Effect of Bisphenol A on Expression of Estrogen-, Retinoid- and Thyroid Hormone-Related Genes in the Green Catfish (*Mystus nemurus*)

Ladda Leelawatwattana[1*], Peasala Sokul[1] and Jiraporn Khwanmunee[2]

ABSTRACT

The impact of bisphenol A (BPA), a chemical known for its endocrine-disrupting ability, has been investigated in various fish species. This study aimed to examine the effect of BPA on the expression of genes associated with estrogen, retinoid, and thyroid hormones in green catfish (*Mystus nemurus*). The isolated cDNA fragments, which were 414, 319, 445, and 366 base pairs in length, exhibited significant similarity to brain cytochrome P450 aromatase (cyp19b), transglutaminase-2 (tgase-2), deiodinase type III (dio3), and thyroid hormone receptor alpha (trα), respectively. When translated into amino acids, these cDNA fragments corresponded to approximately 30%, 15%, 55%, and 30% of the full-length length P450AromB, TGase-2, Dio3, and TRα proteins in different fish species, respectively. At 15 days post-hatching, green catfish were exposed to BPA at concentrations of 0.01, 10, 100, and 1,000 nM for three days. The expression of cyp19 decreased compared to the control group when fish were exposed to BPA at 0.01 and 10 nM. The mRNA expression levels of tgase and tr also decreased across all treatment groups compared to the control group. However, no significant changes were observed in the expression of dio across the investigated doses. The study's findings indicate that exposure to BPA at ecologically relevant concentrations leads to changes in gene expression in green catfish.

Keywords: Bisphenol A, Cytochrome P450 aromatase, *Mystus nemurus*, Thyroid hormone receptor, Transglutaminase

INTRODUCTION

Endocrine-disrupting chemicals (EDCs) are a group of exogenous substances that disrupt the normal functioning of hormones or other components of the endocrine system. This results in detrimental consequences on animals and humans (Bhandari *et al*., 2015; Caballero-Gallardo *et al*., 2016). Various water sources naturally serve as reservoirs, and many EDCs can contaminate these systems. As a result, these chemicals directly impact aquatic organisms, including fish.

BPA (2,2-bis (4-hydroxyphenyl) propane) is a commonly used EDC in industrial applications, primarily as a raw material in the production of polycarbonate plastic and epoxy resins. BPA can enter aquatic environments through various pathways (Lee and Peart, 2000; Kang *et al*., 2007; Pookpoosa *et al*., 2014). BPA is classified as a xenoestrogen, capable of interfering with the estrogen signaling pathway by acting as both an agonist and antagonist of the estrogen receptor, with its effects varying depending on the species exposed (Faheem and Bhandari, 2021). Moreover, owing to its structural similarity to thyroid hormones, BPA can interact with thyroid hormone receptors, leading to disturbances in normal thyroid hormone function (Moriyama *et al*., 2002; Faheem and Bhandari, 2021). Research on the effects of different BPA levels on fish has shown that various BPA concentrations can have significant effects. For instance, exposure of zebrafish to BPA

[1]Division of Health and Applied Sciences, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand
[2]Department of Science and Mathematics, Faculty of Science and Technology, Phuket Rajabhat University, Muang, Phuket, Thailand
[*]Corresponding author. E-mail address: ladda.l@psu.ac.th
Received 22 March 2024 / Accepted 31 October 2024

in dosages of 10,000 mg·kg^{-1} and 2,000 mg·kg^{-1}, which exceed environmental levels, resulted in the feminization of the fish (Drastichová *et al*., 2005). In contrast, BPA concentrations of 1.75, 2.40, and 5.00 µg·L^{-1}, similar to environmental levels found in Austrian water systems (1.6 µg·L^{-1}), impacted male brown trout (*S. trutta f. fario*) semen quality and delayed ovulation in females (Lahnsteiner *et al*., 2005).

The presence of EDCs in water sources can negatively impact the metabolic processes of species inhabiting the contaminated environments and can spread to terrestrial and aerial organisms (Caballero-Gallardo *et al*., 2016). Given the significant effects of EDCs on many organisms within ecosystems, it is crucial to investigate the impact of EDCs and develop effective methods for detecting and monitoring them. To accomplish this, identifying sensitive biomarkers is key to this effort.

Approaches for screening and assessing the adverse impacts of EDCs on the endocrine system have been continually refined (Boonphakdee *et al*., 2019; Robitaille *et al*., 2022). Several fish species, such as zebrafish (*Danio rerio*) (Caballero-Gallardo *et al*., 2016), Japanese medaka (*Oryzias latipes*) (Horie *et al*., 2020), and rare minnow (*Gobiocypris rarus*) (Wang *et al*., 2010), have been utilized as experimental models for the assessment of EDCs. Additionally, research has been undertaken to examine the effects of hazardous compounds, including EDCs, on catfish species (Osman *et al*., 2007; Sayed *et al*., 2012; Aniche *et al*., 2019). One study compared the susceptibility of African catfish (*Clarias gariepinus*) and zebrafish to five substances: chromium (Cr), cadmium (Cd), zinc (Zn), pentachlorophenol, and malathion. The results suggested that African catfish could serve as a model for toxicity testing since it exhibited subchronic values for these compounds similar to those observed in zebrafish (Nguyen and Janssen, 2001), a species recommended as an animal model for EDC testing (Caballero-Gallardo *et al*., 2016). This finding highlights the sensitivity of African catfish to hazardous compounds. Furthermore, African catfish and other catfish species have large body sizes, facilitating tissue sampling and enabling comprehensive histopathological investigations. Since African catfish and other catfish species are commonly found in habitats with varying EDC levels, studying the effects of EDCs on these species can yield ecologically relevant insights that may be applicable to other species in similar environments.

Mystus nemurus (green catfish) is an important and commercially valuable food source in Thailand. Research on green catfish in Thailand primarily focuses on areas such as breeding, nutrition, and growth (Mesomya *et al*., 2002). Our previous study revealed that thyroid hormone receptor in juvenile green catfish responded to triiodothyronine (T3) by enhancing gene expression (Leelawatwattana, 2003). The examination of the green catfish's responsiveness to T3, its natural habitat in Thailand, and the known susceptibility of other catfish species to EDCs (Nguyen and Janssen, 2001) prompted the idea of utilizing green catfish as a model for evaluating the effects of BPA. Hence, this study aimed to investigate the impact of BPA and the sensitivity of green catfish to BPA by evaluating changes in the expression of estrogen-, retinoid-, and thyroid hormone-related genes. This research provides insights into the sensitivity of green catfish to BPA and identifies potential gene marker for BPA testing. These findings could contribute to the future applications in EDC testing.

MATERIALS AND METHODS

Fish rearing

Since estrogen, retinoid, and thyroid hormones play crucial roles in the early development of fish (Ross *et al*., 2000; Yamano, 2005; Mouriec *et al*., 2009; Bondesson *et al*., 2015), we used juvenile green catfish at 15 days post-hatching (body weight ranging between 0.07 g and 0.12 g) to assess the impact of BPA. The larvae were reared indoors in a circular fiberglass tank, starting three days post-hatching. The tank was equipped with a continuous aeration system. From day 3 to day 10 post-hatch, the fish were fed water fleas (*Moina* sp.). Afterward, they were gradually acclimated to a powdered feed and were given a combination of water fleas and powdered feed twice daily until 15 days post-hatch. Residual powdered feed was

removed daily, 80% of the tank water was replaced, and the water pump was cleaned regularly to maintain water quality. The water temperature remained between 28 and 29 °C throughout the fish raring, with a pH ranging from 7.0 to 7.6. Dissolved oxygen levels were maintained between 7.10 to 7.60 $mg \cdot L^{-1}$.

Fish treatment

Fifteen-day-old post-hatch green catfish with normal external anatomy, morphology, and typical swimming performance were selected. They were randomly distributed into glass containers filled with 2 L of BPA (Sigma, United States of America) solution at concentrations of 0.1, 10, 100, and 1,000 nM, at 15 fish/container. The control group was exposed to 0.01% DMSO. These BPA concentrations reflect levels commonly observed in water sources in various countries, including Thailand (Kolpin *et al*., 2002; Crain *et al*., 2007; Flint *et al*., 2012; Pookpoosa *et al*., 2014).

The experiment was conducted in duplicate. Each container was continuously aerated, and fish were fed twice a day. Containers were cleaned daily, and three-fourths of the water, along with the chemical solution, was replaced to maintain the desired BPA concentration. After three days of exposure, all surviving fish were euthanized and stored at -80 °C for further analysis.

Tissue collection for gene isolation

Adult green catfish were anesthetized with clove oil (Labvalley, Thailand). Liver and brain tissues were collected, immediately frozen in liquid nitrogen, and stored at -80 °C for further use.

Isolation of partial fragment of cytochrome P450 aromatase, transglutaminase, deiodinase, and thyroid hormone receptor

Total RNA was extracted from liver tissues (for isolation of transglutaminase, deiodinase, and thyroid hormone receptor genes) or brain tissue (for the cytochrome P450 aromatase gene) using the RNeasy Mini Kit (QIAGEN, Germany) following the manufacturer's protocol with slight modifications. Residual DNA was eliminated using DNase I (New England Biolabs, United States of America) based on the basic protocol recommended by the company, also with minor adjustments. First-strand cDNA was synthesized using a random hexamer (Promega, United States of America) and MMLV-RT (New England Biolabs, United States of America). The DNA fragments of transglutaminase, deiodinase, and thyroid hormone receptor genes were amplified using degenerate primers designed from conserved regions of vertebrate cDNA sequence. The cytochrome P450 aromatase gene was amplified using previously published degenerate primers (Kazeto and Trant, 2005) (Table 1). PCR products of the expected size were purified, ligated into the pGEM®-T Easy vector (Promega, United States of America), and transformed into *E. coli* DH5α. Positive clones were identified through blue/white screening, and individual white colonies were cultured overnight. Plasmid DNA was then purified using a mini-prep kit (Geneaid, Taiwan), and its concentration was determined spectrophotometrically (Hewlett Packard, United States of America). The nucleotide sequences were subsequently determined and analyzed for identity using the NCBI-BLAST tool. The deduced amino acid sequence of the investigated genes in green catfish were aligned with those of representative fish species using the CLUSTAL W algorithm.

Analysis of gene expression by real-time PCR

Real-time PCR was employed to determine the relative expression of the target genes under challenging conditions. Total RNA from whole fish was extracted with RNeasy Mini Kit (QIAGEN, Germany) according to the manufacturer's protocol with minor modifications. The quality and quantity of RNA were determined by agarose gel electrophoresis and absorption spectrophotometry analysis (Hewlett Packard, United States of America). Contaminated DNA was removed by digestion with DNase I (New England Biolabs, United States of America). First-strand cDNA was generated using random primers (Promega, United States of America), followed by amplification of the DNA fragment using specific primers designed for nucleotide sequence of each gene (Table 1). SYBR green

Table 1. Primers used for isolation of partial cDNA fragment and real-time PCR analysis in green catfish.

Gene	Purpose	Nucleotide sequence (5´ →3´)	Direction
Beta-actin (ß-actin)	Real-time PCR	ATCGTGCGTGACATTAAGGAG	Forward
		CGTACAGGTCTTTGCGCATG	Reverse
Cytochrome P450 aromatase (cyp19)	cDNA fragment isolation	CAGTGYRTRYTRGARATG	Forward
		TTCRTCATCRCCRTRGMDATGTG	Reverse
	Real-time PCR	GGCCAAAGGGACAAACATTATTC	Forward
		CGACTCGGCACGTTGTTAT	Reverse
Deiodinase (dio)	cDNA fragment isolation	GCCGTGTGGCACGGNCARAARYTNGA	Forward
		CACCCAGCCGTCGGANGGRTGNGCYTC	Reverse
	Real-time PCR	TGTGGTACAGCCAGAAGTTG	Forward
		CGAAGTTAAGGATGAACGGTCT	Reverse
Transglutaminase (tgase)	cDNA fragment isolation	GGCCACGCGTCGACTAGTAC	Forward
		GCGCTCAATCACCATGTTGCTGTTGGTGT CATGGGC	Reverse
	Real-time PCR	ATGTCTCTGCTGGTGTTCAG	Forward
		TGGCGACTAACTACCTCTCG	Reverse
Thyroid hormone receptor (tr)	cDNA fragment isolation	TGGCCAAGCGGAAGYTNATHGAGA	Forward
		GCCCGCAGGGACATDATYTCATRCA	Reverse
	Real-time PCR	AGGAGGAGATGGTGAAGACG	Forward
		GGGTGATGGCTGGCGTGATG	Reverse

(Takara, Japan) was utilized to detect the PCR products. The target genes and internal control genes were amplified in separate reactions. cDNA samples without reverse transcriptase were used as control for real-time PCR. All reactions were run in duplicate using an MX3005P QPCR system (Stratagene, United States of America).

The baseline was automatically set, and the cycle threshold (Ct) for each sample was determined based on the exponential growth phase and baseline signal from the fluorescence versus cycle number plots. To assess primer efficiency and specificity for each gene, cDNA reverse-transcribed from total RNA extracted from 4-day-old fish (for ß-actin, tr, and tgase) or plasmids containing the specific DNA fragment (for cyp19 and dio) were serially diluted across six different concentrations and subjected to real-time PCR using the specific primers (Table 1). The resulting Ct values were plotted against the logarithm of the RNA quantity used for the cDNA preparation or the plasmid quantity. PCR efficiency (E) was then calculated from the slope of the linear regression line using the equation:

$$E = (10^{(-1/slope)}-1)\times 100$$

Using the efficiency-corrected calculation model (Pfaffl, 2001), the relative expression of target genes in fish exposed to BPA was calculated as a fold change compared to the control group, which was not exposed to BPA. The equation is:

$$\text{Fold change} = \frac{\text{Etarget}^{\Delta Ct\ target\ (control\ -\ sample)}}{\text{Ereference}^{\Delta Ct\ reference\ (control\ -\ sample)}}$$

Where: E is primer efficiency for amplification of each gene;
Target is targeted gene;
Reference is beta-actin;
Sample is fish that is exposed to BPA at different concentrations;
Control is fish that is exposed to 0.01% DMSO.

Statistical analysis

All data are presented as mean±standard error of the mean (SE). Differences in gene expression were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for pairwise comparisons. Statistically significant was determined at $p<0.05$.

RESULTS

Isolation of partial cDNA fragment

Cytochrome P450 aromatase

A cDNA fragment of 414 base pairs (bp) was isolated from the brain of the green catfish. The nucleotide and deduced amino acid sequences shared between 70% and 90% identity with brain cytochrome P450 aromatase (cyp19b/P450aromB for gene and protein product, respectively) found in several teleost species, according to NCBI-BLAST analysis. The green catfish cyp19 gene displayed the highest nucleotide sequence identity to the cyp19b gene of yellow catfish (*Tachysurus fulvidraco*, accession number KP135448.1). In terms of deduced amino acid sequences, it showed the greatest similarity to P450aromB in giant devil catfish (*Bagarius yarrelli*, accession number TSK13468.1) and Southern catfish (*Silurus meridionalis*, accession number AAP83132.1).

The isolated cDNA encodes 138 amino acids, representing approximately 30% of the full P450aromB protein found in other fish species (Kishida and Callard, 2001; Kazeto and Trant, 2005; Rasheeda *et al*., 2010). The deduced amino acid sequence from the aromatase in the green catfish was aligned with the sequences of other representative fishes, demonstrating a significant degree of similarity (Figure 1). Additionally, the isolated cDNA fragment contained key regions such as the I-helix, aromatic region, and heme-binding region, which are characteristic domains of teleost P450arom (Kazeto and Trant, 2005; Rasheeda *et al*., 2010). These findings imply that the aromatase found in the brain of green catfish is a member of the P450arom protein family within fish species.

Transglutaminase

The isolated transglutaminase gene fragment was 319 base pairs in length. NCBI blast analysis revealed that the nucleotide and deduced amino acid sequences are identical to isoform 2 of transglutaminase (tgase-2/TGase-2 for gene and protein product, respectively) in many vertebrates, especially bony fish. The identity between the sequences ranged from 85% to 95% for the nucleotide and from 65% to 86% for the amino acid. The deduced amino acid sequence was most similar to TGase-2 in Mexican tetra (*Astyanax mexicanus*, accession number KAG9277081.1). The cDNA fragment encodes a polypeptide of 106 amino acids, representing approximately 15% of the full TGase-2 protein length reported in previous studies (Yasueda *et al*., 1995; Furnes *et al*., 2014). The amino acid sequence of TGase in the green catfish was aligned with sequences from other fish species, revealing a

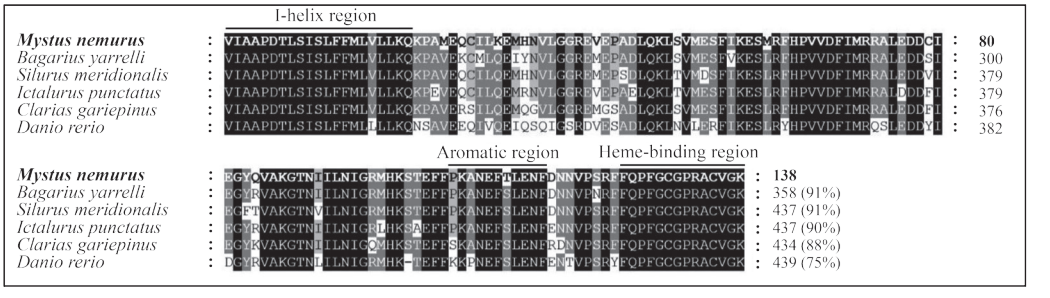

Figure 1. Multiple sequence alignment of deduced amino acid sequence encoded from isolated cDNA of the green catfish cyp19. The representative teleosts include giant devil catfish (*Bagarius yarrelli*, TSK13468.1), Southern catfish (*Silurus meridionalis*, AAP83132.1), channel catfish (*Ictalurus punctatus*, AAL14612.1), African catfish (*Clarias gariepinus*, ADH29766.1) and zebrafish (*Danio rerio*, AAK00642.1). Black shading denotes completely identical residues, while grey highlights signify amino acids conserved across more than four species. The numerical value on the right side is displayed in relation to the total length of the amino acid sequence reported in GenBank, except for the green catfish. The blanket displays the percentage identity of the deduced amino acid sequence.

substantial degree of similarity. Notably, the isolated gene includes the conserved catalytic core region, with cysteine as a critical residue essential for catalytic activity (Yee *et al*., 1994), as illustrated in Figure 2.

Deiodinase

A partial cDNA fragment of deiodinase, measuring 445 bp, was isolated. NCBI blast analysis indicated that the nucleotide sequence exhibited high similarity to the expected iodothyronine deiodinase type 3 (dio3/Dio3 for gene and protein product, respectively) with the highest identity (94%) to the predicted iodothyronine deiodinase 3b of striped catfish (*Pangasianodon hypophthalmus*, accession number XM_026923363.3). The cDNA fragment contained an internal TGA codon encoding selenocysteine (Sec), an amino acid commonly observed in the catalytic core of deiodinase. In addition, the cDNA for green catfish dio encodes 148 amino acid residues, representing approximately 55% of the total amino acids of Dio3 found in other fish species (Sanders *et al*., 1999; Darras and Van Herck, 2012; Jones *et al*., 2013). The deduced amino acid sequences showed 71–75% similarity to Dio3 in several fish species, including goldfish (*Carassius auratus*, accession number ABP64747), red drum (*Sciaenops ocellatus*, accession number AGT55555), and Nile tilapia (*Oreochromis niloticus*, accession number CAA71997). The amino acid sequence of the green catfish Dio was aligned with those of other representative fish species, demonstrating a significant degree of similarity (Figure 3).

Thyroid hormone receptor

The isolated cDNA fragment of the thyroid hormone receptor was 366 bp in length. The sequence exhibited the highest similarity to the alpha isoform of the thyroid hormone receptor (trα) found in various fish species. This fragment represents approximately 30% of the entire TR polypeptide chain, consistent with studies on vertebrates (Kawakami *et al*., 2003; Chen *et al*., 2014). The nucleotide and amino acid sequences exhibited homology ranging from 80% to 96% for nucleotides and 87% to 98% for amino acids when compared to other teleosts. Notably, the green catfish cDNA sequence shared 96% nucleotide identity and 99% amino acid identity with the trα/TRα isoform of yellow catfish (*Tachysurus fulvidraco*) (accession number KC347579 for nucleotide sequence and AGY36893.1 for amino acid sequence). The isolated cDNA also contained the ligand-binding domain characteristic of all TRs. The deduced amino acid sequence of the green catfish TR protein was aligned with those of other

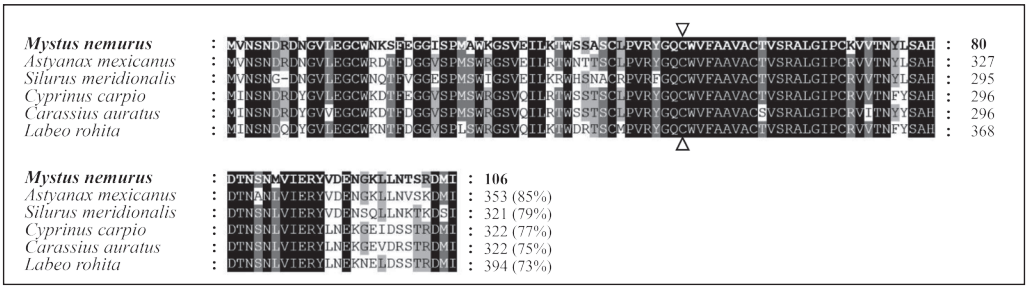

Figure 2. Multiple sequence alignment of the deduced amino acid sequence of the green catfish TGase with the full-length TGase-2 sequences from different teleosts. The representative fishes include Mexican tetra (*Astyanax mexicanus*, KAG9277081.1), Southern catfish (*Silurus meridionalis*, KAI5109184.1), common carp (*Cyprinus carpio*, AAL02240.1), goldfish (*Carassius auratus*, BAF37011.1) and rohu (*Labeo rohita*, KAI2663201.1). Black shading denotes completely identical residues, while grey highlights signify amino acids conserved across more than four species. The numerical value on the right side is displayed in relation to the total length of the amino acid sequence reported in GenBank, except for the green catfish. The triangles indicate the position of the catalytic cysteine residue, which is part of the conserved motif essential for the catalytic function of TGases. The blanket displays the percentage identity of the deduced amino acid sequence.

selected fish species, showing a significant degree of similarity (Figure 4).

Relative expression of genes during the green catfish were challenged with BPA

Primer specificity was confirmed through melting curve analysis, agarose gel electrophoresis of the PCR product, and DNA sequencing. The melting curve analysis displayed a single peak for each gene. Agarose gel electrophoresis revealed a single band corresponding to the expected sizes of 110, 100, 150, 250, and 260 bp for cyp19, tgase, dio, tr, and ß-actin, respectively (supplement figure 1). DNA sequencing of these bands verified that they were the intended target genes. Additionally, when specific primers were used, the PCR efficiencies for cyp19, tgase, dio, tr, and ß-actin were 99.00%, 89.03%, 91.00%, 100.58%, and 110.62%, respectively, with an r^2 value of more than 0.99 for all genes.

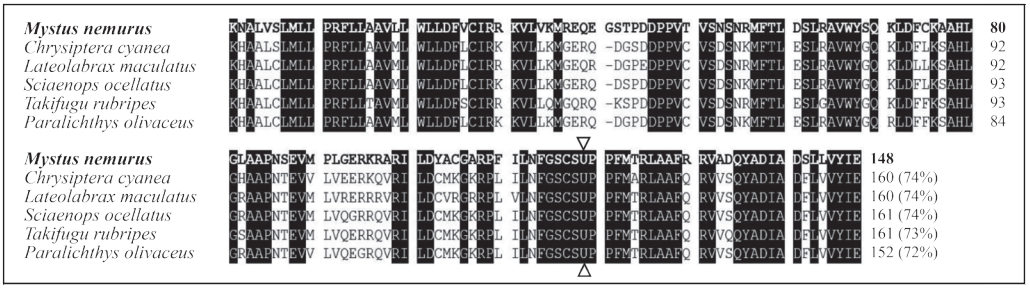

Figure 3. Multiple sequence alignment of the deduced amino acid sequence of the green catfish Dio with the full-length Dio3 sequences from different teleosts. The representative fishes include sapphire devil (*Chrysiptera cyanea*, ADD82415.1), spotted sea bass (*Lateolabrax maculatus*, WKC58919.1), red drum (*Sciaenops ocellatus*, AGT55555.1), Japanese flounder (*Paralichthys olivaceus*, BAG15908.1) and torafugu (*Takifugu rubripes*, NP_001129618.1). Black shading denotes completely identical residues, while grey highlights signify amino acids conserved across more than four species. The numerical value on the right side is displayed in relation to the total length of the amino acid sequence reported in GenBank, except for the green catfish. The triangles indicate the position of selenocysteine (U), which is commonly found in the catalytic core of deiodinase. The blanket displays the percentage identity of the deduced amino acid sequence.

Figure 4. Multiple sequence alignment of deduced amino acid sequence encoded from isolated cDNA of the green catfish TR. The representative teleosts include yellow catfish (*Tachysurus fulvidraco*, AGY36893.1), Mexican tetra (*Astyanax mexicanus*, KAG9268067.1), whitespotted conger (*Conger myriaster*, BAD 27474.1), Japanese eel (*Anguilla japonica*, BAN82439.1) and javeline goby (*Acanthogobius hasta*, AGY 36895.1). Black shading denotes completely identical residues, while grey highlights signify amino acids conserved across more than four species. The numerical value on the right side is displayed in relation to the total length of the amino acid sequence reported in GenBank, except for the green catfish. The blanket displays the percentage identity of the deduced amino acid sequence.

Following verification of primer specificity, the impact of BPA on the expression of the designated genes was evaluated in 15-day-old juvenile green catfish. After three days of exposure to BPA at concentrations of 0.01, 10, 100, and 1,000 nM, the expression of cyp19 was significantly reduced compared to the control group. This reduction was observed in fish exposed to 0.01 and 10 nM BPA (Figure 5). The mRNA expression levels of tgase and tr also decreased in all treatment groups compared to the control group. However, BPA exposure did not significantly affect the expression of dio at any concentrations.

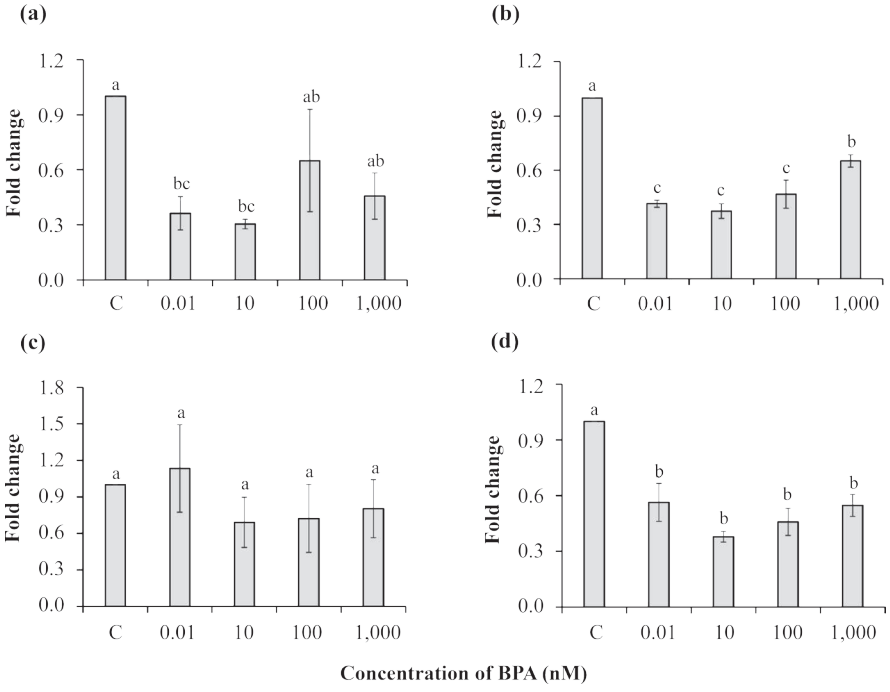

Figure 5. Expression of cyp19 (a), tgase (b), dio (c), and tr (d) in 15-day-old green catfish (fold change) exposed to various concentrations of BPA for 3 days. Data are presented as mean values, with standard error of the mean (SE) indicated by error bars. Different lowercase letters above the bars denote significant differences ($p<0.05$). C in each graph represents the control group.

DISCUSSION

This study examined the impact of BPA on 15-day-old green catfish. After three days of exposure, no significant differences in survival were observed between the BPA-exposed fish and the control group. However, gene expression analysis revealed a positive response to BPA, as evidenced by significant difference in the expression of cyp19, tgase, and tr compared to the control group. Notably, the expression of dio gene did not display a statistically significant difference from the control group, as depicted in Figure 5.

Cytochrome P450 aromatase (P450arom) catalyzes the rate-limiting step in estrogen synthesis, a hormone essential for various developmental and physiological processes (Boon *et al*., 2010). Fish possess two aromatase genes, cyp19a and cyp19b, which encode P450aromA and P450aromB, respectively (Kazeto *et al*., 2001; Piferrer and Blázquez, 2005). cyp19a is primarily expressed in the gonads, while cyp19b is predominantly found in the brain (Piferrer and Blázquez, 2005). This study isolated a partial cyp19 from the brain of green catfish, which exhibited significant similarity to brain cytochrome P450 aromatase in other fish

species, specifically those belonging to the catfish species (Figure 1). Since the isolated cDNA accounts for approximately 30% of the total amino acids in P450aromB, the term cyp19 was used, as it cannot definitively be identified as cyp19b.

Following BPA exposure, the expression level of cyp19 in green catfish exhibited a notable decline, with significant differences at concentrations of 0.01 nM and 10 nM. These findings align with previous study on juvenile rare minnows (*Gobiocypris rarus*), where BPA exposure significantly decreased cyp19b expression (Wang *et al*., 2010). The impact of BPA on cyp19 expression have been inconsistent across studies, with some reporting increases and others reductions. For example, Chung *et al*. (2011) observed increased cyp19b expression in zebrafish embryos exposed to BPA at concentrations above environmental limits (5 μM). Furthermore, BPA was shown to have differential effects on the expression of different cyp19 subtypes. For instance, Murray rainbowfish (*Melanotaenia fluviatilis*) exposed to BPA at 100 and 500 $\mu g \cdot L^{-1}$ for 96 hours exhibited increased expression of cyp19b in the brain and ovaries of female fish, while the expression of cyp19a in the ovaries did not significantly differ from the control group (Shanthanagouda *et al*., 2014). This study highlights the greater sensitivity of cyp19b to BPA, which may be attributed to the presence of complete estrogen-responsive elements (EREs) in the promoter region of cyp19b compared to cyp19a, which lacks full EREs (Guiguen *et al*., 2010; Shanthanagouda *et al*., 2014).

Various factors can modulate aromatase expression, and estrogen is one of the regulators due to the presence of EREs in the cyp19b gene promoter (Piferrer and Blázquez, 2005). Depending on the specific tissue, BPA can bind to the estrogen receptor (ER) and function as an estrogen agonist or antagonist (Faheem and Bhandari, 2021). Furthermore, BPA is recognized as a selective estrogen receptor modulator that interacts distinctively with transcriptional coregulators, influencing its capacity to control the expression of responsive genes (Routledge *et al*., 2000; Welshons *et al*., 2006). Hence, the decrease in cyp19 gene expression in green catfish may be attributed to the regulation of the estrogen receptor induced by BPA.

The expression of cyp19 exhibited notable differences at lower doses of BPA (0.01 and 10 nM), while the higher concentrations (100 and 1,000 nM) did not influence the gene expression (Figure 5). This response characteristic appears to align with the non-monomeric dose-response curve observed in certain EDCs, including BPA (Welshons *et al*., 2003; Vandenberg, 2013). This trait pertains to the interplay between EDC concentration, receptor occupancy, and the subsequent physiological response (Welshons *et al*., 2003).

Retinoids, including vitamin A, are vital for vertebrate development (Ross *et al*., 2000) and detoxification of xenobiotics (Shmarakov, 2015). This study used tgase as a marker to assess impact of BPA on the retinoid system. TGase enzymes facilitate covalent crosslinking of lysine and glutamine residues (Ichinose *et al*., 1990), with TGase-2 being the predominant form in various tissues (Eckert *et al*., 2014). It plays crucial roles in multiple physiological and pathological processes, including bone growth, immune system function, and apoptosis (Joseph *et al*., 1998; Nurminskaya and Belkin, 2012). The catalytic activity of TGases depends on a catalytic triad composed of cysteine, histidine, and aspartate, all situated within the conserved catalytic core domain. These residues are highly conserved among TGase members (Yee *et al*., 1994) and across various organisms, including humans and fish (Lisetto *et al*., 2023). The tgase-2 gene has been successfully cloned from various fish species (Yasueda *et al*., 1995; Furnes *et al*., 2014; Lisetto *et al*., 2023). This study isolated the partial gene of tgase from the liver of the green catfish. It shared high similarity to tgase-2 sequences (Figure 2) which is the predominant form among the various TGase members. Given that the isolated sequence covers only about 15% of the total amino acids in TGase-2, the term tgase was used for this study.

The current investigation revealed that BPA inhibited the expression of tgase in the green catfish at all tested concentrations (Figure 5). The regulation of tgase expression mainly occurs at the transcriptional level. It has been demonstrated that retinoids function as inducers of this process, and the modulation is facilitated by the specific

receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs) (Eckert *et al*., 2014). BPA has a detrimental effect on retinoid metabolism through various mechanisms, including the disruption of rar and rxr expression (Shmarakov, 2015). Nishizawa *et al*. (2003) demonstrated that BPA at an environmentally relevant concentration decreased the expression of rarα and rxrα in the gonads of mouse embryos. Moreover, it has been suggested that BPA interacts significantly with RAR and can serve as either an agonist or antagonist on RAR and RXR-mediated transcription (Shmarakov, 2015). Hence, it is plausible to propose that the decrease in tgase expression in the green catfish may be associated with the disruption caused by BPA on RAR and RXR, subsequently impacting the expression of retinoid-sensitive genes like tgase.

BPA can influence several aspects of the thyroid hormone system, from hormone synthesis to hormone action, through multiple mechanisms (Gorini *et al*., 2020). Thyroid hormones are initially released as prohormones and converted into their active form in peripheral tissues through deiodination, a process facilitated by deiodinase enzymes. Deiodinases are enzymes that maintain the balance of thyroid hormones in the body. They catalyze deiodination, producing either the active or inactive thyroid hormone (Orozco and Valverde-R, 2005). Fish have three types of deiodinases: Dio1, Dio2, and Dio3. Type 1 deiodinase (Dio1) catalyzes the outer ring deiodination of T4 and T3, producing active T3 and an inactive reverse triiodothyronine (rT3). Type 2 deiodinase (Dio2) catalyzes the outer ring deiodination, converting T4 to T3. Conversely, type 3 deiodinase (Dio3) catalyzes the inner ring deiodination of T4 or T3, creating inactive metabolites like rT3 and 3,3′-diiodothyronine (T2), respectively (Orozco and Valverde-R, 2005; Darras and Van Herck, 2012). The current investigation isolated a fragment of the dio gene from the liver of the green catfish. The isolated gene shared similarities with dio3 from various fish species and contained selenocysteine, an amino acid present in an active site unique to all three Dio enzymes (Orozco and Valverde-R, 2005). Although the isolated gene comprises about 55% of the total amino acids in Dio3, it cannot be definitively identified as dio3; hence, the term dio was employed.

BPA at various concentrations had no impact on the expression of dio in green catfish. Research on impacts of BPA on deiodinase expression has yielded diverse outcomes. For instance, Zhang *et al*. (2017) found that exposure of zebrafish to a bisphenol known as BPS did not change the expression of dio3, but they observed a decline in T3 levels along with an elevation in dio1 and dio2 expression. In contrast, Niu *et al*. (2021) found that 10 nM BPA did not affect the expression of dio3 in 52-stage *Xenopus laevis* after a 48-h exposure, though higher concentrations (100 and 1,000 nM) did upregulate gene expression in tadpole brains.

T3 exerts its effect primarily through the genomic pathway, where it binds to TRs. Two main TR subtypes, TRα and TRβ, have been identified in fish, with their expression varying across tissues and developmental stages (Nelson and Habibi, 2006; 2009). The current investigation isolated a partial tr gene from the liver of green catfish, showing high similarity to trα genes from other fish species, particularly those in the catfish family, with an identity exceeding 90%. However, as only 30% of the total amino acids in TRα were identified, it cannot be conclusively confirmed as trα. Therefore, the term tr was used.

BPA significantly decreased the expression of tr at all concentrations tested in green catfish. This finding aligns with Iwamuro *et al*. (2006), who reported that BPA at 10^{-7} M inhibited the expression of trα and trβ in *Xenopus* tail culture. However, other studies have produced different results. For instance, Chan and Chan (2012) found that sub-lethal BPA concentrations did not alter trα and trβ expression in zebrafish embryos or larvae after 96 hours.

Due to its structural similarity to thyroid hormone, BPA can bind to TRs, acting as either a TR agonist or antagonist. The effects of BPA may be biphasic, depending on the concentrations of TR, BPA, and T3 present in the system (Zhang *et al*., 2018). Regarding antagonistic action, prior studies suggest BPA competes with T3 for TR binding, recruiting nuclear corepressor to TR and suppressing transcription of TH-responsive genes (Moriyama *et al*., 2002). Alternatively, BPA may

recruit coactivators to TR, activating TH-responsive gene expression (Zhang *et al*., 2018). These variations in findings likely attributes to differences in BPA concentrations, developmental stage of the fish, and the period of exposure. Nonetheless, this study indicates that BPA can disrupt tr expression in green catfish, potentially impacting the regulation of thyroid hormone-responsive genes.

This study also assessed the survival rates of green catfish after three days of treatment. No significant differences were observed between the BPA-exposed fish and the control group. However, no additional parameters, such as body deformities or immune responses, were monitored. Previous studies have reported adverse effects of BPA on fish health. For instance, Aluru *et al*. (2010) reported that acute exposure to BPA in oocytes of rainbow trout affected offspring long-term health, including delayed growth, altered morphology, reduced body mass, and impaired stress performance. Moreover, acute exposure of *Danio rerio* to environmentally relevant concentrations of BPA induced oxidative stress and upregulation of apoptosis- and inflammation-related genes (Elizalde-Velázquez *et al*., 2023).

Thyroid hormone regulates tr expression, which can influence expression of TH-responsive genes such as growth hormone, which is closely related to fish growth (Sternberg and Moav, 1999). Transglutaminase, involved in apoptosis and immune responses, may also contribute to immune suppression when its expression is altered (Nurminskaya and Belkin, 2012). Cytochrome P450 aromatase, essential for estrogen biosynthesis, plays a critical role in gonad development, reproduction, and bone mineralization (Caballero-Gallardo *et al*., 2016). Disruption of these enzymes can severely impact fish reproductive health. To gain more insights into effects of BPA on green catfish, future research should include observing long term effects on survival rate, change in morphology, effect on immune function, and growth of fish.

The concentration of BPA in water varied across countries (Kolpin *et al*., 2002; Crain *et al*., 2007; Flint *et al*., 2012; Deemoon *et al*., 2016). However, data on BPA levels in water in Thailand is limited. According to Deemoon *et al*. (2016), BPA levels in water samples taken from 12 locations along the Nan River ranged from 34.97 to 1,554.14 $ng \cdot L^{-1}$ (0.15 to 6.81 nM). Additionally, BPA in water from a wastewater treatment plant in Bangkok ranged from 57.5 to 257 $ng \cdot L^{-1}$ (0.25 to 1.13 nM) (Pookpoosa *et al*., 2014). These levels fall within the environmental range, defined as concentrations below 12 $\mu g \cdot L^{-1}$ (0.05 µM) (Flint *et al*., 2012). The BPA concentration in this study (0.01 nM) was lower than these environmental levels, indicating the heightened sensitivity of green catfish to BPA exposure. This study also suggests potential genes, such as tgase and tr, that could serve as biomarkers for BPA contamination in water sources.

CONCLUSIONS

This study demonstrated that green catfish respond to BPA exposure by altering the expression of genes related to estrogen (cyp19), retinoid (tgase), and thyroid hormones (tr). These findings suggest that green catfish are highly sensitive to BPA, even at concentrations commonly found in the environment. Furthermore, green catfish and the specific genes examined in this study could serve as effective biomarkers for monitoring BPA contamination in aquatic environments.

However, it is essential to note that this study used samples from only two replicates, which may have limited the statistical power. To enhance the reliability and significance of future findings, it is recommended that the number of replicates be increased. Additionally, further research is needed to explore the broader implications of BPA exposure, such as its impact on growth, development, morphology, and behavior. Long-term exposure studies and precise quantification of BPA levels in treated water are also essential. Expanding gene analysis would offer a more comprehensive understanding of how BPA induces endocrine disruption in aquatic organisms.

ACKNOWLEDGEMENT

This work was supported by a grant from Prince of Songkla University (Grant No. SCI560 377S).

LITERATURE CITED

Aluru, N., J.F. Leatherland and M.M. Vijayan. 2010. Bisphenol A in oocytes leads to growth suppression and altered stress performance in juvenile rainbow trout. **PLoS One** 5(5): e10741. DOI: 10.1371/journal.pone.0010741.

Aniche, D.C., F.V. Oluwale and B.O. Ogbolu. 2019. Effect of exposure to sub-lethal potassium cyanide on growth rate, survival rate, and histopathology in juvenile Heteroclarias (*Heterobranchus longifilis* × *Clarias gariepinus*). **Journal of Fisheries and Environment** 43(1): 1–10.

Bhandari, R.K., S.L. Deem, D.K. Holliday, C.M. Jandegian, C.D. Kassotis, S.C. Nagel, D.E. Tillitt, F.S. vom Saal and C.S. Rosenfeld. 2015. Effects of the environmental estrogenic contaminants bisphenol A and 17α-ethinyl estradiol on sexual development and adult behaviors in aquatic wildlife species. **General and Comparative Endocrinology** 214: 195–219.

Bondesson, M., R. Hao, C.Y. Lin, C. Williams and J.Å. Gustafsson. 2015. Estrogen receptor signaling during vertebrate development. **Biochimica et Biophysica Acta** 1849(2): 142–151.

Boon, W.C., J.D.Y. Chow and E.R. Simpson. 2010. The multiple roles of estrogens and the enzyme aromatase. **Progress in Brain Research** 181: 209–232.

Boonphakdee, C., Y. Ocharoen, A.P. Shinn, S. Suanla and J. Thamnawasolos. 2019. 18S rRNA, a potential reference gene in the qRT-PCR measurement of bisphenol A contamination in green mussels (*Perna viridis*) collected from the Gulf of Thailand. **Agriculture and Natural Resources** 53: 652–661.

Caballero-Gallardo, K., J. Olivero-Verbel and J.L. Freeman. 2016. Toxicogenomics to evaluate endocrine disrupting effects of environmental chemicals using the zebrafish model. **Current Genomics** 17(6): 515–527.

Chan, W.K. and K.M. Chan. 2012. Disruption of the hypothalamic-pituitary-thyroid axis in zebrafish embryo-larvae following waterborne exposure to BDE-47, TBBPA and BPA. **Aquatic Toxicology** 108: 106–111.

Chen, Q.L., Z. Luo, X.Y. Tan, Y.X. Pan, J.L. Zheng and M. Zou. 2014. Molecular cloning and mRNA tissue expression of thyroid hormone receptors in yellow catfish *Pelteobagrus fulvidraco* and Javelin goby *Synechogobius hasta*. **Gene** 536(2): 232–237.

Chung, E., M.C. Genco, L. Megrelis and J.V. Ruderman. 2011. Effects of bisphenol A and triclocarban on brain-specific expression of aromatase in early zebrafish embryos. **Proceedings of the National Academy of Sciences of the United States of America** 108(43): 17732–17737.

Crain, D.A., M. Eriksen, T. Iguchi, S. Jobling, H. Laufer, G.A. LeBlanc and L.J. Guillette Jr. 2007. An ecological assessment of bisphenol-A: evidence from comparative biology. **Reproductive Toxicology** 24(2): 225–239.

Darras, V.M. and S.L. Van Herck. 2012. Iodothyronine deiodinase structure and function: from ascidians to humans. **Journal of Endocrinology** 215(2): 189–206.

Deemoon, S., C. Sarin, G. Ying, C. Kritsunankul and S. Sriprang. 2016. Occurrence of endocrine disrupting chemicals (EDCs) and estrogenic activity in the Nan River, Phitsanulok, Thailand. **EnvironmentAsia** 9(1): 84–91.

Drastichová, J., Z. Svobodová, M. Groenland, R. Dobšíková, V. Žlábek, D. Weissová and M. Szotkowská. 2005. Effect of exposure to bisphenol A and 17ß-estradiol on the sex differentiation in zebrafish (*Danio rerio*). **Acta Veterinaria Brno** 74: 287–291.

Eckert, R.L., M.T. Kaartinen, M. Nurminskaya, A.M. Belkin, G. Colak, G.V.W. Johnson and K. Mehta. 2014. Transglutaminase regulation of cell function. **Physiological Reviews** 94(2): 383–417.

Elizalde-Velázquez, G.A., L.M. Gómez-Oliván, S.E. Herrera-Vázquez, K.E. Rosales-Pérez, N. SanJuan-Reyes, S. García-Medina and M. Galar-Martínez. 2023. Acute exposure to realistic concentrations of bisphenol A trigger health damage in fish: blood parameters, gene expression, oxidative stress. **Aquatic Toxicology** 261: 106610. DOI: 10.1016/j.aquatox.2023.106610.

Faheem, M. and R.K. Bhandari. 2021. Detrimental effects of bisphenol compounds on physiology and reproduction in fish: a literature review. **Environmental Toxicology and Pharmacology** 81: 103497. DOI: 10.1016/j.etap.2020.103497.

Flint, S., T. Markle, S. Thompson and E. Wallace. 2012. Bisphenol A exposure, effects, and policy: a wildlife perspective. **Journal of Environmental Management** 104: 19–34.

Furnes, C., Ø. Kileng, I. Jensen, P. Karki, L. Eichacker and B. Robertsen. 2014. Isolation and characterization of two cDNAs encoding transglutaminase from Atlantic cod (*Gadus morhua*). **Fish and Shellfish Immunology** 36(1): 276–283.

Gorini, F., E. Bustaffa, A. Coi, G. Iervasi and F. Bianchi. 2020. Bisphenols as environmental triggers of thyroid dysfunction: Clues and evidence. **International Journal of Environmental Research and Public Health** 17(8): 2654. DOI: 10.3390/ijerph 17082654.

Guiguen, Y., A. Fostier, F. Piferrer and C.F. Chang. 2010. Ovarian aromatase and estrogens: a pivotal role for gonadal sex differentiation and sex change in fish. **General and Comparative Endocrinology** 165(3): 352–366.

Horie, Y., N. Kanazawa, C. Takahashi, N. Tatarazako and T. Iguchi. 2020. Bisphenol A induces a shift in sex differentiation gene expression with testis-ova or sex reversal in Japanese medaka (*Oryzias latipes*). **Journal of Applied Toxicology** 40(6): 804–814.

Ichinose, A., R.E. Bottenus and E.W. Davie. 1990. Structure of transglutaminases. **Journal of Biological Chemistry** 265(23): 13411–13414.

Iwamuro, S., M. Yamada, M. Kato and S. Kikuyama. 2006. Effects of bisphenol A on thyroid hormone-dependent up-regulation of thyroid hormone receptor alpha and beta and down-regulation of retinoid X receptor gamma in *Xenopus* tail culture. **Life Sciences** 79(23): 2165–2171.

Jones, R.A., W.B. Cohn, T.C. Miller, J.T. Jaques and D.S. Mackenzie. 2013. Cyclic mRNA expression of thyrotropin subunits and deiodinases in red drum, *Sciaenops ocellatus*. **General and Comparative Endocrinology** 194: 248–256.

Joseph, B., O. Lefebvre, C. Méreau-Richard, P.M. Danzé, M.T. Belin-Plancot and P. Formstecher. 1998. Evidence for the involvement of both retinoic acid receptor- and retinoic X receptor-dependent signaling pathways in the induction of tissue transglutaminase and apoptosis in the human myeloma cell line RPMI 8226. **Blood** 91(7): 2423–2432.

Kang, J.H., D. Asai and Y. Katayama. 2007. Bisphenol A in the aquatic environment and its endocrine-disruptive effects on aquatic organisms. **Critical Reviews in Toxicology** 37(7): 607–625.

Kawakami, Y., M. Tanda, S. Adachi and K. Yamauchi. 2003. Characterization of thyroid hormone receptor alpha and beta in the metamorphosing Japanese conger eel, *Conger myriaster*. **General and Comparative Endocrinology** 132(2): 321–332.

Kazeto, Y., S. Ijiri, A.R. Place, Y. Zohar and J.M. Trant. 2001. The 5'- flanking regions of CYP19A1 and CYP19A2 in zebrafish. **Biochemical and Biophysical Research Communications** 288(3): 503–508.

Kazeto, Y. and J.M. Trant. 2005. Molecular biology of channel catfish brain cytochrome P450 aromatase (CYP19A2): cloning, preovulatory induction of gene expression, hormonal gene regulation and analysis of promoter region. **Journal of Molecular Endocrinology** 35(3): 571–583.

Kishida, M. and G.V. Callard. 2001. Distinct cytochrome P450 aromatase isoforms in zebrafish (*Danio rerio*) brain and ovary are differentially programmed and estrogen regulated during early development. **Endocrinology** 142(2): 740–750.

Kolpin, D.W., E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber and H.T. Buxton. 2002. Pharmaceutical, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000 a national reconnaissance. **Environmental Science and Technology** 36(6): 1202–1211.

Lahnsteiner, F., B. Berger, M. Kletzl and T. Weismann. 2005. Effect of bisphenol A on maturation and quality of semen and eggs in the brown trout, *Salmo trutta f. fario*. **Aquatic Toxicology** 75(3): 213–224.

Lee, H.B. and T.E. Peart. 2000. Bisphenol A contamination in Canadian municipal and industrial wastewater and sludge samples. **Water Quality Research Journal** 35(2): 283–298.

Leelawatwattana, L. 2003. **Effect of thyroid hormones on early development of green catfish (*Mystus nemurus*).** Master Thesis, Prince of Songkla University, Songkhla, Thailand. 118 pp.

Lisetto, M., M. Fattorini, A. Lanza, M. Gerdol, M. Griffin, Z. Wang, F. Ferrara and D. Sblattero. 2023. Biochemical and functional characterization of the three zebrafish transglutaminases 2. **International Journal of Molecular Sciences** 24(15): 12041. DOI: 10.3390/ijms241512041.

Mesomya, W., Y. Cuptapun, P. Jittanoonta, D. Hengsawadi, S. Boonvisut, P. Huttayanon and W. Sriwatana. 2002. Nutritional evaluations of green catfish, *Mystus nemurus*. **Agriculture and Natural Resources** 36(1): 69–74.

Moriyama, K., T. Tagami, T. Akamizu, T. Usui, M. Saijo, N. Kanamoto, Y. Hataya, A. Shimatsu, H. Kuzuya and K. Nakao. 2002. Thyroid hormone action is disrupted by bisphenol A as an antagonist. **The Journal of Clinical Endocrinology and Metabolism** 87(11): 5185–5190.

Mouriec. K., J.J. Lareyre, S.K. Tong, Y. Le Page, C. Vaillant, E. Pellegrini, F. Pakdel, B.C. Chung, O. Kah and I. Anglade. 2009. Early regulation of brain aromatase (cyp19a1b) by estrogen receptors during zebrafish development. **Developmental Dynamics** 238(10): 2641–2651.

Nelson, E.R. and H.R. Habibi. 2006. Molecular characterization and sex-related seasonal expression of thyroid receptor subtypes in goldfish. **Molecular and Cellular Endocrinology** 253(1–2): 83–95.

Nelson, E.R. and H.R. Habibi. 2009. Thyroid receptor subtypes: structure and function in fish. **General and Comparative Endocrinology** 161(1): 90–96.

Nguyen, L.T. and C.R. Janssen. 2001. Comparative sensitivity of embryo-larval toxicity assays with African catfish (*Clarias gariepinus*) and zebra fish (*Danio rerio*). **Environmental Toxicology** 16(6): 566–571.

Nishizawa, H., N. Manabe, M. Morita, M. Sugimoto, S. Imanishi and H. Miyamoto. 2003. Effects of in utero exposure to bisphenol A on expression of RARalpha and RXRalpha mRNAs in murine embryos. **Journal of Reproduction and Development** 49(6): 539–545.

Niu, Y., M. Zhu, M. Dong, J. Li, Y. Li, Y. Xiong, P. Liu and Z. Qin. 2021. Bisphenols disrupt thyroid hormone (TH) signaling in the brain and affect TH-dependent brain development in *Xenopus laevis*. **Aquatic Toxicology** 237: 105902. DOI: 10.1016/j.aquatox.2021.105902.

Nurminskaya, M.V. and A.M. Belkin. 2012. Cellular functions of tissue transglutaminase. **International Review of Cell and Molecular Biology** 294: 1–97.

Orozco, A. and C. Valverde-R. 2005. Thyroid hormone deiodination in fish. **Thyroid** 15(8): 799–813.

Osman, A.G., S. Wuertz, I.A. Mekkawy, H.J. Exner and F. Kirschbaum. 2007. Lead induced malformations in embryos of the African catfish *Clarias gariepinus* (Burchell, 1822). **Environmental Toxicology** 22(4): 375–389.

Pfaffl, M.W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. **Nucleic Acids Research** 29(9): e45. DOI: 10.1093/nar/29.9.e45.

Piferrer, F. and M. Blázquez. 2005. Aromatase distribution and regulation in fish. **Fish Physiology and Biochemistry** 31: 215–226.

Pookpoosa, I., R. Jindal, D. Morknoy and K. Tantrakarnapa. 2014. Occurrence of bisphenol A in some municipal wastewater treatment plants' effluents in Bangkok region. **International Journal of Advances in Agricultural and Environmental Engineering** 1(1): 116–120.

Rasheeda, M.K., P. Sridevi and B. Senthilkumaran. 2010. Cytochrome P450 aromatases: impact on gonadal development, recrudescence and effect of hCG in the catfish, *Clarias gariepinus*. **General and Comparative Endocrinology** 167(2): 234–245.

Robitaille, J., N.D. Denslow, B.I. Escher, *et al*. 2022. Towards regulation of endocrine disrupting chemicals (EDCs) in water resources using bioassays - A guide to developing a testing strategy. **Environmental Research** 205: 112483. DOI: 10.1016/j.envres.2021.112483.

Ross, S.A., P.J. McCaffery, U.C. Drager and L.M. De Luca. 2000. Retinoids in embryonal development. **Physiological Reviews** 80(3): 1021–1054.

Routledge, E.J., R. White, M.G. Parker and J.P. Sumpter. 2000. Differential effects of xenoestrogens on coactivator recruitment by estrogen receptor (ER) alpha and ERbeta. **Journal of Biological Chemistry** 46: 35986–35993.

Sanders, J.P., S. Van der Geyten, E. Kaptein, V.M. Darras, E.R. Kühn, J.L. Leonard and T.J. Visser. 1999. Cloning and characterization of type III iodothyronine deiodinase from the fish *Oreochromis niloticus*. **Endocrinology** 140(8): 3666–3673.

Sayed, A.E.D.H., U.M. Mahmoud and I. Mekkawy. 2012. Toxic effects of 4-nonylphenol on the embryonic development of African catfish *Clarias gariepinus* (Burchell, 1822). **International Journal of Biology and Biological Sciences** 1(2): 34–46.

Shanthanagouda, A.H., D. Nugegoda and J.G. Patil. 2014. Effects of bisphenol A and fadrozole exposures on cyp19a1 expression in the Murray rainbowfish, *Melanotaenia fluviatilis*. **Archives of Environmental Contamination and Toxicology** 67(2): 270–280.

Shmarakov, I.O. 2015. Retinoid-xenobiotic interactions: the ying and the yang. **HepatoBiliary Surgery and Nutrition** 4(4): 243–267.

Sternberg, H. and B. Moav. 1999. Regulation of the growth hormone gene by fish thyroid/retinoid receptors. **Fish Physiology and Biochemistry** 20: 331–339.

Vandenberg, L.N. 2013. Non-monotonic dose responses in studies of endocrine disrupting chemicals: bisphenol A as a case study. **Dose Response** 12(2): 259–276.

Wang, J., X. Liu, H. Wang, T. Wu, X. Hu, F. Qin and Z. Wang. 2010. Expression of two cytochrome P450 aromatase genes is regulated by endocrine disrupting chemicals in rare minnow *Gobiocypris rarus* juveniles. **Comparative Biochemistry and Physiology Part C Toxicology and Pharmacology** 152(3): 313–320.

Welshons, W.V., K.A. Thayer, B.M. Judy, J.A. Taylor, E.M. Curran and F.S. vom Saal. 2003. Large effects from small exposures. I. mechanisms for endocrine-disrupting chemicals with estrogenic activity. **Environmental Health Perspectives** 111(8): 994–1006.

Welshons, W.V., S.C. Nagel and F.S. vom Saal. 2006. Large effects from small exposures. III. endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. **Endocrinology** 147: 56–69.

Yamano, K. 2005. The role of thyroid hormone in fish development with reference to aquaculture. **Japan Agricultural Research Quarterly** 39(3): 161–168.

Yasueda, H., K. Nakanishi, Y. Kumazawa, K. Nagase, M. Motoki and H. Matsui. 1995. Tissue-type transglutaminase from red sea bream (*Pagrus major*). Sequence analysis of the cDNA and functional expression in *Escherichia coli*. **European Journal of Biochemistry** 232(2): 411–419.

Yee, V.C., L.C. Pedersen, I.L. Trong, P.D. Bishop, R.E. Stenkamp and D.C. Teller. 1994. Three-dimensional structure of a transglutaminase: human blood coagulation factor XIII. **Proceedings of the National Academy of Sciences of the United States of America** 91(15): 7296–7300.

Zhang, D.H., E.X. Zhou and Z.L. Yang. 2017. Waterborne exposure to BPS causes thyroid endocrine disruption in zebrafish larvae. **PLoS One** 12(5): e0176927. DOI: 10.1371/journal.pone.0176927.

Zhang, Y.F., X.M. Ren, Y.Y. Li, X.F. Yao, C.H. Li, Z.F. Qin and L.H. Guo. 2018. Bisphenol A alternatives bisphenol S and bisphenol F interfere with thyroid hormone signaling pathway in vitro and in vivo. **Environmental Pollution** 237: 1072–1079.