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RESEARCH ARTICLE

Effects of polycyclic aromatic hydrocarbons on biomarker responses in *Gambusia yucatana*, an endemic fish from Yucatán Peninsula, Mexico

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are petroleum components that, when dissolved in the aquatic environment, can disrupt normal animal physiological functions and negatively affect species populations. *Gambusia yucatana* is an endemic fish of the Yucatán Peninsula that seems to be particularly sensitive to the presence of PAHs dissolved in the water. Here, we examined PAH effects on gene expressions linked to endocrine disruption and biotransformation in this species. Specifically, we examined the expression of vitellogenin I (*vtg1*), vitellogenin II (*vtg2*), oestrogen receptor α (*esr1*), oestrogen receptor β (*esr2*), aryl hydrocarbon receptor (*AhR*) and the cytochrome P4503A (*CYP3A*) genes. We exposed *G. yucatana* to different concentrations of PAHs (3.89, 9.27, 19.51 μg/L) over a period of 72 h and found changes associated with reproduction, such as increases in hepatic expression of *vtg*, *esr*, *AhR* and *CYP3A*, mainly at concentrations of 9.27 and 19.51 μg/L. Our results also indicate that benzo[a]pyrene was probably the main PAH responsible for the observed effects. The genes examined here can be used as molecular markers of endocrine-disrupting compounds, as the PAHs, present in the environment, as gene expression increases could be observed as early as after 24 h. These biomarkers can help researchers and conservationists rapidly identify the impacts of oil spills and improve mitigation before the detrimental effects of environmental stressors become irreversible.

Keywords Water contamination · Environmental stressors · Polycyclic aromatic hydrocarbons · Endocrine disruptors · Biotransformation · Benzo[a]pyrene

Introduction

The release and discharge of petroleum and its chemical products are increasing worldwide (Walter et al., 2019), affecting sea and freshwater ecosystems due to the long-lasting effects of these pollutants (Zabbey and Olsson, 2017). Polycyclic aromatic hydrocarbons (PAHs) are a family of compounds present among the petroleum components. They are

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characterised by the presence of 2 to 10 condensed aromatic rings (Ha et al., 2019; Litasov et al. 2019) and are a matter of significant ecotoxicological concern in aquatic environments (Soltani et al., 2019). They also act as endocrine disruptors (ED) by mimicking or antagonising the action of hormones that occur naturally, thereby impacting the regulation of vital life processes, including development, growth, metabolism and reproduction (Roldán-Wong et al., 2020; Pulster et al., 2019).

Both crude oil and its by-products are highly toxic to fish (Prabowo and Bae 2019). In fish, PAHs can alter the development of secondary sexual characteristics and may impair reproductive functions (Acolas et al., 2019; Toft et al., 2004; Toft and Guillette Jr, 2005). Endocrine-disrupting chemicals (EDCs), such as PAHs, activate oestrogen receptors (ERs), encoded by the esr (oestrogen receptor) genes, which induce the fish liver to produce vitellogenin (Vtg) (Chen et al., 2019), a female-specific hepatic yolk precursor protein and that is not detectable in male plasma. However, after exposure to EDCs, the Vgt level in male plasma increases and remains elevated (Davis et al., 2010).

Also, aryl hydrocarbon receptor (AhR) activation produces cytochrome P4501A (CYP1A) (Marris et al., 2019) and cytochrome P4503A (CYP3A; a subfamily of the CYP1A), metabolising oestrogen, pregnanes and xenoestrogens into more polar substances that are easier for the body to excrete (Rodrigues et al., 2018). Therefore, the messenger RNAs (mRNAs) of these proteins (i.e. Vtg, ER, AhR and CYP3A) could indicate the effects of waters contaminated by PAHs and act as an early biomarker of oil impact on fish species (Nichols et al., 2019).

Biomarkers can be any measurable biomolecules that provide early warning signs of an individual imbalance resulting from exposure to a xenobiotic (Martyniuk et al., 2019; Colin et al., 2016). Conservation actions can highly benefit from these tools that can be used in a predictive manner, allowing bioremediation measures to be taken before irreversible ecological damage and severe consequences occur (Aguilar et al., 2020; Martyniuk et al., 2019; Colin et al., 2016). Biomarkers of environmental contamination by PAHs in fish seem to vary with species, habitat and environment and are often contradictory (Aguilar et al., 2020). Therefore, they need to be species- and region-specific to be efficient, especially to detect an impact on species with restricted geographic distribution (Aguilar et al., 2020).

Here, we assessed the effects of ED caused by PAHs on *Gambusia yucatana*, an endemic fish species from the Yucatán Peninsula (Rodríguez-Fuentes et al., 2016). We examined the transcriptional changes of *vtg1* and *vtg2*, *esr1* and *esr2* and *AhR* and *CYP3A* in the livers of *G. yucatana* exposed to PAHs. This species seems to be sensitive to the presence of petroleum on the water. Chakrabarty et al. (2016) recorded that between 2005 and 2010, before the oil spill in the Gulf of Mexico, researchers collected *G. yucatana* 14 times but only once after this event (between 2010 and 2015), indicating that petroleum had lasting effects on the species' survival. Therefore, identifying the consequences of petroleum and its by-products on *G. yucatana* could help scientists and conservationists detect oil impacts at an early stage and respond immediately to minimise them.

Material and methods

Harvesting and acclimatisation of *G. yucatana*

Juveniles of *G. yucatana* (mean body mass = 389.3 ± 78.0 mg; total length = 3.3 ± 0.2 cm) were collected from a small creek in San Francisco de Campeche City (19° 49′ 39.36″ N, 90° 29′ 42.41″ W). Fish were acclimated to laboratory conditions for 15 days in 20-L aquariums (five individuals per litre of aged dechlorinated tap water) at 26 ± 2 °C under continuous filtration and aeration; 70% of the water was renewed every 3 days, following Osten et al. (2005). Fish were fed twice a day (at 8 AM and 3 PM) with commercial fish food tested by the manufacturer and free of contaminants (TretaMin, by Treta Holding, USA). Uneaten feed and faecal wastes were removed before the next feeding. In the 24 h preceding the experiments, fish were not fed, and during the test period, no food was provided. All procedures followed the guidelines approved by Biological Ethics Committee of Ecology, Fisheries and Oceanography of the Gulf of Mexico Institute (EPOMEX), Campeche University, according to the Federal Mexican Norm: NOM-01992-STPS-1993, with approval date 15 March 2017.

Experimental design

The petroleum (EPOMEX Institute, Mexico) was diluted by weighing 10 g of petroleum into 100-mL glass scintillation vials and adding 50 mL of HPLC-grade acetone to yield a stock solution of 0.5 g/mL. The vials were shaken (Ika vortex 2 shaker, Ident. No. 0025000258 IKA-Werke GmbH & Co, Germany) at 1500 rpm for 10 min (Schwab et al., 1999). Subsequently, the PAH extract was quantified, defined as a mixture of total PAHs that can potentially contain other polar hydrocarbons such as alkanes (not analysed in the present study but may be also partially extracted with acetone). Experimental treatments consisted of two control groups of females, one with acetone and no petroleum extract added, and another with dechlorinated water without acetone to ensure acetone did not affect baseline mortality. Based on a previous LC_{50} bioassay (Aguilar et al., 2020), the approximate value of half of the LC10 was used as the lowest concentration for the bioassays. We examined petroleum extract concentrations of 50, 100 and 200 mg/L in three experimental groups of male fish; acetone (HPLC grade, < 0.5 mL/L) was used as a

carrier in all treatments (Barata et al., 2005). Fish were added with a stocking rate for the in vivo study of one fish per litre of water (n = 15 per treatment) in a static test. The fish were exposed to the experimental conditions for 72 h, and all bioassays were conducted in triplicate. After each full day (24, 48 and 72 h), five fish from each treatment were killed by snap-freezing, and the length and weight of each fish were measured. Subsequently, the livers and fillets were removed and stored at −80 °C until use. The fillets were used to measure the PAHs from the muscles of *G. yucatana* before and after each experimental treatment.

RNA isolation and quantitative polymerase chain reaction analyses

Liver samples were pooled for the analysis, using five per treatment per period of exposure to reduce the errors associated with individual differences. The RNA was isolated according to the manufacturer's protocol (Ultra Clean Tissue & Cells RAN Isolation, Mobio Company) and diluted at 1 mg/mL for reverse transcriptase. The reverse transcriptase reaction mixture was composed of 5 μg of total RNA, 1 μL of RNAase inhibitor, 1 μL of oligo (dT) primer and diethylpyrocarbonate-treated water. The reaction mixture was heated at 70 °C for 10 min and quickly chilled on ice. After cooling, 4 μL of 5× reaction buffer containing 25 mM $MgCl_2$, 2 μL of deoxynucleotide triphosphate (dNTP, 10 mM each), 1 μL of RNAase inhibitor and 1 μL of ReverTra Ace (Bio-Rad) were added to a total volume of 20 μL. The reaction mixture was incubated for 60 min at 42 °C and then heated for 5 min to stop the reverse transcriptase. Amplification reactions were carried out in triplicate according to the instructions of the manufacturer (Bio-Rad). Each quantitative polymerase chain reaction (qPCR) amplification contained 2 μL of the reverse transcriptase reaction mixture as the cDNA template, 5 μL of 10× PCR buffer, 1 μL of Taq polymerase (5 U/μL, Bio-Rad), 3 μL of 25 mM MgCl2, 1 μL of deoxynucleotide triphosphate (10 mM) and 15 pmol of both forward and reverse primers. The total volume of the reaction mixture was 50 μL. The primer pair sequences are shown in Table 1. The reaction was performed in Step-one qPCR (Applied Biosystems) according to Ishibashi et al. (2007). We used the method $2-\Delta\Delta CT$ to calculate the expression of the *β-actin* gene, chosen as the reference gene (Livak and Schmittgen, 2001).

Measurement of PAHs in petroleum extract and quality control

The total PAHs of the extract were quantified following Vallarino and Rendón-von Osten (2017). We used a gas chromatographer GC Varian 3800 equipped with a DB-5 (5%-phenyl)-methylpolysiloxane column of 30 m × 0.25 mm × 0.32 mm with a flame ionization detector, using nitrogen as a gas carrier, following the 8310-method approved by U.S.EPA (1986). The column program was as follows: initial temperature, 60 °C for 5 min, ramp to 300 °C at a rate of 2 °C/min and final hold time of 5 min. The detector was kept at 300 °C with a nitrogen makeup gas flow at 28 mL/min, hydrogen at 30 mL/min and air at 300 mL/min. Each compound was identified using congruence of standard and unknown retention times and was quantified using the integration of peak areas. The limit of detection for individual aromatic compounds was 0.01 μg/g, with recovery yields of 86 and 105%. The PAHs present in the extract (of the 16 major PAHs) were identified by comparing their retention times with those of the aromatic analytical standards by Supelco 48743, according to the priority PAHs from method EPA 610. For every batch of 10 samples, we analysed two quality-control samples and one reagent-blank sample. To monitor extraction and analysis efficiency, the quality-control samples were spiked with each of the standards (Vallarino and Rendón-von Osten, 2017). The bioassays were conducted in triplicate.

Determination of PAHs in the water and in *G. yucatana* muscle

The PAHs in the water and muscle were quantified following Vallarino and Rendón-von Osten (2017). Solvents with a high degree of purity (98% HPLC) were used; the glass material was washed with Extran® MA 02 (Merck, Germany), dried for 4 h at 200 °C and then rinsed with acetone and hexane. The PAH analysis was performed according to Zhang et al. (2007). Samples of *G. yucatana* muscle and water were weighed and oven-dried for 24 h at 45 °C. Extraction was carried out with a mixture of acetone and hexane (1:1) by placing the samples into an ultrasonic water bath for 20 min. The amount of fat was used to determine the concentrations of compounds (μg/g of fat). Subsequently, the samples were purified by filtering them through a glass column packed with silica gel, glass wool and sodium sulphate. The glass vessels were then rinsed with 20 mL of acetone, then with 20 mL of a mixture of dichloromethane and hexane (1:1) and finally with 20 mL of dichloromethane, then evaporated to dryness and resuspended in 50 μL of hexane for analysis by gas chromatography; the PAHs were quantified using a Varian 3800 gas chromatograph equipped with a Ni63 electron capture detector (SGE Analytical Science, USA). The temperature of the injector and the detector was 150 and 300 °C, respectively. The concentration of the PAHs was obtained by calculating the area under the curve with the software Star Chromatography Workstation version 6 and standard calibration. A mixture of the 16 major PAHs standards was used — according to the EPA 610 method of Supelco 48743 — to identify and quantify the PAHs (Vallarino and Rendón-von Osten, 2017) (Table 2).

Table 1 Sequences of primer pairs used in the qPCR study

Primers Name	Sequence		Reference
β-actina	Forward	AGACCACCTACAGCATC	Ishibashi et al. (2008)
(S74868)	Reverse	TCTCCTTCTGCATTCTGTCT	
AhR	Forward	AGGGGCGTCTGAAGTTCC	Mortensen and Arukwe (2008)
	Reverse	GTGAACAGGCCCAACCTG	
CYP3A	Forward	GAAGCTGTGATGCAGATGGA	Ribecco et al. (2012)
	Reverse	TGCTGAACCTTTCAGGTTTG	
ERα	Forward	GTCAGTCGGGTTACTTGGCC	Ishibashi et al. (2008)
(AB033491)	Reverse	CATCACCTTGTCCCAACCTG	
ERβ	Forward	GTGGACTCAACTTTCGGC	Ishibashi et al. (2008)
(AB070901)	Reverse	CACGTCGCAGCAGGATCTT	
vtg1	Forward	TGGAAAGGCTGATG GGGAAG	Ishibashi et al. (2008)
(AB064320)	Reverse	AACTGCAGGCATGGTGAGCC	
vtg2	Forward	GTCTTCAGGAGGTCTTCTTC	Ishibashi et al. (2008)
(AB074891)	Reverse	GGTAGACAATGGTATCCGAC	

Statistical analysis

The analyses were iterated in three replicates for each sample, and the mean of the two closest values was used for statistical interpretation. We tested the differences in individual gene expressions among different concentrations and exposure times, using a two-way analysis of variance (two-way ANOVA) with a permutation test. Also, we tested differences in the joint responses among treatments of all genes, using a permutational analysis of variance (PERMANOVA). The

Table 2 Polycyclic aromatics hydrocarbons (PAH) analyzed in water and muscles of *Gambusia yucatana* per treatment. LOD, limit of detection; LOQ, limit of quantification.

Compound	No. of rings	Molecular weight	LOD	LOQ	Treatment					
					3.89 μg/L		9.27 μg/L		19.51 μg/L	
					Water	Muscle	Water	Muscle	Water	Muscle
			(Water = μg/mL and muscle = μg/g)							
Naphthalene	2	128.2	0.06	0.22	0	0	0	0	0	0
Acenaphthylene	3	152.2	0.02	0.07	0.1822	0	0.3492	0	0.8847	0
Acenaphthene	3	154.2	0.02	0.05	0	0	0	0	0	0
Flourene	3	166.2	0.002	0.008	0	0	0	0	0	0
Phenanthrene	3	178.2	0.009	0.04	0	0	0	0	0	0
Anthracene	3	178.2	0.0009	0.003	0.3092	0	0.7133	0.0144	1.3901	0.0558
Fluoranthene	4	202.3	0.02	0.11	0.5238	0	0.9987	0.0099	2.1003	0.2375
Pyrene	4	202.1	0.07	0.21	0.39285	0	0.7577	0	1.4796	0
Benzo[a]anthracene	4	228.3	0.006	0.009	0.3055	0.0139	0.5481	0	0.9978	0
Chrysene	4	228.3	0.02	0.06	0	0	0	0	0	0
Benzo[b]flouranthene	5	252.3	0.1	0.3	0	0	0	0	0	0
Benzo[k]flouranthene	5	252.3	0.15	0.35	0.3701	0	1.0303	0	1.9003	0
Benzo[a]pyrene	5	252.3	0.0023	0.057	0.6534	0.0594	1.0793	0.0985	2.519	0.1994
Benzo[ghi]perylene	6	276.4	0.35	0.55	0.5987	0	1.0466	0	2.0545	0
Dibenz[a,h]anthracene	5	278.3	0.07	0.27	0.4733	0	1.0295	0	2.1344	0
Indeno[1,2,3-cd]pyrene	6	276.3	0.016	0.05	0.3321	0.0599	0.6771	0	1.4003	0

significance level was set at $p < 0.05$. All tests were performed using the software R version 3.4.3 (2017-11-30).

Results

Health effect assessments

The fish presented toxic responses to the higher concentrations of PAHs. In the first 2 h of exposure, they were excited and swam freely. In the following hours, they swam close to the surface. The next day, their heads and gills were supervascularised, showing a more reddish hue than those of the control group. The reddish tone lasted until the end of the test. Gradually, they became slower and slower, and at the end of the experiment, they were almost immobile at the bottom of the aquarium. However, there was no mortality in any treatment until the end of the exposure period. The control groups without acetone (only with chlorinated water) and with acetone showed no significant differences. For this reason, to simplify the display of the results, we only show the results of the control group with acetone.

Measurement of PAHs in petroleum and petroleum extract, quantity of PAHs in the water and bioaccumulation in *G. yucatana* muscle

The measured PAHs in the petroleum represented 8.73% of the petroleum. The most predominant PAHs were those with five, six and four rings: benzo[a]pyrene (BaP) (15%), benzo[ghi]perylene (14%), fluoranthene (12%) and dibenzo[a,h]anthracene (12%).

The concentrations of total PAHs at 50, 100 and 200 mg/L of PAH extract were 3.89, 9.27 and 19.51 μg/L, respectively. We detected 10 of the 16 major PAHs in the muscle of *G. yucatana* (Table 1). The PAHs with five and four rings showed the highest concentrations, following the order BaP > fluoranthene > pyrene.

The BaP was bioaccumulated in all treatments. The highest level of bioaccumulation (0.2 μg/g) occurred in the highest PAH concentration (19.51 μg/L), with 2.5 μg/L of BaP in the water. Fluoranthene bioaccumulated in two PAH concentrations (9.27 and 19.51 μg/L). Muscle bioaccumulation was 0.01 and 0.20 μg/g, respectively. At these concentrations, there was fluoranthene in the water at concentrations of 1.00 and 2.10 μg/L, respectively.

Anthracene was also bioaccumulated in the muscles of *G. yucatana* at two PAH concentrations (9.27 and 19.51 μg/L). Muscle bioaccumulation was 0.01 and 0.06 μg/g, respectively. In the water, anthracene occurred at concentrations of 0.70 and 1.40 μg/L, respectively. Benz[a]anthracene and ideno[1,2,3-cd]pyrene were bioaccumulated only at the lower PAH concentration (3.89 μg/L). Muscle bioaccumulation was 0.01 and 0.06 μg/g, respectively. In the water, benz[a]anthracene and ideno[1,2,3-cd]pyrene occurred at concentrations of 0.31 and 0.33 μg/L, respectively, at the lower PAH concentration.

Gene expression

The levels of *vtg1* were higher in exposed fish than in the control group at the treatments with 9.27 and 19.51 μg/L ($F = 55.1$, $p < 0.0001$) (Fig. 1a; Table 3). The levels of *vtg2* in exposed fish were higher than those in the control group at 3.89 and 19.51 μg/L ($F = 655.4$, $p < 0.0001$) (Fig. 1b; Table 3).

The *esr1* levels were higher in exposed fish than in the control group at the treatments with 9.27 and 19.51 μg/L ($F = 10.4$, $p < 0.01$) (Fig. 2a; Table 3). The levels of *es2* were higher in exposed fish than in the control group only in the treatment with 9.27 μg/L ($F = 174.1$, $p < 0.0001$) (Fig. 2b; Table 3).

The levels of *CYP3A* were higher in exposed fish than in the control group in the treatment with 19.51 μg/L ($F = 6.8$, $p < 0.0001$) (Fig. 3; Table 3).

The *AhR* levels were higher in exposed fish than in the control group in all treatments ($F = 6{,}226$, $p < 0.0001$) (Fig. 4; Table 3).

Discussion

Whereas previous studies have described the effects of PAHs in the endemic species *G. yucatana* and identified biomarkers of oxidative stress (Aguilar et al., 2020), we expand this knowledge by further identifying molecular markers of endocrine-disrupting compounds in the environment. Combined, these results can help researchers and conservationists identify the early responses of the species to oil

Table 3 Statistics analysis summary table for the assays with specific genes in the liver homogenates of mosquito fish showing the differences between the gene expressions according to the concentrations. For each gene, the degrees of freedom (Df), the *F* statistics (with associated *P* value), sum-of-squares (SS) and mean squares (MS) are shown, as well as the statistics method used. Critical $F = 2.922$

Gene	Df	*F*	*P* value	SS	MS	Method
AhR	3	6226	< 0.001	129131	43044	ANOVA
CYP3A		6.840	< 0.001	333.39	111.130	ANOVA
ERα		10.380	0.0014	92.924	30.9748	ANOVA
ERβ		174.1	< 0.001	1039.18	346.39	ANOVA
vtg1		55.048	< 0.00	77.000	25.6667	ANOVA
vtg2		655.4	< 0.001	42006	14002.0	ANOVA
All genes		1151.96	0.001	172679	57560	Permanova

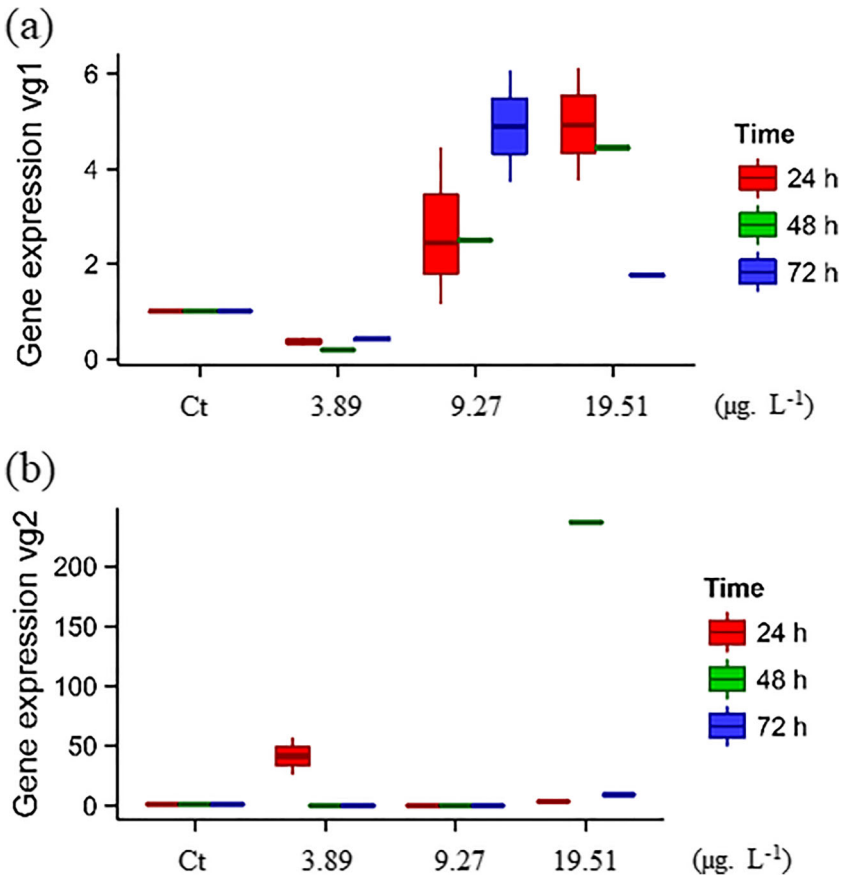

Fig. 1 Expression of **a** vitellogenin 1 (*vtg1*) and **b** vitellogenin 2 (*vtg2*) genes in *Gambusia yucatana* submitted to acute exposure (72 h) to PAHs from crude oil. All data are presented as mean ± standard deviation

pollution and, hence, take rapid mitigation actions. Prompt actions are crucial for *G. yucatana* as its population has been decreasing dramatically in recent years, and petroleum leakage or spill events present a real threat to the species (Chakrabarty et al., 2016).

In our study, the expression of the genes analysed generally peaked at a concentration of 19.51 µg/L after 48-h exposure to PAH extract, but some gene inductions were detectable even at 24 h (Figs. 3 and 4). Therefore, the impacts of oil contaminants could be identified as early as 24 h after the fish had contact with PAH components. Out of the 16 major PAHs, we only detected 10 (Table 2), which seems unusual. However, this discrepancy could be attributed to the fact that PAHs present in complex mixtures vary significantly from oil to oil because of microbial activity, evaporation and/or photodegradation (Bera et al., 2019; Tao et al., 2019).

Rendón-von Osten et al. (2019) have previously shown that oestrogen 17ß-estradiol (E2) caused endocrine disruption in *G. yucatana*. The PAHs can also act in the environment as ED, which interfere with the homeostasis of organisms by mimicking endogenous hormones (Chen et al., 2019; Yang et al., 2020; Zhang et al., 2016). One of the main PAHs that can cause damage is BaP, which can drive endocrine disruption in many vertebrates (Yang et al., 2020; Honda and Suzuki, 2020; Zhang et al., 2016). In all treatments of the present study, the muscles of *G. yucatana* bioaccumulated BaP more frequently than other PAHs (molecular weight = 252.3) (Table 2). The intoxication signs that occurred in our experiment were similar to the physical and behavioural signs of intoxication previously reported for *G. yucatana* (Aguilar et al., 2020) and *Carassius auratus* when exposed to PAHs (Lu et al., 2009).

Expression of *vtg* and *esr* genes

In teleosts, oestrogen steroid hormones influence a wide range of physiological processes, affecting specific ERs of the nuclear receptor superfamily of ligand-activated transcription factors. The ERs ERα and ERβ are activated by E2, inducing the formation of ER homo- or heterodimers that then activate nuclear and extranuclear signalling pathways (Amenyogbe et al., 2020). However, ERs can be activated by EDCs, which can imitate, antagonise or not have an effect on the actions of natural steroid hormones (Carnevali et al., 2018). In relation to PAHs, it has been reported that both exposure to individual compounds and complex mixtures can generate an agonist or antagonistic effect on the expression of genes that encode proteins that act in reproduction, such as Vtg (Vignet et al., 2016). Thus, many authors recognise that both individual PAH compounds and complex mixtures act as ED

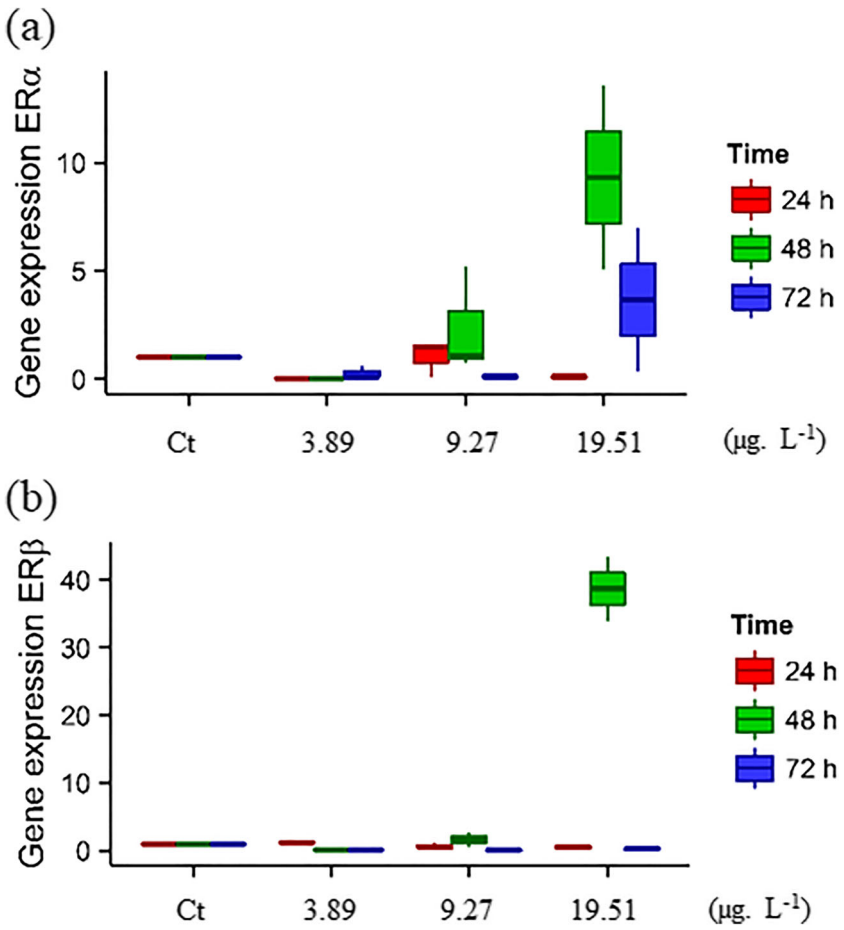

Fig. 2 Expression of **a** estrogen receptor α (*esr1*) and **b** estrogen receptor β (*esr2*) genes in *Gambusia yucatana* submitted to acute exposure (72 h) to PAHs from crude oil. All data are presented as mean ± standard deviation

(Diamante et al., 2017; Booc et al., 2014; Arukwe et al., 2008; Seruto et al., 2005), even though their estrogenic and or anti-oestrogenic properties are not entirely clear.

The synthesis of ERs is induced by oestrogens or EDCs (Amenyogbe et al., 2020). There is also the possibility that AhR, responsible for the synthesis of CYP1A (enzymes involved in PAHs metabolism), affects the expression of *esr*s (Vignet et al., 2016). Some studies have investigated the interactions between ER and AhR, which is another ligand-activated nuclear transcription factor that forms a heterodimeric nuclear complex with AhR nuclear translocator protein, and it appears that these two receptors act in interdependently (Abdelrahim et al., 2006; Liu et al., 2006). The AhR and ER interact physically (Abdelrahim et al., 2006), and it appears that AhR response modulation depends on ER, where ER can increase, decrease or simply not alter the ligand-activated AhR response (Vondráček et al., 2018). The differences in ER-AhR interactions cannot be fully resolved but may be due, in part,

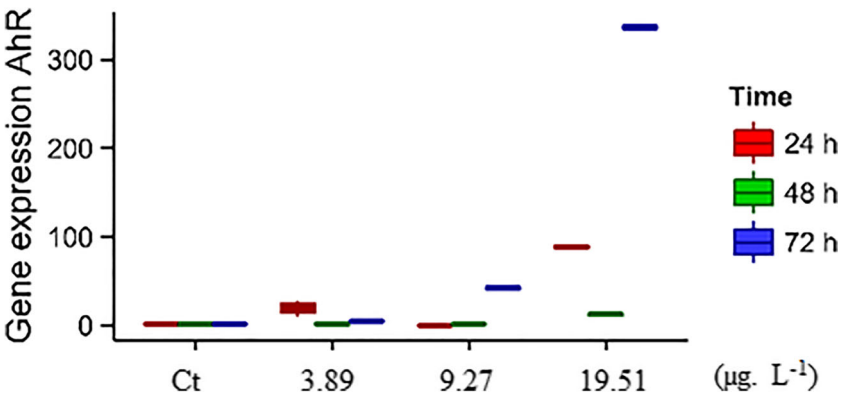

Fig. 3 Expression of aryl hydrocarbon receptor (*AhR*) gene in *Gambusia yucatana* submitted to acute exposure (72 h) to PAHs from crude oil. All data are presented as mean ± standard deviation

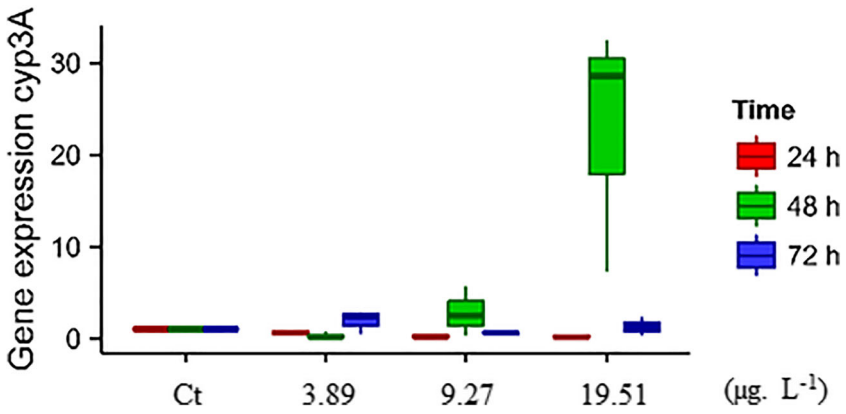

Fig. 4 Expression of cytochrome P4503A (*CYP3A*) gene in *Gambusia yucatana* submitted to acute exposure (72 h) to PAHs from crude oil. All data are presented as mean ± standard deviation

to the cell context and the specific response (Vondráček et al., 2018). The increase in *esr*s expression in fish exposed to EDCs may therefore be generated both by direct induction of the ER signalling pathway in the presence of EDCs and by an increase in AhR expression, indicating that the mechanisms of detoxification and disruption are closely related.

Previous studies have demonstrated that ERs, especially ERα, regulate various oestrogenic actions in female fish, mainly in vitellogenesis and oestrogen overload (Yan et al., 2012; Huang et al., 2010; Marlatt et al., 2010). The Vtg is a phospholipoglycoprotein precursor of egg yolk produced by hepatocytes in females and regulated by E2 (Shaya et al., 2018), which binds to ERs to activate the ER signalling pathway that instructs the liver to synthesise Vtg and secrete it into the bloodstream (Chen et al., 2019). However, when male fish are exposed to E2 or other EDCs, they can bind to ERs, generating an ER homodimer complex that recruits additional transcription factors, which then increase gene transcription and the synthesis of oestrogen-inducible proteins, such as Vtg (Kent et al., 2001). Therefore, exposure to EDCs can lead to the production of Vtg in male fish, which otherwise could not be produced (Burgos-Aceves et al., 2016; Yamamoto et al., 2017).

We observed the peak expression of *esr1* and *esr2* in male specimens of *G. yucatana* at a concentration of 19.51 µg/L, mainly 48 h after exposure to the PAH extract (Fig. 2). Exposure to PAH extract increased the expression of *esr1* more significantly than that of *esr2* in the liver of male *G. yucatana* specimens (Fig. 2). This finding is consistent with Davis et al. (2010), who reported that male specimens of tilapia (*Oreochromis mossambicus*) injected with E2 showed an apparent increase in *esr1* liver expression and a slight effect on *esr2* expression. Likewise, Yan et al. (2012) exposed male goldfish (*C. auratus*) specimens to a combination of E2 and BaP and also found that these substances increased the expression of *esr1* in the liver, with only a slight effect on *esr2*. Our results demonstrate that ERα is the dominant ER that regulates oestrogenic effects, as the positive regulation of *ers1* expression correlated with the increase in Vtg mRNA induction in males of *G. yucatana* (Fig. 1). We detected Vtg induction in males of *G. yucatana*, with the expression of *vtg1* and *2* reaching a peak at the concentration of 19.51 µg/L (Fig. 1). We noticed an increase in *vtg1* expression as early as 24 h after exposure to PAHs at concentrations of 9.27 and 19.51 µg/L and in *vtg2* at a concentration of 3.89 µg/L (Fig. 1).

Previous studies have reported a similar induction of Vtg synthesis, where E2 and EDCs act as agonists, inducing the production of Vtg in male fish. For example, Wang et al. (2015) demonstrated that the injection of E2 induced (10 mg/kg) plasma Vtg in male goldfish (*C. auratus*) after 7 days of exposure. Bowman et al. (2002) reported that the plasma Vtg of males of the largemouth bass (*Micropterus salmoides*) increased 48 h after the injection of E2 (in concentrations of 0.05, 0.5, 5.0 mg/kg). Furthermore, Prasatkaew et al. (2019) showed an increase in plasma Vtg levels in male sea bass (*Lates calcarifer*) 3 days after exposure to 2 mg/kg of E2. Finally, Rodas-Ortíz et al. (2008) found that *O. niloticus* exposed to BaP produced more Vtg, indicating that this EDC has oestrogenic effects. Yan et al. (2012) also observed that BaP induces the expression of *esr*s in male goldfish.

However, several other studies have reported that EDCs also act as antagonists of protein synthesis in the reproduction of female fish, decreasing their reproductive capacity. For example, Gao et al. (2018) exposed adult female zebrafish (*Danio rerio*) to BaP and reported a negative regulation of *ers1*, *ers2*, *vtg1* and *vtg2*. Zheng et al. (2006) observed a decrease in the levels of Vtg and ERs in females of *Sebastiscus marmoratus* after exposure to BaP. Similarly, Smeets (1999) found an antiestrogenic effect of BaP as it decreased the levels of Vtg in the hepatocytes of female carp specimens (*Cyprinus carpio*).

In our experiment, the PAH extract appears to have functioned as a xenoestrogen, inducing the expression of *esr* and *vtg* through the ER signalling pathway in male fish. We believe that PAHs, in males of *G. yucatana*, are critical inducers of Vtg and ERs and potential endocrine disruptors. Due to the higher concentration of BaP, it is possible that this EDC is primarily responsible for our results. However, it is important

to consider that our research evaluated the effects of a PAH extract (and potentially other hydrocarbon substances), and further research into using both BaP and other PAHs in isolation would be necessary to obtain more robust conclusions about the effect of PAHs as endocrine disruptors in *G. yucatana*. Although the estrogenic and/or antiestrogenic properties of PAHs in fish are not entirely clear, PAHs often act as ED in fish, as already mentioned, as they alter the ER signalling pathway either by induction or antagonism. Therefore, the effect observed in our study indicates a serious risk for the populations of *G. yucatana*, since the reproductive success of this species can be affected in the presence of PAHs. In relation to fish, EDCs can seriously alter sex ratios, reduce fecundity, affect reproduction, cause a decline of fecundity and gamete quantity, alter the production of vitellogenin in males, change the intersex condition and collapse fish populations (Belcher et al., 2019; Teta and Naik, 2017).

Expression of *AhR* and *CYP3A* response genes

The gene *AhR* participates in the production of CYP1A phase I biotransformation enzymes, representing a metabolic response to the detoxification of PAHs, which activate AhR, inducing CYP1A expression in fish and mammals (Hahn, 2002; Choi et al., 2011). Although biotransformation generally detoxifies the contaminant, the action of CYP450 complex enzymes can also generate toxic metabolites that contribute to the increased risk of cancer, embryonic deformations and other deleterious effects (Nebert and Karp, 2008). For example, naphthalene generates reactive intermediates (naphthoquinones) after biotransformation of the CYP1A phase, which can cause oxidative stress, DNA damage and lipid peroxidation in the brain and liver of fish and mammals (Bagchi et al., 2002). Here, naphthalene was found in the water but not in the fish tissues. A previous study has reported a strong biotransformation of PAHs at the concentrations of 8.73, 17.46 and 34.95 μg/L in *G. yucatana*, as well as oxidative stress (Aguilar et al., 2020). Based on this, our results lead us to infer that the biotransformation system of *G. yucatana* is activated by PAHs, as expected. However, an increase in the expression of AhR may also be associated with an increase in the expression of *esr*s, as already mentioned. Thus, it is believed that in *G. yucatana*, the increase in AhR expression is also indicative of endocrine disruption caused by PAHs.

The CYP3A—another subfamily of cytochrome P450—participates in the metabolism of steroids and xenobiotics in fish (Hegelund and Celander, 2003; Kullman and Hinton, 2001). Fish CYP3A proteins are mainly expressed in the liver and intestine, suggesting that these enzymes act in the first-pass metabolism of xenobiotics (Della-Torre et al., 2010) and, similar to CYP1A, can metabolize xenobiotics and generate toxic metabolites. The CYP3A metabolises BaP in vertebrates (James et al., 2005), and fish exposed to BaP increase the expression of the *CYP3A* gene (Nebert and Russell, 2002). Although the metabolism of BaP by CYP3A has already been reported, here, exposure to PAH extract induced the expression of *CYP3A*, which indicates that PAHs were metabolised, but also that BaP was the PAH that occurred in greater quantity in the muscles of *G. yucatana* in the treatment of 19.51 μg/L in 48 h of exposure (Table 2). This indicates that this EDC was not completely metabolised. That is, in *G. yucatana*, exposure to PAH extract may generate toxic metabolites, as well as intoxication by the fraction of the non-metabolised components. It is likely that although CYPs are able to biotransform part of the PAHs, another part is bioaccumulated due to the relatively high levels of PAHs bioavailable in the water.

The toxicity as well as the mutagenic and carcinogenic potential of BaP have been described elsewhere (Labib et al. 2013), and several studies have demonstrated that BaP exposure may damage fish in various ways. For example, BaP exposure may result in the generation of ROS, leading to toxic effects via numerous cellular processes (Cui et al., 2019). The lipid metabolism of *Xenopus tropicalis* was impaired by BaP, leading to hepatotoxicity (Regnault et al., 2014). Therefore, the activity of BaP as an endocrine disruptor is generally considered secondary (Booc et al., 2014). However, some studies have already demonstrated that it is a potential endocrine disruptor in fish, and many countries and regions have included BaP in their endocrine-disrupting chemical screening programs (Cai et al., 2018). The detoxification mechanism of BaP is complex; for example, Cai et al. (2018) reported that short-term exposure to BaP can adversely affect detoxification and lipid metabolism in the liver of *Mugilogobius chulae*. Feng et al. (2020) have reported that BaP damages immunity and enhances the consumption of the available energy stored to activate detoxification mechanisms in *G. affinis*. However, according to Booc et al. (2014), there is no doubt that it acts as an endocrine disruptor. In their experiment, the authors exposed males and females of *Fundulus heteroclitus* to BaP and noticed a decrease in the concentration of circulating oestrogen in females and of testosterone in males (Booc et al., 2014).

In view of our findings and the data found in the literature, we can infer that the male specimens of *G. yucatana* positively regulated the CYP3A gene to produce more hepatic CYP3A to metabolise BaP and other components of the PAH extract. We believe that BaP may be one of the main PAHs responsible for inducing the expression of the CYP3A gene in *G. yucatana*; however, we understand that we cannot consider it in isolation since *G. yucatana* was exposed to a complex of PAHs, where BaP was already present at a greater concentration. For this reason, we suggest that future experiments should be conducted to clarify the mechanisms of ED caused by PAHs in *G. yucatana*.

Conclusions

This study demonstrates the effects of PAHs as EDCs on the reproduction of the *G. yucatana*. We found reproductive changes, such as increases in the hepatic expression of *vtg* and *esr*s in male fish (mainly in the higher concentrations). We also found the same pattern of *AhR* and *CYP3A* hepatic expression, demonstrating an increase in the metabolism of xenobiotics of *G. yucatana*.

Based on our results, BaP appears to be the main driver of the effects observed in *G. yucatana*. However, as the experiment was performed using a PAH mixture, we cannot state this conclusively before more specific studies are carried out.

Due to oil exploration in the Gulf of Mexico, a region with a high risk of oil spill accidents, *Gambusia yucatana* may often to be under the action of PAHs dissolved in water. Thus, in addition to acute exposure studies (such as ours), we recommend that future studies on this topic should focus on the effects of chronic exposure to PAHs.

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Data availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate All procedures were following the guidelines approved by Biological Ethics Committee of Ecology, Fisheries and Oceanography of Gulf of Mexico Institute (EPOMEX), Campeche University, according to the Federal Mexican Norm: NOM-01992-STPS-1993 whit approval date 15 March 2017 (please see the letter below).

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

Abdelrahim M, Ariazi E, Kim K, Khan S, Barhoumi R, Burghardt R, Liu S, Hill D, Finnell R, Wlodarczyk B, Jordan VC, Safe S (2006) 3-methylcholanthrene and other aryl hydrocarbon receptor agonists directly activate estrogen receptor α. Cancer Res 66:2459–2467. https://doi.org/10.1158/0008-5472.can-05-3132

Acolas M-L, Davail B, Gonzalez P, Jean S, Clérandeau C, Morin B, Gourves PY, Daffe G, Labadie P, Perrault A, Lauzent M, Pierre M, le Barh R, Baudrimont M, Peluhet L, le Menach K, Budzinski H, Rochard E, Cachot J (2019) Health indicators and contaminant levels of a critically endangered species in the Gironde estuary, the European sturgeon. Environ Sci Pollut Res 27:3726–3745. https://doi.org/10.1007/s11356-019-05139-5

Aguilar L, Dzul-Caamal R, Rendón-von Osten J, da Cruz AL (2020) Effects of polycyclic aromatic hydrocarbons in *Gambusia yucatana*, an endemic fish from Yucatán Peninsula, Mexico. Polycycl Aromat Compd:1–18. https://doi.org/10.1080/10406638.2020.1755322

Amenyogbe E, Chen G, Wang Z, Lu X, Lin M, Lin AY (2020) A review on sex steroid hormone estrogen receptors in mammals and fish. Int J Endocrinol 2020:1–9. https://doi.org/10.1155/2020/5386193

Arukwe A, Nordtug T, Kortner TM, Mortensen AS, Brakstad OG (2008) Modulation of steroidogenesis and xenobiotic biotransformation responses in zebrafish (*Danio rerio*) exposed to water-soluble fraction of crude oil. Environ Res 107:362–370. https://doi.org/10.1016/j.envres.2008.02.009

Bagchi D, Balmoori J, Bagchi M, Ye X, Williams CB, Stohs SJ (2002) Comparative effects of TCDD, endrin, naphthalene and chromium (VI) on oxidative stress and tissue damage in the liver and brain tissues of mice. Toxicology 175:73–82. https://doi.org/10.1016/S0300-483X(02)00062-8

Barata C, Calbet A, Saiz E, Ortiz L, Bayona JM (2005) Predicting single and mixture toxicity of petrogenic polycyclic aromatic hydrocarbons to the copepod *Oithona davisae*. Environ Toxicol Chem 24:2992–2999. https://doi.org/10.1897/05-189r.1

Belcher SM, Cline JM, Conley J, Groeters S, Jefferson WN, Law M, Mackey E, Suen AA, Williams CJ, Dixon D, Wolf JC (2019) Endocrine disruption and reproductive pathology. Toxicol Pathol 47:1049–1071. https://doi.org/10.1177/0192623319879903

Bera G, Doyle S, Passow U, Kamalanathan M, Wade TL, Sylvan JB, Sericano JL, Gold G, Quigg A, Knap AH (2019) Biological response to dissolved versus dispersed oil. Mar Pollut Bull 150:110713. https://doi.org/10.1016/j.marpolbul.2019.110713

Booc F, Thornton C, Lister A, MacLatchy D, Willett KL (2014) Benzo[a]pyrene effects on reproductive endpoints in *Fundulus heteroclitus*. Toxicol Sci 140:73–82. https://doi.org/10.1093/toxsci/kfu064

Bowman C, Kroll K, Gross T, Denslow N (2002) Estradiol-induced gene expression in largemouth bass (*Micropterus salmoides*). Mol Cell Endocrinol 196:67–77. https://doi.org/10.1016/S0303-7207(02)00224-1

Burgos-Aceves MA, Cohen A, Smith Y, Faggio C (2016) Estrogen regulation of gene expression in the teleost fish immune system. Fish Shellfish Immunol 58:42–49. https://doi.org/10.1016/j.fsi.2016.09.006

Cai L, Li J, Yu L, Wei Y, Miao Z, Chen M, Huang R (2018) Characterization of transcriptional responses mediated by benzo[a]pyrene stress in a new marine fish model of goby, *Mugilogobius chulae*. Genes Genomics. https://doi.org/10.1007/s13258-018-0743-8

Carnevali O, Santangeli S, Forner-Piquer I, Basili D, Maradonna F (2018) Endocrine-disrupting chemicals in aquatic environment: what are the risks for fish gametes? Fish Physiol Biochem 44:1561–1576. https://doi.org/10.1007/s10695-018-0507-z

Chakrabarty P, O'Neill G, Hardy B, Ballengee B (2016) Five Years Later: An Update on the Status of Collections of Endemic Gulf of Mexico Fishes Put at Risk by the 2010 Oil Spill. Biodivers Data J 4: e8728. https://doi.org/10.3897/BDJ.4.e8728

Chen J-R, Wu SM, Tsai SC, Hsien F-C, Huang CT (2019) Changes in vitellogenin and estrogen receptor expression and 17β-estradiol concentration in male juvenile tilapia can be used to evaluate endocrine-disrupting chemicals. Comp Biochem Physiol C: 108682. https://doi.org/10.1016/j.cbpc.2019.108682

Choi EY, Lee H, Dingle RWC, Kim KB, Swanson HI (2011) Development of novel CH223191-based antagonists of the aryl hydrocarbon receptor. Mol Pharmacol 81:3–11. https://doi.org/10.1124/mol.111.073643

Colin N, Porte C, Fernandes D, Barata C, Padrós F, Carrassón M, Monroy M, Cano-Rocabayera O, de Sostoa A, Piña B, Maceda-Veiga A (2016) Ecological relevance of biomarkers in monitoring studies of macro-invertebrates and fish in Mediterranean rivers. Sci Total Environ 540:307–323. https://doi.org/10.1016/j.scitotenv.2015.06.099

Cui Q, Chen FY, Chen HY, Peng H, Wang KJ (2019) Benzo[a]pyrene (BaP) exposure generates persistent reactive oxygen species (ROS) to inhibit the NF-κB pathway in medaka (*Oryzias melastigma*). Environ Pollut 251:502–509. https://doi.org/10.1016/j.envpol.2019.04.063

Davis LK, Katsu Y, Iguchi T, Lerner DT, Hirano T, Grau EG (2010) Transcriptional activity and biological effects of mammalian estrogen receptor ligands on three hepatic estrogen receptors in Mozambique tilapia. J Steroid Biochem Mol Biol 122:272–278. https://doi.org/10.1016/j.jsbmb.2010.05.009

Della-Torre C, Corsi I, Nardi F, Perra G, Tomasino MP, Focardi S (2010) Transcriptional and post-transcriptional response of drug-metabolizing enzymes to PAHs contamination in red mullet (*Mullus barbatus*, Linnaeus, 1758): a field study. Mar Environ Res 70:95–101. https://doi.org/10.1016/j.marenvres.2010.03.009

Diamante G, do Amaral e Silva Müller G, Menjivar-Cervantes N, Xu EG, Volz DC, Dias Bainy AC, Schlenk D (2017) Developmental toxicity of hydroxylated chrysene metabolites in zebrafish embryos. Aquat Toxicol 189:77–86. https://doi.org/10.1016/j.aquatox.2017.05.013

Feng Y, Zhou A, Zhang Y, Liu S, Pan Z, Zou J, Xie S (2020) Transcriptomic changes in western mosquito fish (*Gambusia affinis*) liver following benzo[a]pyrene exposure. Environ Sci Pollut Res 27: 21924–21938. https://doi.org/10.1007/s11356-020-08571-0

Gao D, Lin J, Ou K, Chen Y, Li H, Dai Q, Yu Z, Zuo Z, Wang C (2018) Embryonic exposure to benzo(a)pyrene inhibits reproductive capability in adult female zebrafish and correlation with DNA methylation. Environ Pollut 240:403–411. https://doi.org/10.1016/j.envpol.2018.04.139

Ha H, Park K, Kang G, Lee S (2019) QSAR study using acute toxicity of *Daphnia magna* and *Hyalella azteca* through exposure to polycyclic aromatic hydrocarbons (PAHs). Ecotoxicology 28:333–342. https://doi.org/10.1007/s10646-019-02025-1

Hahn ME (2002) Aryl hydrocarbon receptors: diversity and evolution. Chem Biol Interact 141:131–160. https://doi.org/10.1016/s0009-2797(02)00070-4

Hegelund T, Celander MC (2003) Hepatic versus extrahepatic expression of CYP3A30 and CYP3A56 in adult kill fish (*Fundulus heteroclitus*). Aquat Toxicol 6:277–291. https://doi.org/10.1016/S0166-445X(03)00057-2

Honda M, Suzuki N (2020) Toxicities of polycyclic aromatic hydrocarbons for aquatic animals. Int J Environ Res Public Health 17:1363. https://doi.org/10.3390/ijerph17041363

Huang C, Zhang ZB, Wu SM, Zhao YB, Hu JY (2010) In vitro and in vivo estrogenic effects of 17 alpha-estradiol in medaka (*Oryzias latipes*). Chemosphere 80:608–612. https://doi.org/10.1016/j.chemosphere.2010.04.010

Ishibashi J, Yamashita K, Ishikawa T, Hosokawa H, Sumida K, Nagayama M, Kitamura S (2007) The effects inhibiting the proliferation of cancer cells by far-infrared radiation (FIR) are controlled by the basal expression level of heat shock protein (HSP) 70A. Med Oncol 25:229–237. https://doi.org/10.1007/s12032-007-9020-4

Ishibashi H, Yamauchi R, Matsuoka M, Kim JW, Hirano M, Yamaguchi A, Tominaga T, Arizono K (2008) Fluorotelomer alcohols induce hepatic vitellogenin through activation of the estrogen receptor in male medaka (*Oryzias latipes*). Chemosphere 71:1853–1859. https://doi.org/10.1016/j.chemosphere.2008.01.065

James MO, Lou Z, Rowland-Faux L, Celander MC (2005) Properties and regional expression of a CYP3A-like protein in channel catfish intestine. Aquat Toxicol 72:361–371. https://doi.org/10.1016/j.aquatox.2005.03.001

Kent ML, Andree KB, Bartholomew JL, El-Matbouli M, Desser SS, Devlin RH et al (2001) Recent advances in our understanding of the Myxozoa. J Eukaryot Microbiol 48:395–413. https://doi.org/10.1111/j.1550-7408.2001.tb00173.x

Kullman SW, Hinton DE (2001) Identification, characterization, and ontogeny of a second cytochrome P4503A gene from the freshwater teleost medaka (*Oryzias latipes*). Mol Reprod Dev 58:149–158. https://doi.org/10.1002/1098-2795(200102)58:2<149::AID-MRD3>3.0.CO;2-X

Labib S, Guo CH, Williams A, Yauk CL, White PA, Halappanavar S (2013) Toxicogenomic outcomes predictive of forestomach carcinogenesis following exposure to benzo(a)pyrene: relevance to human cancer risk. Toxicol Appl Pharmacol 273(2):269–280. https://doi.org/10.1016/j.taap.2013.05.027

Litasov KD, Inerbaev TM, Abuova FU, Chanyshev AD, Dauletbekova AK, Akilbekov AT (2019) High-pressure elastic properties of polycyclic aromatic hydrocarbons obtained by first-principles calculations. Geochem Int 57:499–508. https://doi.org/10.1134/S0016702919050069

Liu S, Abdelrahim M, Khan S, Ariazi E, Jordan VC, Safe S (2006) Aryl hydrocarbon receptor agonists directly activate estrogen receptor α in MCF-7 breast cancer cells. Biol Chem 387:1209–1213. https://doi.org/10.1515/bc.2006.149

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262

Lu GH, Wang C, Zhu Z (2009) The dose–response relationships for EROD and GST induced by polyaromatic hydrocarbons in *Carassius auratus*. Bull. Environ. Contam Toxicol 82:194–199. https://doi.org/10.1007/s00128-008-9622-3

Marlatt VL, Lakoff J, Crump K, Martyniuk CJ, Watt J, Jewell L, Atkinson S, Blais JM, Sherry J, Moon TW, Trudeau VL (2010) Sex- and tissue-specific effects of waterborne estrogen on estrogen receptor subtypes and E2-mediated gene expression in the reproductive axis of goldfish. Comp Biochem Physiol A Physiol 156:92–101. https://doi.org/10.1016/j.cbpa.2010.01.001

Marris CR, Kompella SN, Miller MR, Incardona JP, Brette F, Hancox JC, Sørhus E, Shiels HA (2019) Polyaromatic hydrocarbons in pollution: a heart-breaking matter. J Physiol 598(2):227–247. https://doi.org/10.1113/JP278885

Martyniuk CJ, Feswick A, Munkittrick KR, Dreier DA, Denslow ND (2019) Twenty years of transcriptomics, 17alpha-ethinylestradiol, and fish. Gen Comp Endocrinol:113325. https://doi.org/10.1016/j.ygcen.2019.113325

Mortensen AS, Arukwe A (2008) Activation of estrogen receptor signaling by the dioxin-like aryl hydrocarbon receptor agonist, 3,3′,4,4′,5-pentachlorobiphenyl (PCB126) in salmon in vitro system. Toxicol Appl Pharmacol 227:313–324. https://doi.org/10.1016/j.taap.2007.11.003

Nebert DW, Karp CL (2008) Endogenous functions of the aryl hydrocarbon receptor (AHR): intersection of cytochrome P450 1 (CYP1)-

metabolized eicosanoids and AHR biology. J Biol Chem 283: 36061–36065. https://doi.org/10.1074/jbc.R800053200

Nebert DW, Russell DW (2002) Clinical importance of the cytochromes P450. Lancet 360:1155–1162. https://doi.org/10.1016/S0140-6736(02)11203-7

Nichols JW, Ladd MA, Hoffman AD, Fitzsimmons PN (2019) Biotransformation of polycyclic aromatic hydrocarbons by trout liver S9 fractions: evaluation of competitive inhibition using a substrate depletion approach. Environ Toxicol Chem 38:2729–2739. https://doi.org/10.1002/etc.4595

Osten JR, Ortíz-Arana A, Guilhermino L, Soares AM (2005) In vivo evaluation of three biomarkers in the mosquito fish (*Gambusia yucatana*) exposed to pesticides. Chemosphere 58:627–636. https://doi.org/10.1016/j.chemosphere.2004.08.065

Prabowo AR, Bae DM (2019) Environmental risk of maritime territory subjected to accidental phenomena: Correlation of oil spill and ship grounding in the Exxon Valdez's case. Res Eng Des 4:100035. https://doi.org/10.1016/j.rineng.2019.100035

Prasatkaew W, Nanthanawat P, Thanomsit C (2019) Assessment of endocrine disrupting chemicals exposure in sea bass (*Lates calcarifer*) and wild fishes using vitellogenin as a biomarker. Environ Asia 12: 69–78. https://doi.org/10.14456/ea.2019.28

Pulster EL, Gracia A, Armenteros M, Carr BE, Mrowicki J, Murawski SA (2019) Chronic PAH exposures and associated declines in fish health indices observed for ten grouper species in the Gulf of Mexico. Sci Total Environ 703:135551. https://doi.org/10.1016/j.scitotenv.2019.135551

Regnault C, Worms IAM, Oger-Desfeux C, MelodeLima C, Veyrenc S, Bayle ML, Combourieu B, Bonin A, Renaud J, Raveton M, Reynaud S (2014) Impaired liver function in *Xenopus tropicalis* exposed to benzo[a]pyrene: transcriptomic and metabolic evidence. BMC Genomics 15:666. https://doi.org/10.1186/1471-2164-15-666

Rendón-von Osten J, Aguayo-Dione G, Dzul-Caamal R, Lara-Flores M (2019) Expression of estrogenic response genes to different concentration of 17ß-estradiol in male mosquito fish (*Gambusia yucatana*). Iran J Fish Sci 18:272–282. https://doi.org/10.22092/ijfs.2018.117433

Ribecco C, Hardiman G, Šášik R, Vittori S, Carnevali O (2012) Teleost fish (*Solea solea*): a novel model for ecotoxicological assay of contaminated sediments. Aquat Toxicol 109:133–142. https://doi.org/10.1016/j.aquatox.2011.12.002

Rodas-Ortíz JP, Ceja-Moreno V, Chan-Cocom ME, Gold-Bouchot G (2008) Vitellogenin induction and increased plasma 17β-estradiol concentrations in male Nile tilapia, *Oreochromis niloticus*, exposed to organochlorine pollutants and polycyclic aromatics hydrocarbons. Bull Environ Contam Toxicol 81:543–547. https://doi.org/10.1007/s00128-008-9556-9

Rodrigues S, Antunes SC, Correia AT, Nunes B (2018) Toxicity of erythromycin to Oncorhynchus mykiss at different biochemical levels: detoxification metabolism, energetic balance, and neurological impairment. Environ Sci Pollut Res 26:227–239. https://doi.org/10.1007/s11356-018-3494-9

Rodríguez-Fuentes G, Marín-López V, Hernández-Márquez E (2016) Cholinesterases in *Gambusia yucatana*: biochemical characterization and its relationship with sex and total length. Bull Environ Contam Toxicol 97:776–780. https://doi.org/10.1007/s00128-016-1939-8

Roldán-Wong NT, Kidd KA, Ceballos-Vázquez BP, Rivera-Camacho AR, Arellano-Martínez M (2020) Polycyclic aromatic hydrocarbons (PAHs) in mussels (*Modiolus capax*) from sites with increasing anthropogenic impact in La Paz Bay, Gulf of California. Reg Stud Mar Sci 33:100948. https://doi.org/10.1016/j.rsma.2019.100948

Schwab AP, Su J, Wetzel S, Pekarek S, Banks MK (1999) Extraction of petroleum hydrocarbons from soil by mechanical shaking. Environ. Sci Technol 33:1940–1945. https://doi.org/10.1021/es9809758

Seruto C, Sapozhnikova Y, Schlenk D (2005) Evaluation of the relationships between biochemical endpoints of PAH exposure and physiological endpoints of reproduction in male California Halibut (*Paralichthys californicus*) exposed to sediments from a natural oil seep. Mar Environ Res 60:454–465. https://doi.org/10.1016/j.marenvres.2005.01.004

Shaya L, Jones DE, Wilson JY (2018) CYP3C gene regulation by the aryl hydrocarbon and estrogen receptors in zebrafish. Toxicol Appl Pharmacol 362:77–85. https://doi.org/10.1016/j.taap.2018.10.021

Smeets J (1999) The anti-estrogenicity of Ah receptor agonists in carp (*Cyprinus carpio*) hepatocytes. Toxicol Sci 52:178–188. https://doi.org/10.1093/toxsci/52.2.178

Soltani N, Moore F, Keshavarzi B, Sorooshian A, Javid R (2019) Potentially toxic elements (PTEs) and polycyclic aromatic hydrocarbons (PAHs) in fish and prawn in the Persian Gulf, Iran. Ecotoxicol Environ Saf 173:251–265. https://doi.org/10.1016/j.ecoenv.2019.02.005

Tao W, Lin J, Wang W, Huang H, Li S (2019) Biodegradation of aliphatic and polycyclic aromatic hydrocarbons by the thermophilic bioemulsifier-producing *Aeribacillus pallidus* strain SL-1. Ecotoxicol Environ Saf 109994:109994. https://doi.org/10.1016/j.ecoenv.2019.109994

Teta C, Naik YS (2017) Vitellogenin induction and reduced fecundity in zebrafish exposed to effluents from the City of Bulawayo, Zimbabwe. Chemosphere 167:282–290. https://doi.org/10.1016/j.chemosphere.2016.10.011

Toft G, Guillete LJ Jr (2005) Decreased sperm count and sexual behavior in mosquitofish exposed to water from a pesticide-contaminated lake. Ecotoxicol Environ Saf 60:15–20. https://doi.org/10.1016/j.ecoenv.2004.07.010

Toft G, Baatrup E, Guillete LJ Jr (2004) Altered social behaviour and sexual characteristics in mosquitofish (*Gambusia holbrooki*) living downstream of a paper mill. Aquat Toxicol 70:213–222. https://doi.org/10.1016/j.aquatox.2004.09.002

U.S.EPA (1986) Method 8310: Polynuclear Aromatic Hydrocarbons. www.epa.gov/osw/hazard/testmethods/sw846/pdfs/8310.pdf. Accessed 30 June 2019.

Vallarino A, Rendón-von Osten J (2017) Comparison of organochlorine and PAHs residues in terns eggs from two natural protected areas in the Gulf of Mexico. Mar Pollut Bull 116:48–55. https://doi.org/10.1016/j.marpolbul.2016.12.044

Vignet C, Larcher T, Davail B, Joassard L, Le Menach K, Guionnet T, Lyphout L, Ledevin M, Goubeau M, Budzinski H, Bégout M-L, Cousin X (2016) Fish reproduction is disrupted upon lifelong exposure to environmental PAHs fractions revealing different modes of action. Toxics 4:26. https://doi.org/10.3390/toxics4040026

Vondráček J, Pivnička J, Machala M (2018) Polycyclic aromatic hydrocarbons and disruption of steroid signaling. Curr Opin Toxicol 11-12:27–34. https://doi.org/10.1016/j.cotox.2018.12.003

Walter JM, Bagi A, Pampanin DM (2019) Insights into the potential of the Atlantic cod gut microbiome as biomarker of oil contamination in the marine environment. Microorganisms 7:209. https://doi.org/10.3390/microorganisms7070209

Wang J, Bing X, Yu K, Tian H, Wang W, Ru S (2015) Preparation of a polyclonal antibody against goldfish (*Carassius auratus*) vitellogenin and its application to detect the estrogenic effects of monocrotophos pesticide. Ecotoxicol Environ Saf 111:109–116. https://doi.org/10.1016/j.ecoenv.2014.10.007

Yamamoto FY, Garcia JRE, Kupsco A, Oliveira Ribeiro CA (2017) Vitellogenin levels and others biomarkers show evidences of endocrine disruption in fish species from Iguaçu River - Southern Brazil. Chemosphere 186:88–99. https://doi.org/10.1016/j.chemosphere.2017.07.111

Yan Z, Lu G, He J (2012) Reciprocal inhibiting interactive mechanism between the estrogen receptor and aryl hydrocarbon receptor signaling pathways in goldfish (*Carassius auratus*) exposed to 17β-

estradiol and benzo[a]pyrene. Comp Biochem Physiol C Toxicol Pharmacol 156:17–23. https://doi.org/10.1016/j.cbpc.2012.03.001

Yang Y, Zhou Y, Pan L, Xu R, Li D (2020) Benzo[a]pyrene exposure induced reproductive endocrine-disrupting effects via the steroidogenic pathway and estrogen signaling pathway in female scallop Chlamys farreri. Sci Total Environ:138585. https://doi.org/10.1016/j.scitotenv.2020.138585

Zabbey N, Olsson G (2017) Conflicts – oil exploration and water. Glob Challenges 1600015:1–10. https://doi.org/10.1002/gch2.201600015

Zhang YX, Tao S, Cao J, Coveney RM (2007) Emission of polycyclic aromatic hydrocarbons in China by county. Environ Sci Technol 41:683–687. https://doi.org/10.1021/es061545h

Zhang J, Terrones M, Park CR, Mukherjee R, Monthioux M, Koratkar N, Kim YS, Hurt R, Frackowiak E, Enoki T, Chen Y, Chen Y, Bianco A (2016) Carbon science in 2016: Status, challenges and perspectives. Carbon 98:708–732. https://doi.org/10.1016/j.carbon.2015.11.060

Zheng RH, Wang CG, Zuo ZH, Chen YX, Zhao Y (2006) Combined effect of tributyltin and benzo[a]pyrene on the levels of sex hormone and vitellogenin in female *Sebastiscus marmoratus*. J Environ Sci 18:359–363