



# Enzymatic, morphological, and genotoxic effects of benzo[a]pyrene in rainbow trout (*Oncorhynchus mykiss*)

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## Abstract

Fish have defense systems that are capable of repairing damages caused by xenobiotics like benzo[a]pyrene (BaP), so the aims of this study were to identify BaP toxicity in melanomacrophages (MMs) cytoskeleton, evaluate the melanin area in MMs, and analyze genotoxicity. Rainbow trout juveniles (n = 24) were split in 48h and 7d treatments that received 2 mg/kg of BaP. After the experiment, blood samples were collected and liver was removed, to proceed with the analysis: EROD activity, MMs melanin area quantification, melanosomes movements, and a genotoxicity test. The results revealed increased in EROD activity after 48-h and 7-day BaP exposure. The group 7d displayed a reduction in MMs pigmented area, melanosomes aggregation, in addition to an increased frequency of micronucleus. By means of the EROD assay, it was possible to confirm the activation of BaP biotransformation system. The impairment of the melanosomes' movements possibly by an inactivation of the protein responsible for the pigment dispersion consequently affects the melanin area and thus might negatively impact the MMs detoxification capacity. In addition to this cytotoxicity, the increased frequency of micronucleus might also indicate the genotoxicity of BaP in this important fish species.

**Keywords** Cytoskeleton · Melanomacrophage · EROD · Morphological alteration · Liver · Erythrocytes abnormalities

## Introduction

Polycyclic aromatic hydrocarbons (PAHs) originated from incomplete combustion processes of carbonaceous materials, during energy generation and other industrial processes (Curtis et al. 2011), such as the combustion of fossil fuels, petroleum spills, and industrial effluents (Whyte et al. 2000; Santana et al. 2018). Although natural sources of PAHs exist, such as forest fires and natural petroleum sources, the main source of contamination is anthropogenic (Whyte et al. 2000;

Santana et al. 2018). Fish and other aquatic organisms can be exposed to PAHs through the gills (respiration), guts (ingestion), or by means of contact through its integument (dermal contact) (Logan 2007). Several PAHs, such as benzo[a]pyrene (BaP), are of great concern when it comes to the health of aquatic organisms.

Benzo[a]pyrene is a hepatotoxic (Pastore et al. 2014; Regnault et al. 2014, 2016; Fanali et al. 2018) and genotoxic PAH (Mouchet et al. 2005; Fanali et al. 2018), being widely studied due to its bioconcentration capacity (Connel 1990; Logan 2007), extensive distribution (Douben 2003; Head et al. 2015), toxicity (Brandt et al. 2002; Head et al. 2015; Santana et al. 2018), and persistence in the environment (Brandt et al. 2002; Collier et al. 2013).

Aquatic organisms have molecular and cellular defense systems (e.g., detoxifying enzymes and molecules) to protect themselves against harmful effects of xenobiotics (Sturve et al. 2014). Cellular and molecular damages caused by exposure to these substances are often used in monitoring and assessment programs addressing the environmental impact of pollutants (van der Oost et al. 2003; Sturve et al. 2014). Effects of pollutants on both biochemical and physiological functions in fish that result in sub-lethal disturbances can be

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used as early warning biomarkers, indicating possible alterations in reproduction, growth, and even survival (Forlin et al. 1986; Haux and Forlin 1988; Sturve et al. 2014).

Biological responses to PAHs toxicity can be assessed by measuring the induction of hepatic CYP1A activity. CYP1A expression is induced through the aryl-hydrocarbon (Ah) receptor that binds dioxin-like compounds, planar PCBs, and PAHs (Goksøyr and Förlin 1992; Abrahamson et al. 2007; Sturve et al. 2014). CYP1A activity can be measured by means of the ethoxyresorufin-O-deethylase (EROD) activity, a widely used technique in fish species (Whyte et al. 2000; Bonacci et al. 2003; Sturve et al. 2014). Thus, EROD activity serves as a sensitive biomarker for PAHs including BaP (Pacheco and Santos 1998; Whyte et al. 2000).

Melanomacrophages (MMs) are hepatic phagocytic macrophages present in fish, amphibians, and reptiles (Wolke 1992; Fishelson 2006; Bach et al. 2018) that produce and store melanin (Agius and Roberts 2003; Ribeiro et al. 2011), which enables an antioxidant function to these cells (Fenoglio et al. 2005; Bach et al. 2018). Melanin neutralizes free radicals, cations, and other toxic agents produced during the degradation of phagocytosed cell material (Zuasti et al. 1989). The MMs' melanin production is performed by the melanosome (Sichel et al. 1997), an organelle that contains melanogenic enzymes, that is capable of producing melanin (Colombo et al. 2011).

MMs accumulate exogenous materials from both natural and experimental origins (Steinel and Bolnick 2017) and are also responsive in face of the action of xenobiotics (De Oliveira et al. 2017; Fanali et al. 2017, 2018), thus being involved in detoxification processes, due to a combination of enzymatic biotransformation and antioxidant actions (Fenoglio et al. 2005; Bach et al. 2018). This gives them the ability to detoxify cytotoxic substances through the melanic biopolymer (Fenoglio et al. 2005; Bach et al. 2018).

Pigment cells are excellent models to study the transport of organelles, as these are responsible for the translocation of pigment granules in response to specific chemical signals (Aspengren et al. 2006). Toxicological effects in components of the cytoskeleton such as microtubules and actin filaments were previously studied in pigmented cells called melanophores, seen that these components are evolutionarily well-conserved (Aspengren et al. 2006, 2012; Hedberg and Wallin 2010). However, effects on the MMs cytoskeleton have never been used to assess effects of contaminants on melanosome movement.

Environmental stressors and contaminants are able to impair the cytoskeleton, thus compromising the aggregation and dispersion of melanin granules (Aspengren et al. 2006, 2008; Hedberg and Wallin 2010). Such an effect on the cytoskeleton is known to jeopardize the detoxification function of MMs, as it affects the cell's integrity. Additionally, genotoxic substances like BaP can damage the genetic material of cells

through interactions with the DNA structure (Kaur et al. 2018), which may result in the formation of micronucleus (MN) (Kaur et al. 2018) and other nuclear erythrocytes abnormalities (Pacheco and Santos 1998; Peixoto et al. 2019).

Rainbow trouts (*Oncorhynchus mykiss*) have been used as model fish species in several studies concerning the effects of toxic substances in their metabolic activity and cytotoxicity (Laville et al. 2004; Schreer et al. 2005; Ellesat et al. 2010). The aims of the present study were to evaluate the effects of BaP in the MMs melanin area, in order to deduce how the compound can affect the cell's functionality, and to identify toxic effects in actin filaments and microtubules. BaP-mediated activation of the biotransformation system was assessed by measuring hepatic EROD activity. In addition, genotoxicity was analyzed by erythrocytes abnormalities (anucleate, binucleated, MN, and bud).

## Material and methods

All procedures described in this study were conducted according to Swedish ethics guidelines, in accordance with the Ethics permit 15986-2018, and animal handling followed the NIH Guide for Care and Use of Laboratory Animals.

### Experiment with benzo[a]pyrene

A total of 24 rainbow trout juveniles (about 1 year) with an average weight and length of 70 g and 190 mm, originating from Vänneåns Laxodling, Halmstad, Sweden, were used for the experiments. The experimental design consisted of two treatments (control and BaP — Sigma Aldrich, St Louis) and two exposure times (48 h and 7 days), with 6 animals in each group. Thus, our experimental design was represented by: control 48 h (n = 6), BaP 48 h (n = 6), control 7 days (n = 6), and BaP 7 days (n = 6).

The group 48 h received a single intraperitoneal injection of BaP (2 mg/kg, dissolved in peanut oil), while the group denominated 7 days received 3 injections with 2-day intervals. The control groups received the same injections, which contained only the peanut oil. Fish were kept without feeding under a natural night and daylight regime (12 h/12 h), in a 40 L glass tanks with flow through system, de-chlorinated, and constant aerated water at 12 °C that was filtered through a bio-filter. The chosen concentration of BaP was based on the study developed by Padrós et al. (2003), with adaptations.

At the end of the experiment, the animals were euthanized with a blow to the head. Blood was collected from the caudal vein and the liver excised. The middle part of the liver (0.88 g) was shock-frozen and stored on liquid nitrogen until preparation for enzymatic analysis. For quantification of melanin, fragments of liver (5 mm) were fixed in Metacarn (60% methanol, 30% chloroform, 10% acetic acid). For cytoskeleton

analysis, fragments of liver (5 mm) were frozen at  $-20\text{ }^{\circ}\text{C}$  until preparation for analysis.

### EROD assay

Liver pieces ( $n = 24$ ; average weight 0.36 g) were homogenized with glass/teflon in a cold homogenizing buffer solution (0.1 M Na/P — phosphate buffer containing 0.15 M KCl, pH 7.4). The homogenate was centrifuged at  $10,000\times g$  during 20 min at  $4\text{ }^{\circ}\text{C}$ . The supernatant was centrifuged again for  $100,000\times g$  for 60 min at  $4\text{ }^{\circ}\text{C}$ , in order to prepare the microsomal fraction. The pellet (microsomes) was re-suspended in the homogenizing buffer solution containing 20% glycerol and stored at  $-80\text{ }^{\circ}\text{C}$ .

The EROD activity was measured in the microsomal fraction of the liver, according to the method described by Forlin et al. (1986) using rhodamine as standard. The reaction mixture contained sodium phosphate buffer (0.1 M, pH 8.0), ethoxyresorufin (0.5 mM), and 25 to 50 ml of sample in a final volume of 2 ml. The reaction began with the addition of 10 ml of NADPH (10 mM). The increase in fluorescence was monitored at 530 nm (excitation) and 585 nm (emission). EROD activity was expressed as picomoles of resorufin formed per minute and per milligram of protein (pmol/min/mg protein). For this purpose, the protein content was quantified according to Lowry et al. (1951) using bovine serum albumin (BSA) as standard.

### Quantification of melanin

In regard to the histological analysis, one liver fragment for each fish was fixed in Metacarn for 3 h, dehydrated in alcoholic series, embedded in paraffin, and stained with Hematoxylin-Eosin. Under a light microscope with the aid of an image capture system, 25 random pictures were taken from liver fragments. Then, the quantification of the pigmented area was carried out by the difference in color intensity observed in MMs as suggested by Santos et al. (2014), using the software Image Pro-Plus (version 6.0).

### Cytoskeleton analysis

Liver samples ( $n = 24$ ) were frozen at  $-20\text{ }^{\circ}\text{C}$  prior to analysis, and after thawing, fragments of the organ were placed in 2 ml of an EDTA solution (2 mM) for up to 24–48 h at  $4\text{ }^{\circ}\text{C}$ , in order to dissociate liver cells and separate MMs. This step allows cells to be dissociated by mechanical action of continuous suctioning of its contents. Subsequently, the content was centrifuged at  $1500\times g$  for 10 min at  $4\text{ }^{\circ}\text{C}$  for pellet formation. Pellets containing MMs were dissolved in KCl (0.56% w/v) for 20 min and mixed during 10 s to suspend the pellet.

Regarding microtubules immunostaining process, the cells were fixed in ice methanol for 6 min and then washed three

times during 5 min with PBS (phosphate buffered saline), before being incubated with the primary antibody Rabbit polyclonal to beta tubulin-loading control (Abcam) for 60 min at room temperature. The cells were then washed three times during 5 min with PBS and incubated in the darkness with the secondary antibody Goat polyclonal to rabbit IgG-H&L (Alexa Fluor® 488) (Abcam) for 45 min. Finally, cells were again washed three times during 5 min with PBS and underwent a final rinse in miliQ water, in the attempt to avoid the formation of salt crystals before they were allowed to dry (Hedberg and Wallin 2010).

The analysis of actin filaments was performed by initially rinsing cells in PBS, followed by fixation in a 3.7% formaldehyde solution for 10 min, then washing it twice with PBS, and permeabilized with 0.1% Triton X-100 for 5 min. The slides were then washed again twice for 5 min in PBS and blocked with 1% BSA for 20 min. The cells were then stained with Rhodamine-Phalloidin (Thermo-Fisher) for 20 min, washed three times for 5 min in PBS, rinsed in miliQ water, and dried (Hedberg and Wallin 2010).

A total of 50 cells per animal were counted under a Nikon Eclipse E100 fluorescence microscope, using the ACT-1 (version 2.0) software. The quantification was made by the measurement of the fluorescent cell area, using the software Image Pro-Plus (version 6.0).

### Nuclear abnormalities analysis

Upon euthanasia, blood was collected from the caudal vein with a heparinized syringe and needle, dripped onto a slide, and blood smears were made. After drying, slides were fixed in methanol for 20 min and stained with Giemsa 7.5%, for 30 min. The following nuclear abnormalities in erythrocytes were analyzed: anucleate, binucleated, bud, and MN. For each animal, 1000 erythrocytes were counted, as suggested by Pérez-Iglesias et al. (2014).

### Statistical analysis

The experimental design consisted of two treatments (control and one BaP concentration) and two exposure times (48 h and 7 days), which characterizes a  $2 \times 2$  factorial design. In each experimental group, six animals were used for the quantification of EROD activity, cytoskeleton, MMs, and erythrocytes abnormalities.

A Kruskal-Wallis test was applied in the EROD assay, in order to determine whether significant differences among groups existed, seen that the data did not present a normal distribution.

A linear mixed-effects model (package lme4; Bates et al. 2015) (Zuur et al. 2009) with restricted maximum likelihood (REML; Bolker et al. 2009) considering treatment and time of exposure as fixed factors along with their interaction was applied to model

microtubules, actin filaments, and MMs area, considered as continuous responses variables. Sampling units (cells for microtubules and actin filaments; pictures for MMs area), in which we estimated the response variables, were nested within each animal (true replicate). To control the dependency among 50 cells or 25 pictures from the same animal (Crawley 2012: 703), we included a random intercept for animal (categorical with 6 levels; Moen et al. 2016). Then, in order to test the model’s assumptions, we used diagnostic plots with the R package sjPlot (Lüdecke 2016) and to assess for differences between treatment and exposure time, we used least-squares means with R package lsmeans (Lenth 2016). Microtubule analysis data were log-transformed, aiming to attend the assumptions of normality and homogeneity. Then, the statistical models were summarized and, as suggested by Kenward and Roger (1997), P values were estimated based on conditional F-tests, with the approximations of degrees of freedom being made with the aid of the sjPlot.

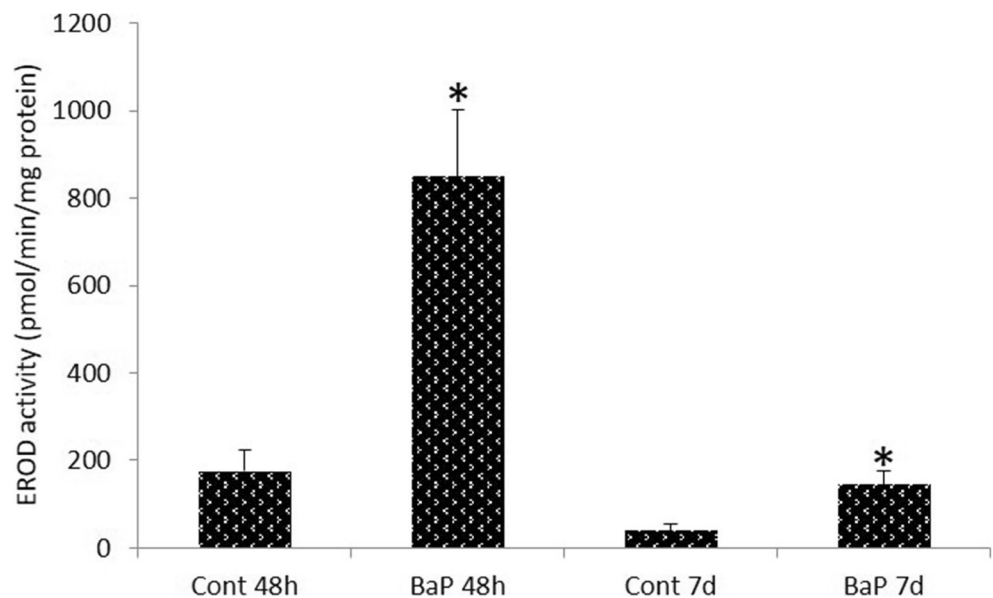
A generalized linear model (GLM) was used to model erythrocytes abnormalities. A binomial distribution and log link function included treatment and exposure time, along with their interactions. To test model assumptions, we used diagnostic plots from the R (Team Core 2016) sjPlot package (Lüdecke 2016). All analyses were performed using software R v. 3.3.2 (R Core Team 2016).

## Results

### EROD activity

Results show that EROD activity was significantly induced after both exposure times, 48 h and 7 days. After 48 h, the activity increased from  $175.8 \pm 48.8$  to  $849.8 \pm 153.6$  pmol/min/mg, i.e.,  $4.84\times$  ( $p < 0.05$ ) (Fig. 1). Similarly, the

**Fig. 1** EROD activity in rainbow trouts exposed to 2 mg/kg of benzo[a]pyrene for 48 h (48h; n = 6 individuals) and 7 days (7d; n = 6 individuals). The asterisk indicates significant differences between the control and the treated groups for the same time interval ( $P < 0.05$ ). Cont = peanut oil, BaP = benzo[a]pyrene



activity increased from  $38.5 \pm 16.5$  to  $144.2 \pm 30.3$  pmol/min/mg after 7 days, i.e.,  $3.74\times$  ( $p < 0.05$ ) (Fig. 1).

### Melanin area of melanomacrophages

The presence of BaP did not exert significant effects on the area of melanin ( $p > 0.05$ ) after 48 h. After 7 days of exposure, the melanin area of MMs was reduced from  $91.0 \pm 31.9$  to  $64.4 \pm 28.3 \mu\text{m}^2$ , which represents a reduction of 29.2% ( $F = 0.8965$ ,  $p < 0.01$ ) (Fig. 2).

### Cytoskeleton

After 48 h of exposure, we did not observe significant effects of BaP in fluorescent cell area ( $p > 0.05$ ). After 7 days, the fluorescent cell area corresponding to actin filaments decreased from  $7915.9 \pm 1305.9$  to  $5058.0 \pm 901.1 \mu\text{m}^2$ , which means that melanin granules were 36.1% ( $F = 26.0756$ ,  $p < 0.01$ ) more aggregated in the treated group in comparison to the control (Fig. 3). In relation to microtubules, no significant differences were observed among groups ( $p > 0.05$ ) (Fig. 4).

### Nuclear abnormalities

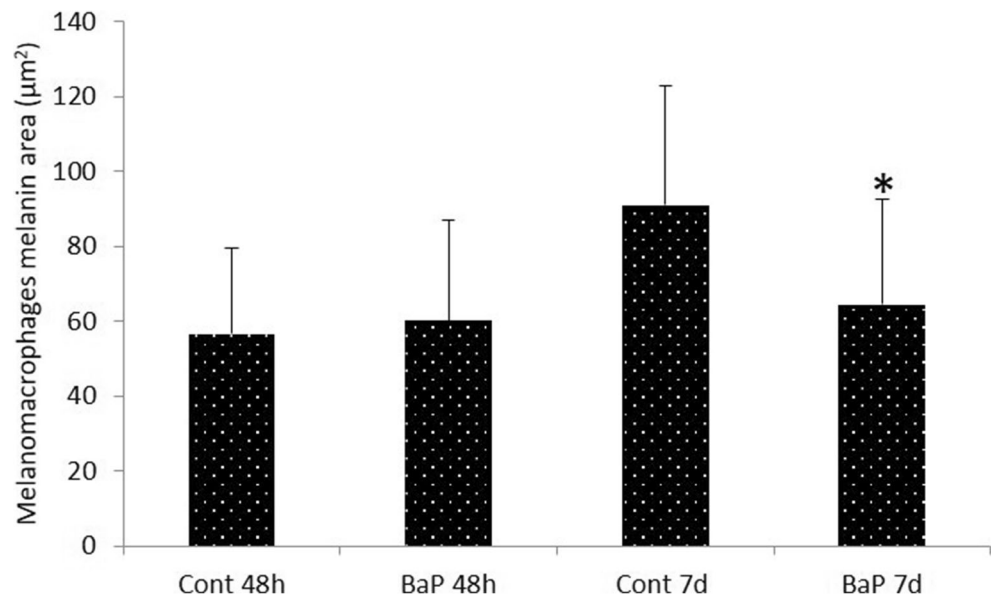
Regarding the group exposed for 48 h to BaP, no significant differences were found in relation to nuclear abnormalities. Differently, after 7 days of exposure, a 30% higher frequency of MN was observed ( $p < 0.05$ ) (Fig. 5).

## Discussion

Results from the present study show that EROD activity was increased in the BaP exposed animals, possibly due to an



**Fig. 2** Melanin area of melanomacrophages in rainbow trouts exposed to 2 mg/kg of benzo[a]pyrene for 48 h (48h; n = 6 individuals; 150 measurements) and 7 days (7d; n = 6 individuals; 150 measurements). The asterisk indicates significant differences between the control and the treated groups for the same time interval ( $P < 0.05$ ). Cont = peanut oil, BaP = benzo[a]pyrene

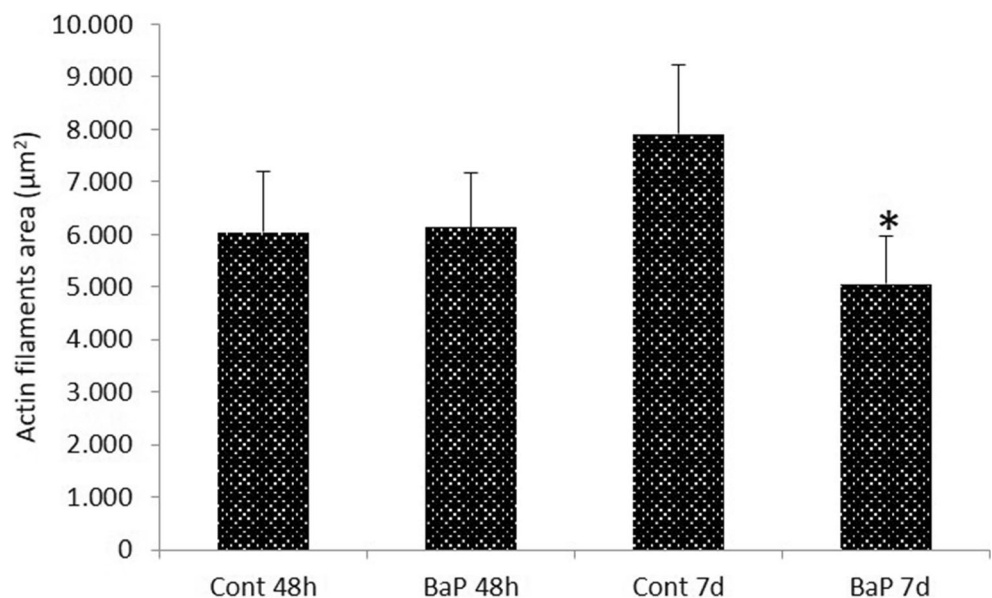


induction of CYP1A, results that are supported by other studies. When Medaka (*Oryzias latipes*) were exposed to 0.5 and 5 µg/L BaP for 48 h, it resulted in increased EROD activity (Pannetier et al. 2019) and studies with flounder (*Platichthys flesus*) and rainbow trout (*Oncorhynchus mykiss*) showed the same response after several days of exposure with 10 and 50 mg/kg BaP (Malmström et al. 2004). Eelpouts (*Zoarces viviparus*) exposed to three different doses of bunker oil containing around 25% PAHs (10, 100, and 1000 µg L<sup>-1</sup>) also exhibited increased EROD activity (Sturve et al. 2014). Curtis et al. (2011) showed increase hepatic microsomal EROD activity after 3 and 14 days in rainbow trout exposed to 3 µg BaP/g fish/day. Ethoxyresorufin-O-deethylase is characterized to be sensitive to induction by Ah receptor binding chemicals such as PAHs (Pacheco and Santos 1998). BaP

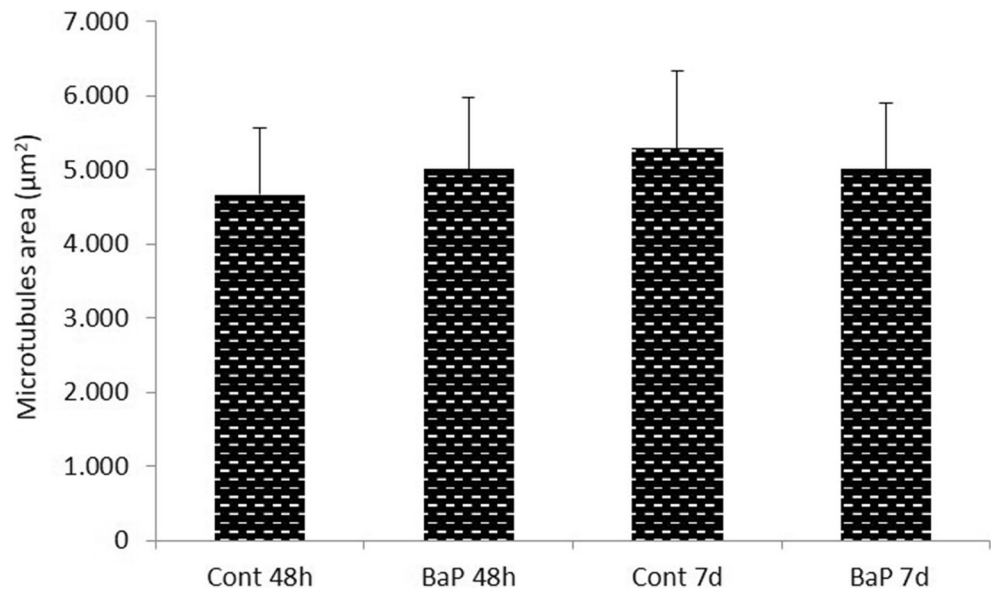
binds to the Ah receptor, which is translocated to the nucleus, where the transcription of CYP1A occurs, an enzyme responsible for EROD activity (Whyte et al. 2000).

The increase in EROD activity demonstrates the induction of CYP1A, which in turn is responsible for the metabolism of BaP (Whyte et al. 2000). In the metabolism of lipophilic xenobiotics such as BaP, the formation of water-soluble products of low toxicity is expected (Goksøyr and Förlin 1992; Bonacci et al. 2003). However, during the processes of hepatic biotransformation, which is mostly catalyzed by cytochrome P450 enzymes such as CYP1A (Caruso and Alaburda 2008; Wakx et al. 2016), higher concentrations of toxic byproducts such as 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetra (a) pyrene (BPDE) may be formed (Madureira et al. 2014).

**Fig. 3** Fluorescent cell area corresponding to actin filaments of rainbow trouts exposed to 2 mg/kg of benzo[a]pyrene for 48 h (48h; n = 6 individuals; 300 measurements) and 7 days (7d; n = 6 individuals; 300 measurements). The asterisk indicates significant differences between the control and the treated groups for the same time interval ( $P < 0.05$ ). Cont = peanut oil, BaP = benzo[a]pyrene



**Fig. 4** Fluorescent cell area corresponding to microtubules of rainbow trouts exposed to 2 mg/kg of benzo[a]pyrene for 48 h (48h; n = 6 individuals; 300 measurements) and 7 days (7d; n = 6 individuals; 300 measurements). No statistical differences were observed ( $P > 0.05$ ). Cont = peanut oil, BaP = benzo[a]pyrene



These compounds can lead to increased oxidative stress and genotoxic effects (McFarland et al. 1999; Freire et al. 2019).

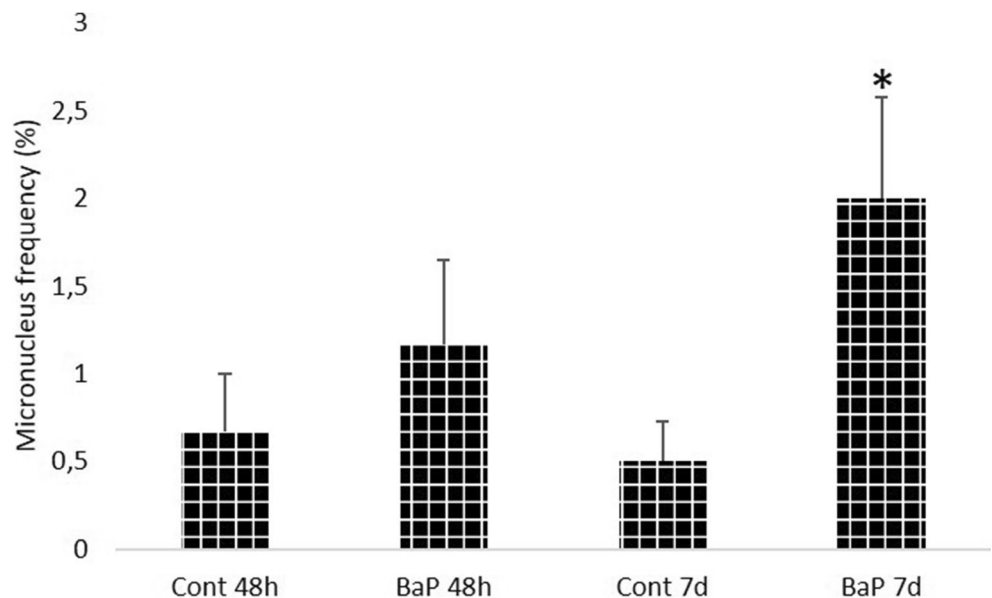
After 7 days of BaP exposure, the melanosomes of MMs were more aggregated, according to the smaller fluorescent cell area corresponding to actin filaments; additionally, the melanin area of MMs was found to be reduced. The basic principle for the intracellular transport of melanosomes involves microtubules and actin filaments, cytoskeletal components as a molecular engine on melanosomes, and the presence of the kinesin and dynein proteins, as a mode for transporting (Alberts et al. 2002). The cytoplasmic protein dynein is involved in the movement of melanosomes by microtubules towards the nuclei, while kinesin disperses the granules throughout

microtubules (Bagnara and Matsumoto 2006; Sköld et al. 2002, 2016).

In the anuran *Xenopus laevis*, the removal of actin filaments from melanophores inhibits the dispersion of melanosomes (Aspengren et al. 2006; McGuire et al. 1972), and those filaments are essential for pigment dispersion in these cells (Rogers et al. 1999). Actin filaments may be disrupted in dispersed melanophores, which leads to melanosomes aggregation in the cell center (Rogers et al. 1998, 1999; Aspengren et al. 2006). Drugs (e.g., cytochalasin B) are also capable of inducing pigment aggregation by causing actin disruption (Koyama and Takeuchi 1980; Aspengren et al. 2008).

Even though the rupture of actin filaments leads to the aggregation of pigments in anurans, the opposite happens in fish (Sköld et al. 2002; Aspengren et al. 2008). However, the

**Fig. 5** Micronucleus frequency in rainbow trouts exposed to 2 mg/kg of benzo[a]pyrene for 48 h (48h; n = 6 individuals; 6000 cells counted) and 7 days (7d; n = 6 individuals; 6000 cells counted). The asterisk indicates significant difference between the control and the treated groups for the same time interval ( $P < 0.05$ ). Cont = peanut oil, BaP = benzo[a]pyrene



motor protein kinesin, which is involved in the dispersion of melanosomes in fish (Rodionov et al. 1991), is regulated and activated by the cyclic adenosine monophosphate (cAMP) signal cascade (Sköld et al. 2002), a second messenger responsible for regulating pigment translocation in most melanophores (Tuma and Gelfand 1999). Low levels of cAMP lead to kinesin motor inactivation and cell aggregation (Rozdzial and Haimo 1986; Sammak et al. 1992; Sköld et al. 2002). Yeo et al. (2017) reported that BaP administration decreased cAMP levels in tumors with adjacent lung tissues, while in our study, BaP may have triggered a similar response in relation to low levels of cAMP, which prevented kinesin activation and maintained the melanosomes of MMs aggregated.

In relation to the reduction of the melanin area of MMs, Pronina et al. (2014) reported similar results in the common Roach (*Rutilus rutilus*) from a lake contaminated with cyanobacteria microcystin, while Payne and Fancey (1989) observed a decreased number of MMs in the liver of *Pseudopleuronectes americanus* kept in tanks with high levels of PAHs. The anuran *Hypsiboas albopunctatus* exposed to 7 mg/kg BaP for 3 days (Fanali et al. 2017) and *Physalaemus cuvieri* and *Leptodactylus fuscus* anurans exposed to 2 mg/kg BaP for 7 days (Fanali et al. 2018) showed the same reduction of melanin area. A possible explanation for these results was suggested by Joo et al. (2015), who observed that BaP inhibits the tyrosinase activity, an enzyme responsible for the regulation of melanogenesis, induced by the melanocyte stimulating hormone (a-MSH) (Park et al. 2009; Videira et al. 2013), and consequently decreases melanin synthesis (Joo et al. 2015). Another explanation is based on the results obtained in our study regarding the analysis of actin filaments, where it was possible to observe an aggregation of melanosomes in the center of the cell, which may have triggered a reduction of melanin area of the MMs.

BaP exposure also led to an increased frequency of MN after 7 days of exposure. MN analysis is a widely applied method to study genotoxicity since it is a convenient, sensitive, and easy applicable method, in particular for genotoxicological studies with aquatic organisms (Kaur et al. 2018). A recent study indicated increased frequency of MN in brown trouts (*Salmo trutta fario*) captured in a polluted river contaminated mostly by PAHs (Hariri et al. 2018). In vitro studies utilizing the rainbow trout liver cell line RTLW1 revealed that the exposure to the PAHs carbazole, acridine, and dibenzothiophene resulted in increased MN frequency, suggesting genotoxic impact (Brinkmann et al. 2014). In Nile tilapia (*Oreochromis niloticus*) exposed to nitrated PAHs, MN frequencies were significantly higher in the exposure groups after 7 and 14 days (Bacolod et al. 2017). Similarly, two anuran species (*Physalaemus cuvieri* and *Leptodactylus fuscus*) exposed to 2 mg/kg

BaP had increased frequency of MN after 7 days (Fanali et al. 2018). Taken together, the results presented in this study and previously published studies clearly show that BaP has genotoxic potential.

## Conclusion

The reduced melanin area of MMs is associated with the aggregation of melanosomes, evidenced by the decrease in the fluorescent cell area, corresponding to the actin filaments. This is the first study that evaluated cytoskeleton components in the attempt of explaining the reduced area of MMs after exposure to a contaminant, when the expected would be an increase in this cell's area, due to its detoxification function. Our hypothesis was partly corroborated, as we demonstrated that BaP has an influence on the cytoskeleton and alters the movement of melanosomes, not by disrupting the components of the cytoskeleton but possibly by an inactivation of the protein responsible for the pigment dispersion. Further studies are necessary to elucidate these mechanisms. Considering that BaP induced an increase in the frequency of MN, it was concluded that this contaminant is genotoxic to rainbow trouts at a level of 2 mg/kg.

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**Author contribution** LZF: conceptualization, investigation, methodology, data collection, writing original draft, and review. CO: conceptualization, resources, writing original draft, review, and editing. JS: methodology, resources, formal analysis, review, and editing. All authors read and approved the final manuscript.

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

## Declarations

**Ethical approval** All procedures described in this study were conducted according to Swedish ethics guidelines, in accordance with the Ethics permit 15986-2018.

**Consent to participate** Not applicable.

**Consent to publish** Not applicable.

**Competing interests** The authors declare no competing interests.

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