Journal of Fisheries
Volume 14 Issue 2 August 2026
Peer Reviewed | Open Access

eISSN 2311-3111
pISSN 2311-729X

DOI: https://doi.org/10.17017/j.fish.964

Original Article

The impact of 4-nonylphenol on the expression of the vitellogenin gene and the alterations in steroid hormones in male Sobaity seabream (*Sparidentex hasta*)

Alireza Golchin Manshadi[1] • Zahra Negintaji[2] • Amirhossein Rezazadeh Shirazi[2]

[1] *Department of Aquatic Animal health, Kaz .C, Islamic Azad University, Kazerun, Iran*
[2] *Faculty of Veterinary Medicine, Kaz .C, Islamic Azad University, Kazerun, Iran*

Correspondence
Alireza Golchin Manshadi; Department of Aquatic Animal health, Kaz .C, Islamic Azad University, Kazerun, Iran
dr.golchin@iau.ac.ir

Manuscript history
Received 2 April 2025 | Accepted 19 June 2026 | Published online 1 July 2026

Citation
Manshadi AG, Negintaji Z, Shirazi AR (2026) The impact of 4-nonylphenol on the expression of the vitellogenin gene and the alterations in steroid hormones in male Sobaity seabream (*Sparidentex hasta*). Journal of Fisheries 14(2): 142218. DOI: 10.17017/j.fish.964

Abstract
The study aimed to investigate the effects of 4-nonylphenol exposure on the expression of the vitellogenin gene (Vg) and sexual steroid hormones in male Sobaity seabream (*Sparidentex hasta*). A total of 78 fish individuals were randomly assigned to six tanks, receiving doses of 10, 50, and 100 µg g^{-1} of 4-nonylphenol, as well as 2 µg g^{-1} of 17β-estradiol (E2) via intraperitoneal injection. The negative control group received a solvent mixture (ethanol and olive oil) with no injections. Fish sampling for blood and liver analysis occurred on days 0, 7, and 14. After RNA extraction from liver tissue and cDNA synthesis, variations in Vg expression were analyzed in relation to the beta-actin gene. The results showed a significant increase in Vg expression in the treatment groups compared to control groups. Sequencing confirmed the presence of the Vg gene, with a fragment size of 174 nucleotides. Additionally, plasma levels of sexual steroid hormones were measured using radiomonoactivity, which revealed a decrease in testosterone levels associated with varying concentrations of 4-nonylphenol. In contrast, E2 levels in fish exposed to 4-nonylphenol increased after 7 and 14 days. The findings suggest that exposure to 4-nonylphenol activates Vg production, potentially adversely affecting puberty, sexual development, sexual behaviors, and reproductive success in Sobaity seabream.

Keywords: 17β-estradiol; endocrine-disrupting compound; gene expression; Sobaity seabream; testosterone; vitellogenin

1 | INTRODUCTION

The increase in industrialization and human population growth has raised concerns about environmental contaminants, including nonylphenol. This pollutant, commonly found in industrial and urban sewage, can disrupt the endocrine systems of aquatic organisms, affecting critical processes such as growth, metabolism, and reproduction. Many industrial chemicals are known endocrine-disrupting compounds (Liu *et al*. 2017), which can interfere with hormonal receptors, disrupt hormone release, or impede hormonal responses (Flint *et al*. 2012). Notably, 4-nonylphenol ethoxylate possesses estrogenic properties and genotoxic tendencies, posing a risk to the endocrine systems of living organisms, particularly aquatic species. Sexual steroids are especially vulnerable to such disruptions, with 4-nonylphenol being capable of binding to estrogen receptors and disrupting fish endocrine systems.

Nonylphenol in aquatic environments arises from the microbial degradation of nonylphenol ethoxylate, a significant group of non-ionic surfactants used in a wide range of products, including detergents, emulsifiers, and industrial solvents (De La Parra-Guerra and Acevedo-Barrios 2023). Its environmental resilience and lipophilic nature lead to biological accumulation in aquatic organisms. However, there is limited understanding of the impacts and mechanisms of action of phenolic compounds in non-mammalian vertebrates compared to mammals. Given that aquatic species are continuously exposed to various pollutants, their study is essential. Evidence suggests that the interactions of phenolic compounds in these organisms resemble those in mammals (Canesi and Fabbri 2015), but further research is needed, especially in environments like lakes and seas that serve as ultimate reservoirs for pollutants.

The coastal regions of the Persian Gulf are crucial habitats for Sobaity seabream, where influxes of sewage from petrochemical facilities heighten the risk of exposure to endocrine-disrupting compounds, potentially leading to reproductive disorders and declines in reproduction rates and population numbers. This study aims to investigate the effects of nonylphenol on the endocrine system of fish, using vitellogenin (Vg) as a biomarker for detecting endocrine disruptors and assessing the reproductive health of aquatic organisms, while also identifying the presence of environmental pollutants.

2 | METHODOLOGY

2.1 Fishing and fish maintenance

In order to conduct the current investigation, marine fish were captured from the sea by the fishermen of the Imam Khomeini Port Marine Fish Research Station using the methods of passive fishing and angling. The captured fish were swiftly transferred to seawater tanks containing filtered and UV-treated water, where they were kept for a week to ensure their well-being. Subsequently, 78 fish individuals of similar size, with an average weight of 60±4.67 g, were selected and evenly distributed into 6 tanks of 300 L each (13 fish per tank). The tanks were gradually aerated, allowing the fish to acclimate to the sea water conditions (salinity 45±1 ppm, temperature 25±1°C, pH = 8.1) over a period of 10 days. Physicochemical parameters such as temperature, salinity, and pH were monitored daily. The fish were fed daily with commercial sea bass food provided by the 21-beyza company. To prevent the accumulation of uneaten food and fish waste at the bottom of the tanks, approximately 30% of the tank water was replaced with fresh sea water daily by siphoning. The fish were deprived of food 24 hours prior to sampling (Ishibashi *et al*. 2016).

2.2 Exposure of fish to 4-nonylphenol

Stock solutions were prepared by dissolving the required dose (based on the average fish weight) in 99% ethanol and then diluting with olive oil at a 1:9 ratio (Merck, Germany). To improve solubility, the stock solutions were stirred at 37°C overnight. After anesthetizing the fish with 0.2% 2-phenoxy ethanol, they were subjected to three treatment groups receiving 10, 50, and 100 micrograms of 4-nonylphenol per gram of body weight via intraperitoneal injection over two weeks. Injections were given at half-dose twice a week, specifically on days 0, 4, 8, and 11, between 8 and 10 am (Pait and Nelson 2003). A separate group was administered 2 micrograms per gram of E2 to demonstrate the estrogenic effects of 4-nonylphenol as a positive control. The control group received a solvent mixture (ethanol and olive oil) with no injections to serve as a negative control (Mollegaard *et al*. 2000).

2.3 Fish sampling

Before the injection, a random fish selected from each tank, anesthetized with 2-phenoxy ethanol, and weighed for hematopoietic procedures. The initial weight measurements served as a zero-day sample for comparison with control groups after the experiment. Fish sampling occurred on the 7th and 14th days post-injection (Mollegaard *et al*. 2000). Blood samples were centrifuged at 1000× g for 10 minutes, and plasma was collected in micro tubes. The livers were excised to determine their weights for calculating the hepatosomatic index (HSI), and samples were stored at –80°C for gene expression analyses. RNA was extracted from liver tissue using the guanidinium thiocyanate-phenol-chloroform method, and its quantity and quality were assessed using UV spectrophotometry and agarose gel electrophoresis (Wang and Stegemann 2009). To synthesize single-stranded cDNA from fish liver RNA, the CinnaGen cDNA synthesis kit was used following the manufacturer's instructions, with samples stored at –20°C until PCR. Degenerate primers were designed using gene sequences from the National Center for Biotechnology Information (NCBI) and PRIMER3 software, based on mRNA sequences from *Sparus aurata*, *Lithognathus mormyrus*, and *Pagrus major*. The forward and reverse primers were AACAGAGCGGTGGGCATC and TGCAGATTCCACAGGTCTGTCC, respectively. PCR was conducted for both treated samples and controls, targeting the vitellogenin gene and using β-actin as an internal control to assess gene expression and determine optimal primer binding temperatures with a BioRad thermocycler. The quality of the PCR products was evaluated through 1% agarose gel electrophoresis (Primrose and Twyman 2006). Following this, specific primers for vitellogenin were developed for real-time PCR, where Vg expression was quantified using the Cyber Green method with three replicates. To optimize RT-PCR conditions, varying concentrations of cDNA from different treatments were amplified using both target and reference gene primers. A standard curve was created to assess the efficien-

cy and reproducibility of the test for each primer, with efficiency calculated using the following formula:

Efficiency = $10^{-1/slope}$

2.4 Methodology for determining the CT $E^{-\Delta CT}$ relative threshold cycle

A relative approach is commonly used to assess variations in expression after specific treatments. This method involves the use of an internal control gene, or reference gene, which is typically constitutively expressed and vital for functions. Internal standards help minimize fluctuations in RNA values that may arise during reactions, as well as errors in device performance and sample handling, and are expected to show consistent expression across all tissues. The β-Actin gene is frequently used as the reference gene. By comparing treated samples with the internal control and control groups, researchers can observe fluctuations in the expression of target genes (Liu and Saint 2002).

2.5 ΔΔCt Model

In the ΔΔCt model, the ratio of the expression of the target and reference genes in treated and control samples is measured, assuming uniform amplification efficiency of the target gene primers.

ΔCt control = target control - reference control
ΔCt sample = target sample - reference sample
ΔΔCt = ΔCt sample - ΔCt control

In the aforementioned equations, the samples consist of fish that have not been exposed to 4-nonylphenol or E2. The sample refers to the treated fish. The target gene is the Vg, and the reference gene is the housekeeping gene. The equation $R = 2^{-\Delta\Delta CT}$ is used to determine the relative expression level of the samples. To measure the concentrations of steroid hormones (testosterone and E2) in plasma, radioimmunoassay was employed using the ImmunoTech kit. Hormone levels were quantified in nanograms per liter based on the competitive reaction between the hormone in the serum and the hormone labeled with radioactive Iodine 125, which binds to antihormone antibodies in the solid phase. The resulting radiation was quantified using the LKB gamma counter (Suman *et al*. 2021).

2.6 Data analysis

Software SPSS (version 20) was used for data analysis, presenting results as mean ± standard error. The Shapiro-Wilk test assessed data normality, and upon confirming normal distribution, two-way ANOVA was employed to evaluate the significant effects of different treatments, timing, and their interactions. One-way ANOVA followed by the Duncan test was then used to identify significant differences among treatments. The independent-samples t-test compared average data for treatments on days 7 and 14. A significance level of $p < 0.05$ was set for all tests. Changes in steroid hormones and relative expression levels of Vg compared to control groups were analyzed using ANOVA and the Duncan post-test. Graphs and tables were created using Microsoft Excel 2013.

3 | RESULTS

3.1 Impact of 4-nonylphenol on behavioral alterations

No mortalities were recorded during the experimental period. Fish treated with concentrations of 100 µg g^{-1} of 4-nonylphenol exhibited aberrant behaviors such as leaping above the water surface, heightened respiratory rate (manifested by accelerated opening and closure of the gill covers), and instances of unsteady swimming. Additionally, an escalation in mucus secretion was observed in the 4-nonylphenol-treated fish.

3.2 Effect of 4-nonylphenol on HSI

The findings revealed a significant increase in the HSI of treated fish after one week at doses of 50 and 100 µg g^{-1} of 4-nonylphenol compared to both the solvent control and control groups ($p < 0.05$). Moreover, the HSI measurements in the second week of the experiment demonstrated a dose-dependent response, with a noteworthy elevation compared to the control and solvent control groups at doses of 50, 10, and 100 µg g^{-1} of 4-nonylphenol ($p < 0.05$; Figure 1A). Simple linear regression was employed to investigate the correlation between increased HSI and the expression of the Vg in the fish liver. As illustrated in Figure 1B, there is a direct and significant relationship ($r = 0.83$) between the expression of the Vg and the HSI value, with both factors exhibiting a concurrent increase ($p < 0.05$).

3.3 Vitellogenin gene expression

3.3.1 Quantitative and qualitative analysis of extracted RNA

The ratio of RNA absorption intensity at 260 and 280 Nm, as measured by the biophotometer, fell within the range of 1.7–1.9, indicating optimal purity and quality with no presence of protein or phenolic contamination in the extracted RNA. The presence of ribosomal RNA bands S18 and S28 signifies the integrity and quality of the RNA (Figure 1C). Notably, the absence of distinct S18 and S28 bands suggests RNA degradation due to RNase activity.

3.4 Results of RT-PCR reaction and sequencing of the Vg

Figure 1D presents the outcomes of the RT-PCR reaction conducted on the vitellogenin and beta-actin genes to identify these genes, as well as to assess the impact of 4-nonylphenol and E2 on Vg expression. The sequencing results confirmed the identity of the target gene (Vg of Sobaity Seabream) in the study, with the gene fragment size measuring 174 nucleotides.

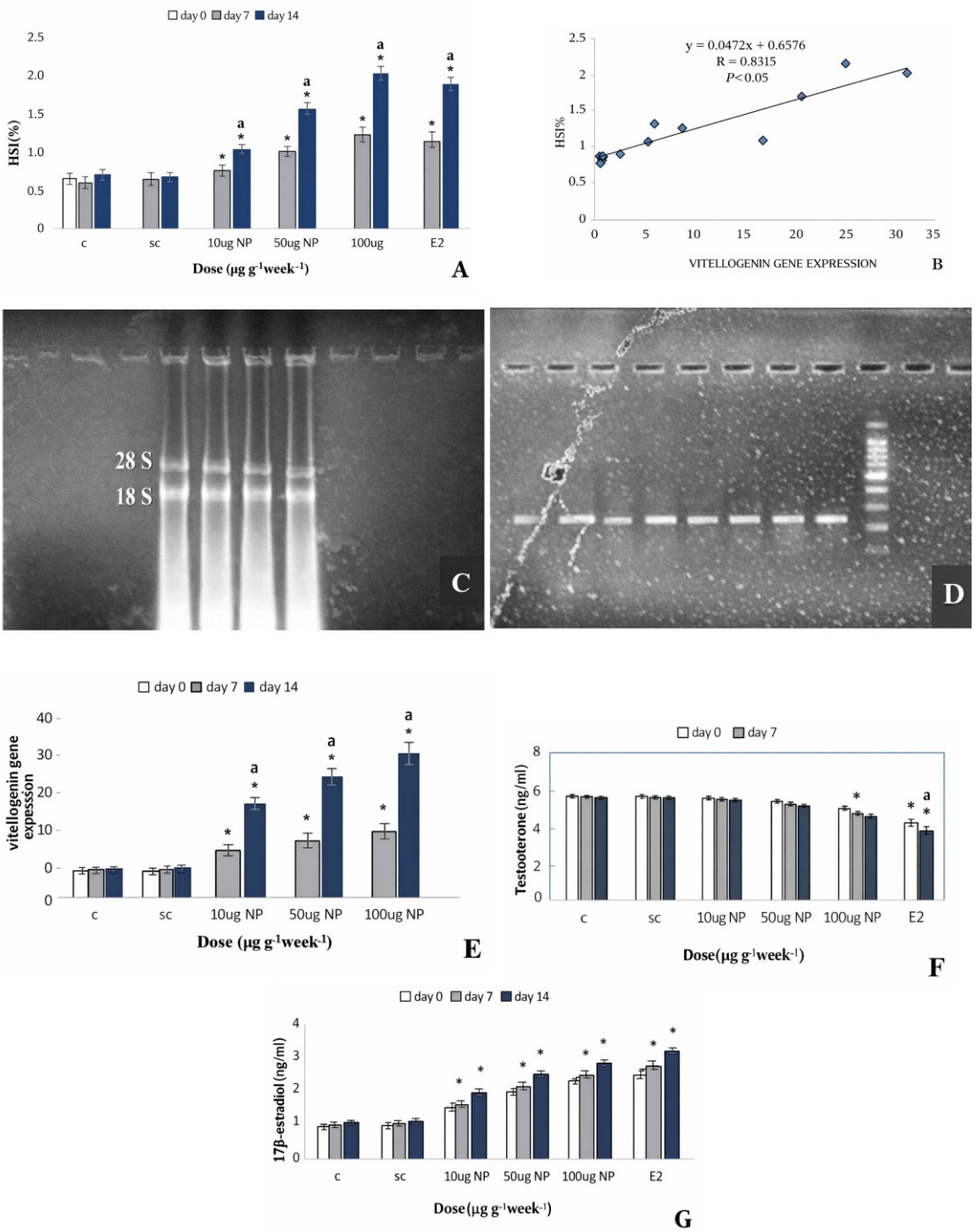

FIGURE 1 (A): Sobaity Seabream Following Exposure to 4-nonylphenol. Statistical Significance: Asterisks (*) denote statistically significant differences from the control group ($p < 0.05$). (a) Indicates a significant difference in HSI relative to day 7 within the same dosage. C: negative control treatment without injection, SC: negative injection control treatment with vector material, E2: 17β-estradiol. (B): Correlation between changes in vitellogenin (Vg) expression and HSI values in seabream exposed to 4-nonylphenol. (C): RNA quality extracted from the liver tissue of Sobaity seabream. (D): Amplification results of the vitellogenin and beta-actin genes via RT-PCR; Row 1: Molecular marker; Rows 2-6: Vitellogenin genes under 4-nonylphenol treatment; Row 7: Positive control; Rows 8-9: Beta-actin genes. (E): Ratio of Vg expression to beta-actin internal control gene, with significant deviations from the control group (*) and noteworthy differences at the same dose as on day 7 (a). (F): Plasma testosterone concentrations in control and treated groups after 7 and 14 days of exposure to different doses of 4-nonylphenol, indicating significant differences from the control group (*) and within the same dose compared to day 7 (a). (G): Changes in E2 (estradiol) concentration in the plasma after exposure to varying concentrations of 4-nonylphenol, with significant differences from the control group (*).

3.5 Expression relative to beta-actin internal control gene

Figure 1E displays the alterations in Vg expression relative to control samples. The expression levels of the Vg in Sobaity Seabream exhibited a significant increase after 7 days of exposure to doses of 10, 50, and 100µg g^{-1} of 4-nonylphenol and 2µg g^{-1} of E2 compared to control groups ($p < 0.05$). Following 14 days of exposure, the heightened expression of the Vg in all treatment groups surpassed that of the control groups significantly. Furthermore, a marked disparity in Vg expression was noted across all doses utilized compared to the previous sampling period ($p < 0.05$). Hence, the modulation of Vg expression in response to 4-nonylphenol in Sobaity Seabream is contingent upon both dosage and duration.

3.6 Steroid hormones

To assess the impact 4-nonylphenol on steroid hormones, the concentrations of testosterone and E2 in the plasma of Sobaity seabream were evaluated. Following exposure to 4-nonylphenol, plasma testosterone levels showed a dose-dependent decrease over time, with a statistically significant decline observed only at the 100 µg g^{-1} dose compared to control groups ($p < 0.05$). Differences in testosterone levels between treated and control groups were not significant at various sampling times, except for fish injected with 2 µg g^{-1} of estradiol. Furthermore, no significant variations in plasma testosterone levels were found between fish in the 10 and 50 µg g^{-1} treatment group and the control groups (Figure 1F).

Changes in E2 levels in the plasma of fish across various experimental treatments are illustrated in Figure 1G. A notable disparity in E2 levels in the plasma of treated Sobaity seabream compared to control groups was observed. The levels of this hormone displayed a dose-dependent increase in fish treated with 4-nonylphenol compared to control groups after 7 and 14 days of exposure ($p < 0.05$). This increase was noticeable even at the lowest dose of 4-nonylphenol (10 µg g^{-1}). However, there were no significant differences among the treated groups, solvent control, and control at different sampling times.

4 | DISCUSSION

4.1 Effect of 4-nonylphenol on HSI of Sobaity Seabream

The study explored how -nonylphenol affects the hepatosomatic index (HSI) of Sobaity seabream. It found that HSI increased in a dose- and time-dependent manner in response to 4-nonylphenol and a dose of estrogen (E2). This rise in HSI indicates heightened metabolism and liver activity as the fish process pollutants, possibly due to increased liver cell number or size. Liver cell size can vary with physiological conditions, including states of underemployment or hyperactivity. Estrogenic endocrine-disrupting compounds may raise HSI by boosting vitellogenin production. Yang *et al*. (2017) reported increased hepatosomatic index (HSI) in both male and female zebrafish exposed to bisphenol B for 21 days across different concentrations. HSI is broadly recognized as a universal biomarker for the adverse effects of endocrine-disrupting compounds (EDCs). Mollegaard *et al*. (2000) showed higher HSI in Atlantic salmon exposed to bisphenol A, estradiol (E2), and nonylphenol, while Pait and Nelson (2003) observed similar increases in male killifish at comparable bisphenol A doses. Overall, the rise in HSI with 4-nonylphenol and E2 may reflect heightened metabolism and liver cell hypertrophy driven by vitellogenin production.

4.2 Vitellogen gene expression

Vitellogenin (Vg) is a yolk protein precursor predominantly produced in the liver of female oviparous vertebrates in response to estradiol, but it can also be induced in males and immature fish by xenoestrogens and antiandrogens, making Vg a useful biomarker for monitoring such compounds in aquatic environments. Naderi *et al*. (2015) showed that 4-nonylphenol and estradiol stimulate Vg production in immature yellowfin seabream. The current study examined hepatic Vg expression in male Sobaity seabream exposed to varying doses of 4-nonylphenol and estradiol, finding a progressive time-dependent increase in Vg, with higher 4-nonylphenol doses prompting earlier Vg expression. Saravanan *et al*. (2019) also reported increased Vg expression in two marine fish species subjected to different 4-nonylphenol doses. Zhong *et al*. (2014) reported substantial increases in vitellogenin (Vg) expression at the protein level in male zebrafish exposed to 17α-ethinylestradiol. In the current study, melting curve analysis of Vg expression confirmed primer specificity with a single amplification peak. Sobaity seabream is a protandrous hermaphrodite, capable of changing from male to female, a process influenced by xenoestrogens. Therefore, induction of Vg production in response to nonylphenol could affect sex ratios and reproductive success in this species. The rapid Vg response to 4-nonylphenol suggests that Sobaity seabream could be a valuable model for marine toxicology studies. The dose- and time-dependent Vg response also supports its use as a biomarker for monitoring aquatic ecosystems and assessing reproductive health in marine fish breeding facilities exposed to xenoestrogens (Zhong *et al*. 2014).The study found a strong positive correlation ($r = 0.83$) between higher vitellogenin (Vg) expression and the hepatosomatic index (HSI) in Sobaity seabream exposed to increasing doses of 4-nonylphenol, indicating that both Vg and HSI are reliable indicators of vitellogenin production under xenoestrogen exposure.

4.3 Sex steroid hormones

Exposure to 4-nonylphenol can disrupt sex steroid hormones in fish. Previous studies show this compound

modulates hormone levels across species. In the present work, plasma testosterone in Sobaity seabream significantly decreased after exposure to 100 µg g^{-1} 4-nonylphenol ($p < 0.05$). Comparable findings were reported by Suman *et al*. (2021), who observed reduced testosterone and increased estradiol (E2) in catfish (*Heteropneustes fossilis* and *Clarias batrachus*) after 30 days of exposure. The observed rise in E2 in Sobaity seabream may reflect decreased activity of estradiol-metabolizing enzymes in the presence of 4-nonylphenol, contributing to altered hormonal balance.

5 | CONCLUSIONS

Our findings show that 4-nonylphenol induces vitellogenin production in male Sobaity seabream. The hepatosomatic index (HSI) increased across varying doses of 4-nonylphenol and with a single estradiol dose, suggesting elevated metabolism and liver activity to process these compounds, along with enhanced liver cell activity associated with vitellogenin production. RT-PCR analysis revealed a progressive, time-dependent rise in liver Vg expression in response to 4-nonylphenol and estradiol, underscoring the sensitivity and utility of RT-PCR for quantifying vitellogenin. Additionally, exposure to 4-nonylphenol altered sex steroid hormones, showing decreased testosterone and increased plasma E2 levels. Sequencing of Vg specific to Sobaity seabream provides a valuable resource for future work on the reproductive biology of this species and the impact of xenoestrogens. Overall, vitellogenin production in male or immature Sobaity seabream emerges as a robust biomarker for exposure to xenoestrogens, particularly 4-nonylphenol, with practical applications for monitoring these compounds in aquatic environments and marine fish populations..

ETHICAL APPROVAL

All applicable international, national and/or institutional guidelines for the care and use of animals were followed in this study.

CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

Conceptualization, AGM and ZN; methodology and validation, AGM and ZN; formal analysis, AGM; investigation AGM; resources, ZN; data curation, AGM; writing original draft preparation, AGM and AMS; writing review and editing, AGM, ZN and ARS. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of the study will be made available on a reasonable request from the corresponding author.

REFERENCES

Canesi L, Fabbri E (2015) Environmental effects of BPA: focus on aquatic species. Dose-Response 13(3): 1559325815598304.

De la Parra-Guerra AC, Acevedo-Barrios R (2023) Studies of endocrine disruptors: nonylphenol and isomers in biological models. Environmental Toxicology and Chemistry 42(7): 1439–1450.

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

Ishibashi H, Uchida M, Koyanagi A, Kagami Y, Kusano T, … Ishibashi Y (2016) Gene expression analyses of vitellogenin, choriogenin and estrogen receptor subtypes in the livers of male medaka (*Oryzias latipes*) exposed to equine estrogens. Journal of Applied Toxicology 36(11): 1392–1400.

Liu H, Yang X, Yin C, Wei M, He X (2017) Development of predictive models for predicting binding affinity of endocrine disrupting chemicals to fish sex hormone-binding globulin. Ecotoxicology and Environmental Safety 136: 46–54.

Liu W, Saint DA (2002) A new quantitative method of real time reverse transcription polymerase chain reaction assay based on simulation of polymerase chain reaction kinetics. Analytical Biochemistry 302: 52–59.

Mollegaard NE, Bailly C, Waring MJ, Nielsen PE (2000) Quinoxaline antibiotics enhance peptide nucleic acid binding to double-stranded DNA. Biochemistry 39(31): 9502–9507.

Naderi M, Safahieh A, Madiseh SD, Zolgharnein H, Ghatrami ER (2015) Induction of vitellogenin synthesis in immature male yellowfin seabream (*Acanthopagrus latus*) exposed to 4-nonylphenol and 17β-estradiol. Toxicology and Industrial Health 31(3): 209–220.

Pait AS, Nelson JO (2003) Vitellogenesis in male *Fundulus heteroclitus* (killifish) induced by selected estrogenic compounds. Aquatic Toxicology 64(3): 331–342.

Primrose SB, Twyman RM (2006) Principles of gene manipulation and genomics. 7th ed. Malden, MA , Oxford : Blackwell Pub.

Saravanan M, Nam SE, Eom HJ, Lee DH, Rhee JS (2019) Long-term exposure to waterborne nonylphenol alters reproductive physiological parameters in economically important marinefish. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 216: 10–18.

Suman A, Radha C, Geeta JG (2021) 4-Nonylphenol affects the structure and function of testis in catfish *H. fossilis* and *C. batrachus*. Journal of Scientific Research

65(5): 155–163.

Wang L, Stegemann JP (2009) Extraction of high-quality RNA from polysaccharide matrices using cetyltrimethylammonium bromide. Biomaterials 31(7): 1612–1618.

Yang Q, Yang X, Liu J, Ren W, Chen Y, Shen S (2017) Exposure to bisphenol B disrupts steroid hormone homeostasis and gene expression in the hypothalamic–pituitary–gonadal axis of zebrafish. Water, Air and Soil Pollution 228: 112.

Zhong L, Yuan L, Rao Y, Li Z, Zhang X, … Dai H (2014) Distribution of vitellogenin in zebrafish (*Danio rerio*) tissues for biomarker analysis. Aquatic Toxicology 149: 1–7.

AG Manshadi https://orcid.org/0000-0002-2627-3491
Z Negintaji https://orcid.org/0009-0002-8559-7231
AR Shirazi https://orcid.org/0009-0002-5730-0971