ± 2.6 ng/mL), significantly differing from the other treatments. The T3:T4 ratio was significantly higher in the T4 group (1.4 ± 0.0) compared to all other groups (Fig. 1C; $P < 0.05$). The Control, MMI, and T4+MMI groups displayed similar T3:T4 ratios (approximately 1.1–1.2), with no significant differences among them.

3.3. Histological analysis

The oocyte developmental profile in female goldfish ovaries showed marked differences among the treatment groups, supported by both numerical data and histological observations (Figs. 2 and 3). In the control group, oocytes were predominantly at the cortical alveoli stage (O2, 55.2 %), with moderate proportions at the perinucleolar (O1, 26.0 %), primary yolk (O3, 12.5 %), and secondary yolk (O4, 6.3 %) stages, reflecting a typical progression of oocyte development under normal conditions (Fig. 3A). In contrast, the T4-treated group exhibited a significant shift toward advanced oocyte stages, particularly the O4 stage (34.4 %), accompanied by reductions in early-stage oocytes (O1 at 17.8 % and O2 at 37.8 %), suggesting that exogenous T4 promotes oocyte

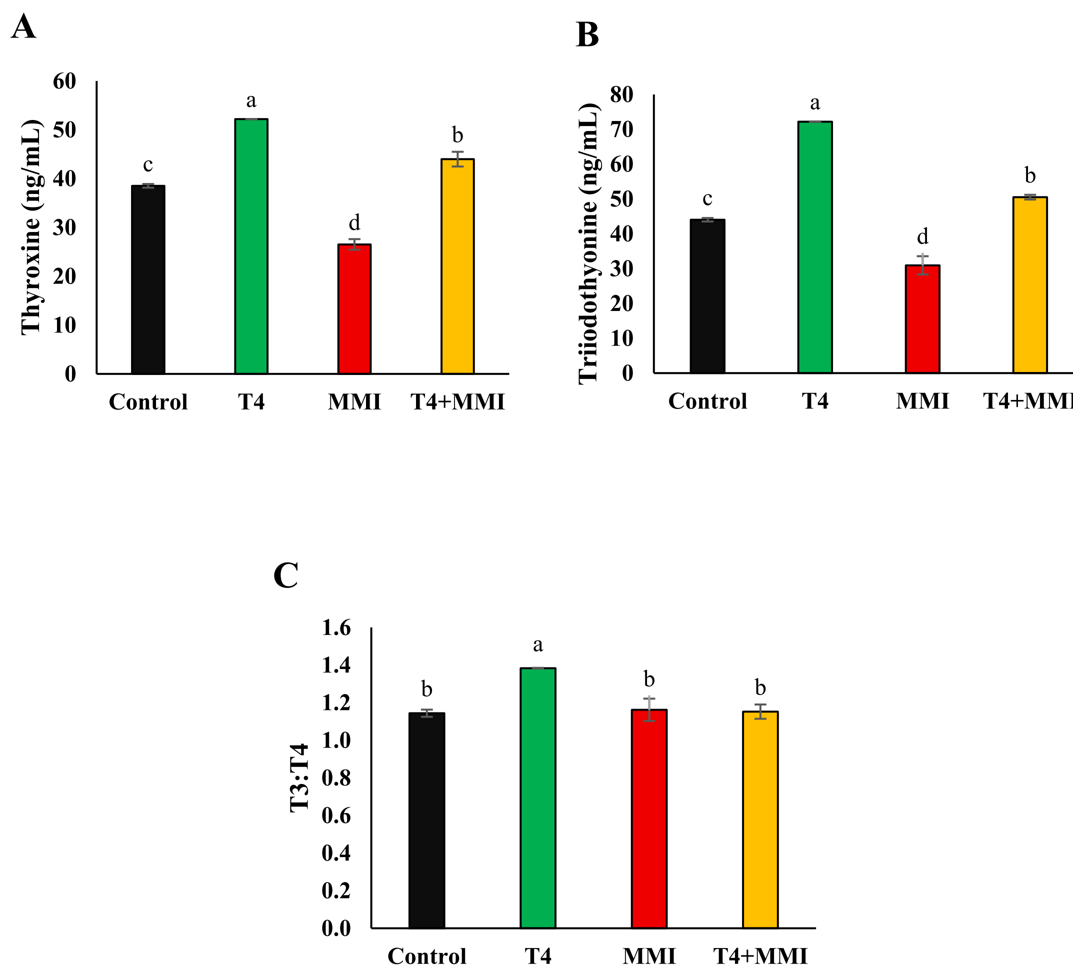

Fig. 1. Effects of thyroid-modulating compounds on serum THs levels and the T3:T4 ratio in female goldfish (*Carassius auratus*). (A) Plasma thyroxine (T4) levels (ng/mL), (B) plasma triiodothyronine (T3) levels (ng/mL), and (C) the T3:T4 ratio after a 28-day experimental period. Data are presented as mean ± SE (n = 10). Different letters indicate significant differences among groups ($P < 0.05$).

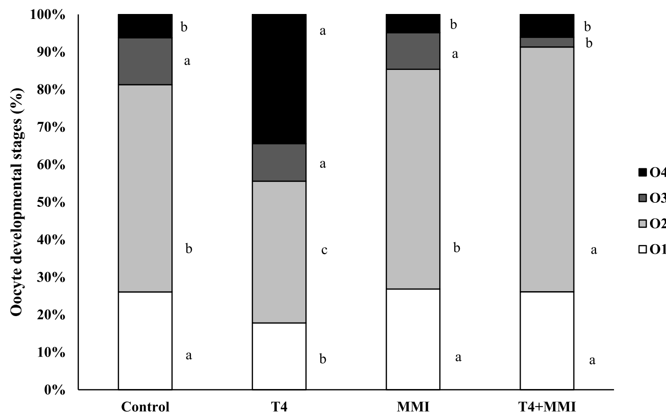

Fig. 2. Effects of thyroid-modulating compounds on the percentage distribution of oocyte developmental stages in female goldfish (*Carassius auratus*) after a 28-day experimental period. The bar chart represents the mean percentage of oocytes at each developmental stage (O1–O4) within each treatment group (n = 10). O1 indicates the least developed oocytes, while O4 represents the most advanced. Different letters indicate significant differences among groups for each oocyte stage ($P < 0.05$).

maturation and vitellogenesis. Histologically, these ovaries displayed enhanced yolk vesicle accumulation and a prominent zona radiata (Fig. 3B). In the MMI-treated group, oocyte development was clearly suppressed, with a majority of oocytes arrested at the O2 stage (58.5 %), and only 4.9 % progressing to the O4 stage. The proportion of O1 oocytes (26.8 %) remained similar to the control, indicating a lack of developmental progression. Histological sections confirmed poor yolk deposition and reduced cytoplasmic differentiation in oocytes (Fig. 3C), highlighting the inhibitory effect of MMI on ovarian maturation. The T4 + MMI combined treatment group exhibited intermediate effects. While a large proportion of oocytes remained at the O2 stage (65.2 %), a slight improvement in maturation was observed, with 6.1 % reaching the O4 stage higher than in the MMI group but still substantially lower than in the T4 group alone. Oocyte morphology appeared moderately improved, with limited yolk vesicle formation and partial structural organization (Fig. 3D).

The effects of different treatments (Control, T4, MMI, and T4+MMI) on oocyte diameter in female goldfish were significant (Fig. 4; $P < 0.05$). Oocyte diameter was highest in the T4 group (160.3 ± 11.3 μm), which was significantly larger than in all other groups. The control group exhibited an intermediate oocyte diameter (93.0 ± 5.7 μm), while the MMI (79.4 ± 4.3 μm) and T4+MMI (77.4 ± 2.8 μm) groups had significantly smaller oocyte diameters compared to the control group.

The histopathological analysis of female goldfish liver revealed distinct alterations across the different treatment groups. In the control group, the liver exhibited normal histoarchitecture with well-preserved hepatocytes showing a uniform cytoplasmic appearance and only mild vacuolation; there were no signs of necrosis, cell death, or inflammatory hepatitis, and the hepatic sinusoids and vasculature remained intact (Fig. 5A). In the T4-treated group, the liver structure was largely maintained, with hepatocytes displaying hypertrophy and a noticeable reduction in cytoplasmic vacuolation compared to controls; no necrosis or inflammation was observed, and the hepatocytes appeared

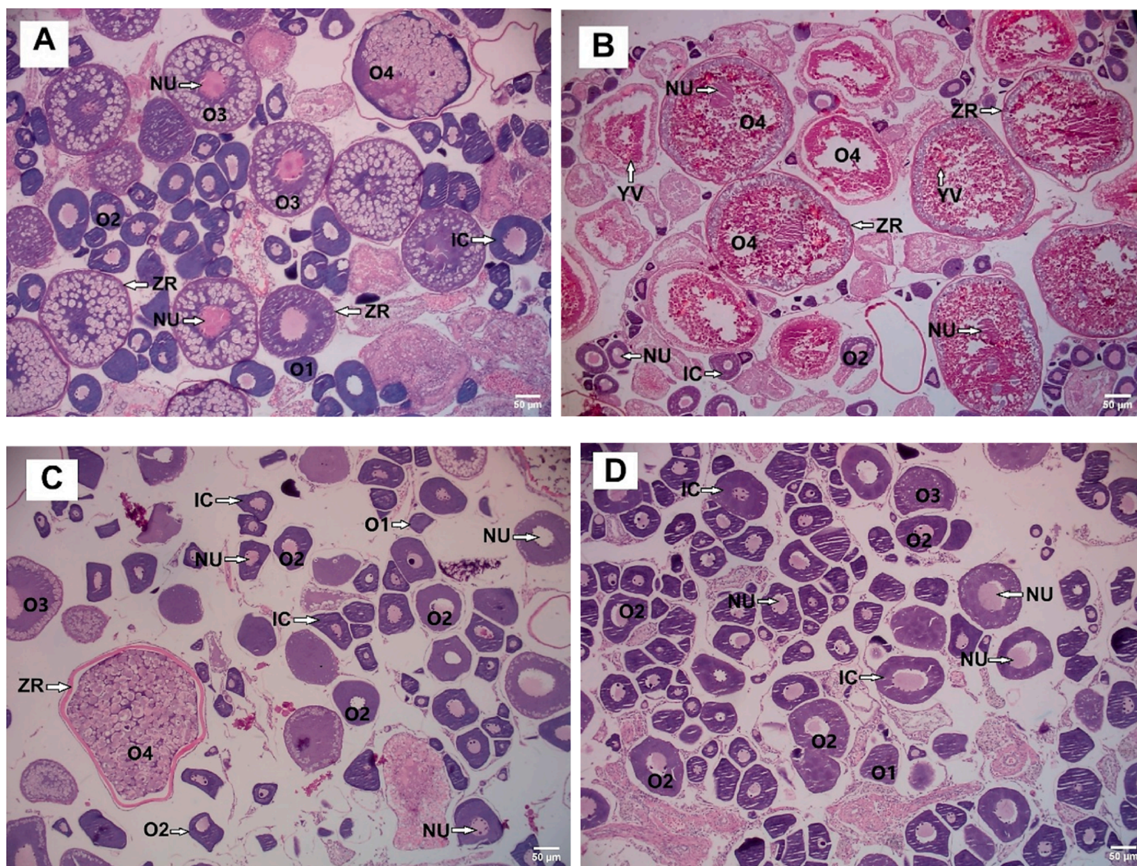

Fig. 3. Effects of thyroid-modulating compounds on the histological sections of ovaries in female goldfish (*Carassius auratus*). Oocyte in control treatment (A), T4 treatment (B), MMI treatment (C) and T4 + MMI treatment (D). O1: perinucleolar oocyte; O2: the cortical alveoli oocyte O3: primary yolk oocyte; O4: second yolk oocyte; YV; yolk vesicle; NU: nucleus; IC: internal cytoplasm; ZR: zona radiata. Ovarian scale bar is 50 μm (n = 10).

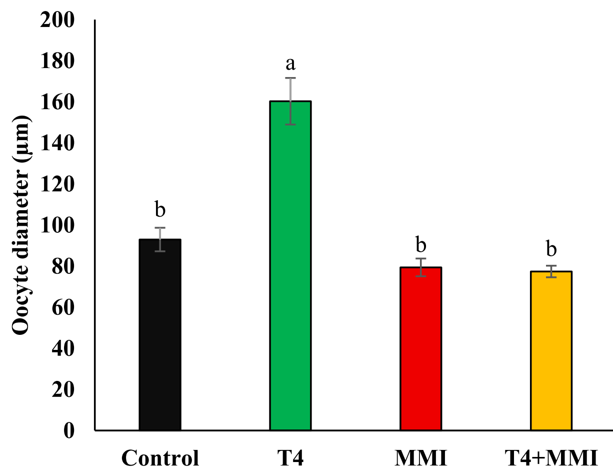

Fig. 4. Effects of thyroid-modulating compounds on ovaries diameter in female goldfish (*Carassius auratus*). Oocyte diameter (μm) was measured after a 28-day experimental period. Data are presented as mean ± SE (n = 50). Different letters indicate significant differences among groups ($P < 0.05$).

compact with preserved tissue integrity (Fig. 5B). In contrast, the MMI-treated group showed severe hepatic alterations, including widespread cytoplasmic vacuolation, prominent necrosis, and extensive cell death. This group also exhibited marked inflammatory hepatitis characterized by dense infiltration of inflammatory cells throughout the tissue, irregular hepatic sinusoids, and vascular changes, resulting in significant structural disorganization and cellular degeneration (Fig. 5C). The combined T4+MMI treatment group demonstrated intermediate hepatic changes, with necrosis and cell death present but less severe than in the MMI-only group. Inflammatory hepatitis persisted, though with moderate inflammatory cell infiltration, and the hepatocytes retained a more organized structure relative to the MMI group, showing only mild signs of cellular stress (Fig. 5D). Overall, these results highlight a gradient of hepatic damage across the treatments, with MMI inducing the most severe pathology, T4 exhibiting protective effects, and the combined treatment partially mitigating MMI-induced liver injury.

4. Discussion

This study demonstrates that MMI, an antithyroid pharmaceutical, acts as a potent endocrine disruptor, inducing severe hypothyroidism that impairs growth, metabolic homeostasis, reproductive maturation, and hepatic health in female goldfish, and that these perturbations map onto specific mechanistic pathways linking thyroid status to reproductive maturation and hepatic function. Rather than repeatedly restating that MMI causes hypothyroidism, we focus below on how thyroid disruption plausibly produced the observed changes in oocyte development and liver pathology, and on how exogenous T4 modulated those pathways. Fish treated with T4 showed the highest values for final weight, biomass, WG, SGR, and BWI, along with improved FCR and CF. Conversely, MMI treatment produced marked growth suppression. Mechanistically, these effects are consistent with thyroid hormone regulation of the growth hormone (GH)–insulin-like growth factor-1 (IGF-1) axis: THs enhance GH/IGF-1 signaling to stimulate protein synthesis, cell proliferation and nutrient

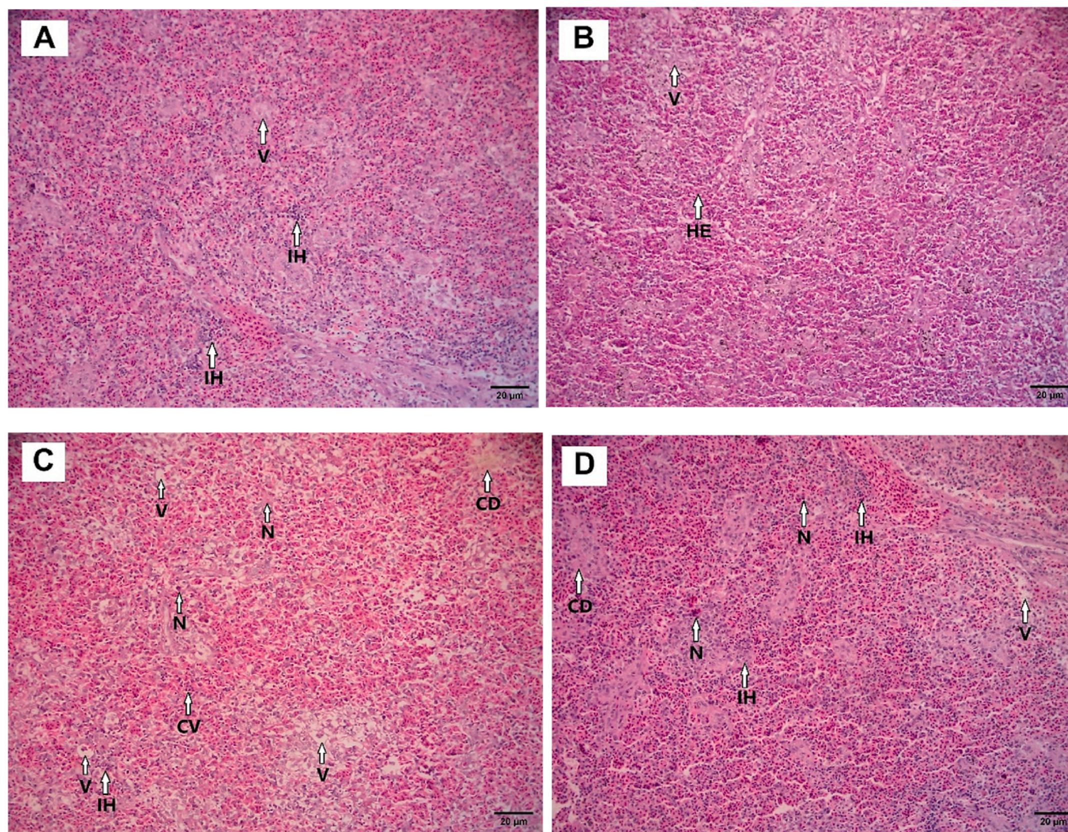

Fig. 5. Effects of thyroid-modulating compounds on histopathological changes in the liver of female goldfish (*Carassius auratus*). Liver tissue in control treatment (A), T4 treatment (B), MMI treatment (C), and T4+MMI treatment (D). CV: cytoplasmic vacuolation; V: vacuolation; CD: cell death; IH: inflammatory hepatitis; N: necrosis; HE: hepatocytes cell. scale bar is 20 μm (n = 10).

utilization, whereas thyroid blockade reduces anabolic signaling and feed efficiency [3,81–83]. Our results align with previous findings in red sea bream (*Pagrus major*) and sterlet sturgeon (*Acipenser ruthenus*), where THs promoted growth, appetite, and digestive efficiency [25,84]. Similar effects were observed in other fish species, suggesting a conserved mechanism by which THs enhance protein synthesis and interact with GH/IGF-1 signaling [85–88]. The growth suppression seen in MMI-treated fish supports the ecotoxicological paradigm that endocrine-disrupting chemicals can impair critical life-history traits like growth, with dire consequences for individual fitness and population sustainability [89–95]. The improved FCR in the T4-treated group reflects enhanced nutrient utilization and metabolic stimulation, which are hallmark effects of thyroid hormones [75,86]. Conversely, the elevated FCR observed in the MMI group likely stems from reduced feed intake and metabolic suppression associated with hypothyroidism. From an ecological perspective, an impaired FCR suggests that wild fish exposed to MMI would be less efficient at converting energy from natural prey sources, potentially reducing their competitive advantage and survival. The intermediate FCR in the T4+MMI group suggests partial mitigation of MMI-induced effects by T4, in line with previous thyroid restoration models [96–98]. This study demonstrates that THs supplementation can counteract the negative effects of thyroid disruption in aquaculture. THs are essential for regulating growth, metabolism, and feed efficiency in goldfish, with T4 enhancing and MMI impairing these processes. The findings highlight THs' role in GH-IGF-I signaling and nutrient metabolism. Future research should investigate the molecular basis of TH action, their interaction with other growth regulators, and the environmental impact of thyroid-disrupting chemicals.

Biochemical shifts support a mechanistic link to hepatic metabolism. Elevated plasma glucose, total protein and albumin in the T4 group reflect upregulated hepatic gluconeogenesis and protein synthesis under hyperthyroid conditions [26,99–103]. In contrast, MMI-treated fish had reduced glucose levels, painting a picture of a metabolically compromised organism, ill-equipped to meet energy demands [104–108]. These proteins also reflect immune competence and osmotic balance [109] and are valuable indicators of liver function [110,111]. The suppression of these proteins by MMI indicates not only impaired liver function but also potential negative impacts on immune competence and osmoregulation, key factors for survival in the wild [109–111]. Triglyceride levels increased significantly in T4-treated fish, supporting the role of THs in stimulating lipolysis and fatty acid metabolism [99,100,112,113]. THs enhances lipase activity and lipid redistribution [114,115], whereas MMI reduced these levels, reflecting inhibited lipid metabolism. Again, the T4+MMI group showed intermediate values, indicating partial mitigation of MMI's suppressive effects by T4. While MMI may have reduced T4's full effects, further research is needed to clarify how THs regulate metabolism, especially in liver function, immunity, and glucose balance. Understanding their interaction with hormones like insulin and cortisol, as well as the impact of environmental stressors, is vital for improving fish health and sustainable aquaculture.

Endocrine data showed that T4-treated fish had the highest plasma T4 and T3 levels, consistent with previous studies demonstrating increased circulating THs following exogenous T4 administration [3,54,116]. The intermediate hormone levels in the T4+MMI group suggest partial inhibition of synthesis or conversion, while MMI alone resulted in the lowest hormone levels, due to inhibition of thyroid peroxidase, which is essential for TH biosynthesis [117–120]. The elevated T3:T4 ratio in the T4 group reflects increased deiodination activity, a hallmark of hyperthyroid states [99,121,122]. Future research should examine how long-term, low-level environmental exposure to MMI affects these endocrine endpoints in wild fish populations, as well as explore compensatory responses and ecological impacts of thyroid disruption in both wild and farmed fish.

Reproductive data showed that T4 accelerated oocyte progression (O3–O4), whereas MMI caused arrest at the cortical alveoli (O2) stage. THs can influence folliculogenesis both directly and indirectly: directly via thyroid hormone receptors (TRα/β) expressed in ovarian tissue and indirectly via modulation of pituitary gonadotropins and sex-steroid synthesis. For example, THs alter pituitary tshβ and gonadotropin subunit expression and modulate aromatase (cyp19a1a) activity, thereby affecting estradiol-driven vitellogenesis [3,21,25,123,124]. This arrest in vitellogenesis represents one of the most significant threats posed by emerging endocrine disruptors the impairment of reproductive capacity and potential population-level decline [125–127]. The intermediate oocyte stages in the T4+MMI group further demonstrate partial reversal of MMI's inhibitory effects. In our study, the MMI-induced lowering of circulating THs likely reduced hepatic vitellogenin induction (via decreased ERα priming) and/or suppressed ovarian steroidogenesis, thereby arresting vitellogenesis and oocyte growth. Conversely, exogenous T4 restored components of this axis, partially rescuing oocyte maturation. These mechanistic links are consistent with goldfish-specific reports showing THs influence hepatic ERα/vtg expression and pituitary–gonadal signaling [128–130]. While this study focused on female goldfish, further research is warranted to investigate the effects of MMI and T4 on male reproductive endpoints, including spermatogenesis, steroidogenesis, and testicular histopathology, to provide a comprehensive understanding of endocrine disruption in both sexes.

Histology revealed normal hepatic architecture in controls and T4-treated fish (mild hypertrophy), but extensive vacuolation, necrosis and inflammatory infiltration in MMI-treated fish. Several mechanistic pathways could account for this hepatotoxicity: (i) hypothyroid-induced impairment of hepatic energy metabolism leading to lipid accumulation and steatosis (vacuolation); (ii) reduced antioxidant enzyme expression and increased oxidative stress causing hepatocyte injury and necrosis; and (iii) disrupted phase I/II biotransformation capacity (e.g., UDP-glucuronosyltransferases) impairing detoxification and promoting inflammatory responses. THs regulate mitochondrial function, antioxidant defenses and detoxification enzyme expression; MMI-induced TH deficiency therefore plausibly precipitates metabolic collapse and inflammatory hepatocellular damage. The partial improvement in the T4+MMI group suggests that restoration of TH signaling mitigates metabolic stress and supports hepatocyte maintenance [131–138].

Taken together, the results indicate a coherent mechanistic sequence in which MMI inhibits thyroid peroxidase, leading to a marked reduction in systemic thyroid hormone availability. This decline limits thyroid-dependent signaling in peripheral tissues, particularly the liver, where reduced deiodinase activity and diminished TR-mediated transcription impair components of the GH–IGF-1 axis. As a result, hepatic protein synthesis, lipid metabolism, and vitellogenin production are substantially suppressed, ultimately contributing to arrested vitellogenesis and delayed oocyte maturation. At the same time, impaired hepatic metabolism and weakened antioxidant capacity create conditions that favor cytoplasmic vacuolation, hepatocellular necrosis, and inflammatory infiltration. Exogenous T4 counteracts several of these disruptions by restoring circulating thyroid hormones and re-establishing thyroid-responsive gene activity in both central and peripheral tissues, thereby partially reversing reproductive and hepatic impairments. This mechanistic framing offers a more informative interpretation of the observed phenotype than

Table 3
Investigation of THs exposure across aquatic species, summarizing species details (weight/age), treatment methods, doses, exposure duration, and key results for comparison of THs effects.

Species	Weight or Age	Method of Administration	Doses	Work Interval	Results	References
Little Yellow Croaker (*Larimichthys polyactis*)	23.1 g (initial)	Dietary (T3, T4, PTU)	T3: 20 ng/g, T4: 20 ng/g, PTU: 5000 ng/g	28 days	• T3, T4, PTU reduced growth; T4 increased intestinal microbiota diversity; Vibrio and Sediminibacterium impacted SCFA profiles	[2]
Japanese medaka (*Oryzias latipes*)	Embryos and 1 dph larvae (NIES-R strain)	Waterborne exposure to MMI (TPO inhibitor)	0 (control), 12.5, 25, 50, 100 mg/L	10 days (9 embryonic + 1 larval)	•T4 levels decreased in a dose-dependent manner • No significant dose-related effects on T3 levels or TH/growth-related gene expression • Swim bladder inflation showed statistical differences but remained ~90 % in all groups; not dose-dependent	[142]
Zebrafish (*Danio rerio*)	57 dpf (132 males, 132 females)	Dietary T4, CO, and T4 + CO	T4: 10 mg/kg, CO: 10 mg/kg	28 days	• T4 boosted growth, reproduction, and survival; CO impaired reproduction and raised cortisol levels	[3]
Three-spot gourami (*Trichogaster trichopterus*)	Adult females (~75 fish)	Intramuscular injection (every 2 days)	0 (control), 0.025, 0.05, 0.1 mg/kg BW	20 days	• GSI decreased significantly in a dose-dependent manner (lowest: 3.33 %) • Declines in sex steroid hormones (E2, T, 17-OHP) at higher doses • GOT and GPT elevated at 0.05 and 0.1 mg/kg, indicating hepatotoxicity • Histology showed ovarian arrest at early oocyte stages and liver damage (necrosis, hypertrophy, inflammation)	[143]
Rohu (*Labeo rohita*)	Adult females	Intramuscular injections (LHRHa, T4)	LHRHa: 0.4 mL/kg; T4: 1, 10 μg/kg	12 h post-injection	• T4 (10 μg/kg) improved spawning success, fecundity, GSI, fertilization, hatching, and larval survival. Increased DHP, reduced estradiol, and downregulated Zp2, Cyp19a1a, and SF-1 expression.	[88]
Red Tilapia (*Oreochromis mossambicus* × *O. urolepis hornorum*)	1 g (fingerlings)	Waterborne (T4, Sodium Perchlorate)	T4: 10 nM/L, Perchlorate: 30 mg/L	30 days (+30 days recovery)	• T4 boosted growth, ovarian development, and skewed sex ratio to females; Perchlorate impaired liver histology, delayed maturity, and skewed sex ratio to males.	[144]
African clawed frog (*Xenopus laevis*)	Stage 52 Tadpoles	Water exposure with T3 + MMI or SP	MMI: 0, 5, 25, 125 mg/L; SP: 0, 20, 100, 500 mg/L	96 h	• MMI & SP blocked T3-induced weight loss and morphological changes; Concentration-dependent effects observed.	[50]
Pufferfish (*Takifugu rubripes*)	20–80 days post-hatch	MMI (oral), T4 (immersion)	MMI: 1000 μg/g diet, T4: 2 nmol/L	55 days	• MMI induced 100 % masculinization by suppressing foxl2, cyp19a1a, and stimulating dmrt1; T4 did not induce feminization.	[145]
Sterlet sturgeon	Female broodstock (708 ± 37 g)	Intraperitoneal T4 injections	Control (CO), T1: 1 mg/kg, T10: 10 mg/kg	170 days (4 injections)	• T10 raised RBC, Hb, Hct, immune parameters (C3, C4, IgM), and weight gain; Decreased liver enzymes (ALP, AST, LDH); Elevated cortisol and glucose.	[26]
Seahorse (*Hippocampus barbouri*)	Juveniles (0–8 weeks)	Artemia enriched with T4, KI, CLO, CLO + T4, CLO + KI	T4: 0.5 ppm; KI: 0.5 g/L; CLO: 5 ml/L; CLO + T4 or CLO + KI at same concentrations	8 weeks	• CLO + T4 produced the highest wet weight (0.14 g), CLO + KI highest length (3.9 cm). Twice-weekly CLO + T4 was as effective as daily feeding.	[146]
Zebrafish	Embryos (0–120 hpf)	Water exposure (PTU, MMI, T4)	PTU: 7.6–8.6 μM; MMI: 372–765 μM; T4: 30 nM	48–120 h	• PTU and MMI induced gene expression changes, craniofacial malformations, and reduced locomotion. T4 co-treatment rescued THs levels.	[97]
Yellow catfish (*Pelteobagrus fulvidraco*)	Juvenile (1.8 ± 0.0 g)	Dietary MMI (0 or 2 g/kg diet)	0 g/kg (Control), 2 g/kg (MMI-treated)	8 weeks	• MMI reduced growth, plasma T3/T4 levels, hepatic lipid content, and lipogenic enzyme activity; Increased lipolysis-related gene expression.	[95]
Goldfish	Adults	Osmotic-pump implant (chronic delivery)	T4 implant; PTU implant (doses per methods)	12 days	• T4 increased feeding motivation (behavioral index), central TSHβ and DIO2, hepatic DIO2, and hypothalamic DIO3; produced hyperthyroid axis changes. • PTU had no detectable effect, consistent with limited PTU sensitivity in fish deiodinases.	[7]
Goldfish	Juveniles	Dietary (T3 inclusion)	T3: 0, 1.25, 6.25 ppm in feed	60 days	• Both T3 diets increased SGR, % body weight gain, food conversion efficiency and protein efficiency ratio vs control. • Final weight higher at 6.25 ppm; disease resistance after *Aeromonas* challenge greatest at 1.25 ppm. • Lower T3 dose was most beneficial for survival.	[139]
Goldfish	Adults	Intraperitoneal injection; pituitary cell culture	T3 injections: 0, 2.5, 25, 250 ng per fish (and in vitro T3 exposure)	Blood/tissue sampling at 6–24 h	• T3 reduced pituitary *tshβ* mRNA (all stages) and *lhβ* mRNA (early recrudescence) in culture. • In vivo T3 reduced circulating 17β-estradiol (E2) in males (early/mid) and reduced testosterone in both sexes (mid).	[128]

(continued on next page)

Table 3 (*continued*)

Species	Weight or Age	Method of Administration	Doses	Work Interval	Results	References
Goldfish	Male adult	Waterborne exposure	T3: 20 nM, 100 nM (±1 nM E2)	3 days	• In follicle culture T3 suppressed E2 secretion → T3 impairs gonadotropin signaling and steroidogenesis. • T3 decreased testicular ERα and ERβ1 transcripts; TRα-1/β unchanged. • Hepatic DIO2 downregulated and DIO3 upregulated by T3. • Demonstrates rapid reprogramming of TH- and estrogen-related gene expression.	[129]
Goldfish	Adults	Single IP injection	T3 (single IP dose; assay 24–48 h)	24–48 h post injection	• Hepatic ERα (*esr1*) and vitellogenin (*vtg*) mRNA upregulated; ERβ1 (*esr2a*) downregulated. • Suggests T3 primes liver for vitellogenesis via ERα.	[130]

reiterating that MMI induces hypothyroidism, because it explicitly links endocrine suppression to metabolic dysfunction, reproductive impairment, and histopathological injury.

Table 3 summarizes various studies on the effects of THs exposure across different species, revealing its impacts on growth, reproduction and physiology. The present findings are consistent with earlier goldfish studies, which show that exogenous THs tend to suppress reproduction while enhancing growth. For example, Allan and Habibi found that T3 injections inhibit gonadotropin and steroid production in goldfish [128], and Nelson and Habibi reported that T3 elevates liver ERα and vitellogenin expression [130], suggesting a shift toward somatic (hepatic) metabolic functions. Likewise, dietary T3 markedly increased goldfish growth efficiency [139]. Conversely, inducing hypothyroidism delays sex differentiation in cyprinids: in zebrafish (a close relative), methimazole treatment skewed genetic males toward ovarian development by slowing testis formation [140]. These patterns – TH-induced suppression of gonadal activity and enhanced growth – echo the general principle that thyroid status mediates energy allocation between growth and reproduction in cyprinid fishes [128,141]. In summary, our results fit within the goldfish/cyprinid literature: exogenous THs bias physiology toward growth (with elevated hepatic ERα/vtg and reduced pituitary LH and sex steroids [128,130], whereas hypothyroid treatments (goitrogens) are expected to retard reproductive development.

5. Limitations and future directions

Coconut oil was used as the IP vehicle because it supports the gradual release of lipophilic compounds and maintains solubility after ethanol evaporation, consistent with previous applications in teleost studies [147,148]. Although oil-based vehicles can induce peritoneal irritation or local inflammatory responses [149], our carrier-only group exhibited no detectable changes in endocrine, growth, reproductive, or hepatic endpoints, indicating that coconut oil did not measurably affect the outcomes. We consider it suitable for this pharmacological design, while acknowledging that subtle vehicle-related effects cannot be entirely excluded. Future studies should incorporate an additional aqueous or sham control when feasible to better distinguish vehicle from compound effects.

Our T4 treatment protocol (10 mg/kg IP, single dose) reflects established thyroid-manipulation methods in teleost studies designed to induce a controlled, acute elevation in circulating thyroid hormones. Although this pulse-dose produces a transient supraphysiological state rather than sustained hyperthyroidism, it is widely used to investigate thyroid-dependent mechanisms influencing growth, metabolism, and reproductive maturation. While such acute perturbation limits direct extrapolation to chronic environmental conditions, it remains an appropriate approach for mechanistic evaluation of thyroid–gonadal interactions.

It should be emphasized that the T4 (10 mg/kg) and MMI (20 mg/kg) treatments represent supraphysiological, pharmacological pulse doses rather than environmentally realistic exposures. Although such high-dose IP protocols are widely used to generate controlled endocrine disruption, they do not reflect the chronic, low-ng/L concentrations reported for MMI in surface waters [41–43]. Thus, while the study clarifies MMI's capacity to disrupt thyroid function and the degree to which exogenous T4 can counteract these effects, environmental extrapolation should be made cautiously. Future research should apply chronic, lower-dose waterborne or dietary exposures, quantify free and bound THs, and include molecular markers to more accurately evaluate ecological risk.

It should be noted that we measured total plasma T4 and T3 (bound + unbound) rather than free hormone. In teleosts, most THs are bound to transport proteins such as transthyretin, albumin, and lipoproteins, which regulate distribution, half-life, and tissue delivery [150,151]. The supraphysiological IP dose of T4 likely exceeded binding capacity, producing a brief surge in free T4 followed by redistribution and clearance; thus, total TH levels mainly reflect the injected load rather than sustained bioactive hormone availability over the 28 days. Although the resulting growth, metabolic, and histological effects confirm a strong pharmacological response, these data cannot be directly extrapolated to chronic environmental exposure. Future studies should quantify free TH or partition bound versus unbound fractions to better evaluate biologically relevant hormone dynamics.

A key limitation of this study is the absence of molecular endpoints for thyroid-axis or reproductive gene expression. While plasma TH levels and histological data demonstrate endocrine disruption, they do not clarify potential changes in core regulators such as deiodinases (*dio1–3*), thyroid hormone receptors (*TRα/β*), transport proteins (*ttr*), or steroidogenic and vitellogenic genes (*cyp19a1a*, *foxl2*, *vtg*). As a result, the mechanistic pathways remain partly inferential. Future work should incorporate tissue-specific molecular analyses (qPCR, RNA-seq, protein markers) alongside oxidative-stress and lipid-metabolism indicators to validate the proposed endocrine–metabolic–reproductive linkages and more precisely attribute hepatic damage and oocyte arrest to specific molecular disruptions.

6. Conclusion

This study demonstrates that MMI produces pronounced endocrine disruption and multi-organ pathology in female goldfish, with clear impairments of growth, metabolic homeostasis, reproductive maturation, and liver integrity. Partial mitigation by T4 indicates that thyroid signaling centrally mediates these effects

and that interactions among thyroid-active compounds can alter outcomes. Collectively, the results identify MMI as an emerging contaminant of concern and support the need for enhanced environmental monitoring and targeted ecological risk assessment at environmentally relevant concentrations.

CRediT authorship contribution statement

Hamed Abdollahpour: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Milad Karimzadeh:** Visualization, Validation, Resources, Formal analysis, Data curation, Conceptualization. **Naghmeh Jafari Pastaki:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hosseinali Zamani:** Validation, Software, Resources, Methodology, Formal analysis, Data curation, Conceptualization.

Consent for publication

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Declaration of competing interest

The authors declare no conflicts of interest related to this work and confirm the absence of any commercial or associative interests that could represent a conflict in connection with the submitted manuscript. We also gratefully acknowledge the three anonymous reviewers of Emerging Contaminants for their valuable comments and constructive suggestions, which significantly improved the quality of this article.

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Data availability

Data supporting the findings of this study can be obtained from the corresponding author (H.A.) upon reasonable request.

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