Physiological changes in reproductively active rainbowfish (*Melanotaenia fluviatilis*) following exposure to naphthalene

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ABSTRACT

Naphthalene makes up a substantial fraction of polycyclic aromatic hydrocarbons (PAHs) in crude oil and is an important by-product of industry; however, few studies have investigated the toxicity of naphthalene to aquatic organisms. We examined the toxicity of increasing concentrations (0, carrier control, 130, 200 and 400 µg/l) of naphthalene to adult rainbowfish (*Melanotaenia fluviatilis*) for 3 and 14 days to determine its potential to act as an endocrine disruptor. After exposure for 3 days, no changes in sex steroids were measured. After 14 days, a decrease of serum estradiol in females and an increase in serum testosterone in males was observed. These results suggest that naphthalene has the potential to act as an endocrine disruptor, although since no changes in plasma vitellogenin concentrations were observed in either sex, it is unlikely that naphthalene is acting as a xenoestrogen. There was a positive correlation between the incidences of deformities in larval offspring with increasing naphthalene concentrations, suggesting parental transfer of the toxicant. Egg production, hatchability and larval lengths remained unaltered, whilst few changes were measured in γ -glutamyltranspeptidase (GTP), an enzymatic indicator of spermatogenesis. Contrary to other PAHs, hepatic ethoxyresorufin-O-deethylase (EROD) activities declined with increasing exposure concentration, suggesting that naphthalene was either having a cytotoxic effect or disrupting enzyme synthesis.

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1. Introduction

The majority of studies investigating the toxicity of polycyclic aromatic hydrocarbons (PAHs) have tended to focus on benzo(a)-pyrene. Like benzo(a)pyrene, naphthalene is released into the environment from both point and non-point sources. Up to 1.5% of crude oil is made up of PAHs, of which 65% is made up of naphthalene and its derivatives. Naphthalene is abundant in coal and coal tar, and is produced from coal tar fractions by crystallisation and distillation (Gavin et al., 1996). Release of naphthalene occurs during wastewater disposal from chemical production processes, refineries and oil production, and due to leaching of treated wood products (Gavin et al., 1996). Non-point source releases of naphthalene are from traffic, gasoline and lubricants, and boat traffic. Consequently, naphthalene is commonly found in soils, sediments, surface water and groundwater (Kanga et al., 1997).

Naphthalene is one of the lighter PAHs, being only a di-aromatic hydrocarbon and having a low molecular weight PAH (molar mass = 128.171 g/mol). Consequently, naphthalene is

highly volatile. It is a non-polar compound, with an octanol–water partition coefficient of $\log K_{ow}$ 3.01–3.45, suggesting that it is moderately hydrophobic, having a tendency to adsorb to particulate matter, including soils and sediments, and may potentially accumulate in biota. In Australia, little information is available regarding ambient concentrations of naphthalene in the environment. Only very low concentrations of between 19 and 26 ng/l were found in the Brisbane River estuary (Kayal and Connell, 1989). Given the physical characteristics of naphthalene and low emissions of naphthalene from industry, we have found that it is unlikely to reach high concentrations in freshwater systems and cause acute toxicity (Pollino and Holdway, 2002b). However, there is little information describing the risk of fish populations in areas of chronic contamination and in the vicinity of an oil spill.

PAHs have been identified as potential endocrine disruptors (Cooper and Kavlock, 1997). Evidence of this has been found in a previous study where exposure to PAHs caused a decline in estradiol in the flounder (*Platichthys flesus*) (Monteiro et al., 2000), possibly due to a reduction in the conversion of testosterone to estradiol, as mediated by aromatase. Similarly, exposure of the Atlantic croaker (*Micropogonias undulatus*) to naphthalene alone (reported as 0.5 and 1.0 ppm) and water-soluble fraction of crude oil (reported as 2.5% and 5.0% water-soluble fraction) blocked sexual maturation and impaired ovarian recrudescence, with a

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decline in plasma level of gonadal hormones following naphthalene exposure (Thomas and Budiantara, 1995). An assay measuring *in vitro* germinal vesicle breakdown found that some of naphthalene's reproductive toxicity was due to either membrane damage or interference with hormone membrane receptors on target tissues. Orally administered naphthalene was found to accumulate in the ovaries of the spawning English sole (*Parophrys vetulus*) (Reichert and Varanasi, 1982). A more recent study by Tintos et al. (2007) also found a decline in plasma estradiol following injections of naphthalene to rainbow trout (*Oncorhynchus mykiss*). In addition, previous studies have found that PAH exposure of fish can alter the survival and viability of their offspring (Hose et al., 1981).

The mediating factors for determining the toxicity of PAHs to fish are the exposure level, the rate of metabolism of the parent compound and the form of the metabolite, as enzymatically transformed PAHs are often more toxic than the parent compound. Metabolism of PAHs occurs predominantly via the cytochrome P450 family of detoxification enzymes. Studies with benzo[a]pyrene have demonstrated the potential for induction of the cytochrome P4501A (CYP1A1) detoxification enzymes to be induced on exposure and for toxicologically active derivatives to be formed (Wu et al., 2003). The biotransformation of PAHs can increase CYP1A activity by 10–100 times (Livingstone, 1998). Although no mechanistic links between the cytochrome P450-mediated metabolism of PAHs and adverse effects on reproduction have been established, other studies have established a relationship between detoxification enzyme activity and adverse reproductive effects (Meucci and Arukwe, 2006; Arukwe et al., 2008).

In this study, we investigated the effect of naphthalene exposure on reproductive variables of the adult crimson-spotted rainbowfish (*Melanotaenia fluviatilis*; also referred to as the Murray rainbowfish) using a range of bioindicators. Biochemical reproductive parameters measured were estradiol, testosterone, plasma vitellogenin and γ -glutamyltranspeptidase (GTP) and organism reproductive indicators were egg production, egg hatchability and larval lengths. GTP enzymes have a role in spermatogenesis and are often used as indicator of dysfunction in mammals (Kumar et al., 2000). GTP enzymes have been successfully used as an indicator of testicular activity in fish, specifically the male eelpout (*Zoaces viviparous*) (Christiansen et al., 1998) and the crimson-spotted rainbowfish (*M. fluviatilis*) (Pollino and Holdway, 2003; Pollino et al., 2007). The ethoxyresorufin-O-deethylase (EROD), a catalytic measure of cytochrome P4501A induction, was measured to determine whether enzyme activity could be related to reproductive parameters. We have shown previously that crimson-spotted rainbowfish (Pollino et al., 2007) are a suitable indicator species for endocrine disruption.

2. Materials and methods

The crimson-spotted rainbowfish, *M. fluviatilis*, was used as the experimental animal in this study. We assure that our study was conducted in accordance with university, State and international (American Fisheries Society, 2004 American Fisheries Society, VIII. Storage or disposition of experimental animals: euthanasia. In: American Fisheries Society, Editor, *Guidelines for the Use of Fishes in Research*, American Fisheries Society, Bethesda, Maryland (2004)) guidelines for the protection of human subjects and animal welfare.

Nominal naphthalene exposure concentrations were 0, 0 (CC), 320, 560 and 1000 $\mu\text{g/L}$. These concentrations are likely to be higher than found in the environment; however, generally little monitoring information is available regarding ambient naphthalene concentrations, including after an oil spill. Naphthalene was introduced to solutions using ethanol as a carrier. A volume of 100 $\mu\text{L/L}$ was used for carrier controls (CC) and exposure concentrations, meeting standard guideline requirements (ASTM, 2006). Exposures were conducted as 50% static replacement tests and were conducted in quadruplicate. Samples for

analysing test solution concentrations were collected prior to, and following solution replacement.

Naphthalene concentrations were measured in water samples using a UV spectrophotometer (Cary 50, Varian), and water samples were analysed within 1 h of collection. Test solutions (3 mL) were placed in a quartz cuvette and measured at 220 nm (Stene and Lonning, 1985) and solution concentrations were calculated from a standard curve prepared daily. Mean (SE) test concentrations used were 0 (0), 0 (0) (CC), 130 (39.2), 200 (49.9) and 400 (43.1) $\mu\text{g/L}$ ($n = 28$). Mean ($\pm$ SE) pH, dissolved oxygen, conductivity and temperature measurements were 7.0 (0.2), 6.1 mg/L (0.7), 109 μS (11) and 24.8 $^{\circ}\text{C}$ (0.3), respectively.

Rainbowfish stock was originally collected from the Murray River in Victoria (Australia). The laboratory stock was the fifth generation removed from the wild and was maintained at the RMIT-University wet laboratory. Adult rainbowfish were fed twice daily on three types of feed: commercial flake, newly hatched live brine shrimp and frozen adult brine shrimp. The water and room temperature were maintained at 25 $^{\circ}\text{C}$. Rainbowfish are sexually dimorphic. Groups ($n = 20$) of 2 male and 3 female sexually mature adult fish were randomly assigned to one of twenty 28 L glass aquaria. This ratio of sexes has been used as a standard in previous studies using this species. The weight of female fish ranged between 3 and 5 g, whilst males ranged between 6 and 8 g. Lengths of females ranged from 3.3 to 5.3 cm and males ranged from 4.2 to 6.0 cm. Replicates A and B were sampled after 3 days of exposure, while replicates C and D were sampled after 14 days of exposure. The aquaria were aerated.

Total egg numbers were counted daily throughout the exposure period. Artificial plastic spawning grass substrates were placed in each tank for collection of eggs. Eggs were collected by hand, counted and dipped in 4 mg/L malachite green for 5 min to prevent fungal growth (Pollino and Holdway, 2003b). Eggs were distributed to Petri dishes and randomly placed in an incubator maintained at 25 $^{\circ}\text{C}$. Dead eggs were removed up until hatch and the developmental stages and state of embryos were recorded. Post-hatch, the percentage hatch was calculated and larval lengths were measured using a Wild binocular microscope.

Adults were sampled for blood and tissues at days 3 and 14 for biochemical analyses. Fish were anaesthetized using Tricaine Methane Sulphonate ([MS-222], Sigma-Aldrich, Castle Hill, NSW, Australia). Blood was collected by caudal severance using heparinised capillary tubes. Fish were then euthanized by spinal severance. Blood was centrifuged at 2500 g and the plasma was collected and stored at -80°C . Serum was collected for measurement of vitellogenin and steroids. Testes were collected for γ -GTP analyses and livers for EROD analyses. Tissues were frozen in liquid nitrogen and stored at -80°C .

Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia).

2.1. Vitellogenin methodology

Vitellogenin was measured using the alkali-labile protein phosphorous method (phosphoprotein), which has been shown to be a reliable indicator of vitellogenin (Kramer et al., 1998). The assay was optimised for rainbowfish (Pollino and Holdway, 2003a). The plasma volume used for the assay was 10 μL . To isolate vitellogenin, 2.5 mL of 10% trichloroacetic acid was added to 10 μL of plasma overnight and the precipitate was collected centrifugally. The precipitate was incubated in 5% trichloroacetic acid for 30 min at 90 $^{\circ}\text{C}$ and washed successively with hot absolute alcohol, chloroform-ether-alcohol (1:2:2), acetone and ether. The precipitate was dissolved and incubated at 100 $^{\circ}\text{C}$ in 0.5 mL 2 N NaOH and neutralised with an equivalent amount of concentrated HCl. Ortho-phosphate concentration was determined by the colourimetric assay of the acidified phosphomolybdate complex using a commercial kit (Sigma Cat. No. 670-A, Australia). Using a spectrophotometer, absorbance was measured at 660 nm (Varian, Mountain View, CA, USA) within 10 min of colour development.

2.2. Sex steroids methodology

Sex steroids were measured via radioimmunoassay (Fitzpatrick et al., 1986). Radiolabels that were purchased from Amersham (Buckinghamshire, UK) and antibodies were supplied by Colorado State University (Fort Collins, CO, USA).

Estradiol was measured in females and testosterone was measured in males. Each plasma sample (25 μL) was extracted twice with diethyl ether (2 mL). Extracts were combined and reconstituted. Antibody (100 μL) was added to all tubes except total count and non-specific binding tubes. Each tube received the appropriate antibody dilution as optimised previously (Pollino and Holdway, 2002a). Tubes were incubated overnight at 4 $^{\circ}\text{C}$ with tritiated steroid (100 μL , 10,000 cpm) in buffer (1,2,6,7,16,17- ^3H estradiol, 1,2,6,7- ^3H testosterone). Following incubation, cold well-mixed dextran-coated charcoal suspension (0.5 mL for estradiol and 1 mL for testosterone) was added to all tubes, except for the total count tubes. Tubes were incubated in an ice bath for 10 min, and then centrifuged. The sample liquid (500 μL) was decanted into scintillation vials with 5.2 mL of scintillation cocktail and counted in a scintillation spectrophotometer (Packard Instrument Company Inc., Meriden, CT, USA).

2.3. GTP methodology

The methodology for GTP analysis was adapted for rainbowfish as detailed in Pollino and Holdway (2002a). To isolate the enzyme, a sample of between 25 and 35 mg wet weight of testis was weighed, homogenised (Heidolph D1AX 900, Germany) in phosphate buffer and centrifuged. The supernatant was collected and assayed for GTP using MPR2 kit (Boehringer Mannheim, Germany). A change in absorbance was measured at 410 nm over 5 min using a UV spectrophotometer (Varian, Mountain View, CA, USA).

2.4. EROD methodology

Hepatic EROD was measured using the procedure of Burke and Mayer (1974), as modified for rainbowfish by Pollino and Holdway (2003b). Briefly, the post-mitochondrial supernatant was prepared in 0.1 M phosphate buffer, which contained 1 mM dithiothreitol, 1 mM EDTA, 0.1 M potassium chloride and 0.1 M phenanthroline. The homogenate was centrifuged at 10,000g for 20 min in the Beckman TLX benchtop ultracentrifuge. The supernatant was collected and stored at -80°C for no longer than 7 days. Test and blank tubes were prepared containing 500 μL of 0.1 M Tris buffer, 250 μL of NADPH regenerating system, 50 μL of supernatant and 100 μL of 12 mg/mL albumin. A volume of 0.1 mL, 0.01 mM

ethoxyresorufin was added to start the reaction and maintained at 30°C for 10 min. The reaction was stopped with methanol and fluorescence was measured with a spectrofluorimeter (Hitachi, F-4500) at excitation wavelength 530 nm and emission wavelength 585 nm.

2.5. Determination of protein concentration

Protein concentrations were determined according to Lowry et al. (1951).

2.6. Statistical analyses

All statistics were performed using the statistical software Statistica (Statsoft, USA). Data were assessed for normality and homogeneity of variance. Non-normal data were transformed as required. Analysis of variance (ANOVA) and Dunnett's test was used to determine significance ($p \leq 0.05$) between samples (Zar, 1999).

3. Results

3.1. Egg production and larval fitness

Although cumulative egg production was reduced at the highest test concentration, these changes were not statistically significant (Fig. 1). Despite hatchability being unchanged, larval deformities were observed in the offspring (Table 1). The percentage of deformed larvae increased over the 14-day exposure period. The major deformities observed were pericardial edema, although some larvae (approximately 10%) also had spinal deformities (Fig. 2). There were no differences in the larval lengths recorded throughout the exposure period (results not shown).

3.2. Sex steroids, vitellogenin and γ -GTP

Both estradiol in females and testosterone in males were altered as a result of the exposure to naphthalene (Table 2). Although there were no changes in plasma estradiol at day 3,

Fig. 1. The number of eggs produced prior to and each day of the 14-day exposure period.

Table 1

Reproductive and biochemical parameters of adult rainbowfish following 3 and 14 days of exposure to various concentrations of naphthalene.

	Naphthalene concentration ($\mu\text{g/L}$)				
	0	CC	130	200	400
Day 3					
Estradiol (f)	483 (52.1) ^a	319 (32.4) ^{ac}	489 (85.0) ^a	300 (84.9) ^{ac}	404 (134) ^a
Testosterone (m)	287 (46.8) ^a	200 (23.16) ^{ab}	135 (25.0) ^b	231 (39.0) ^{ab}	201 (50.5) ^{ab}
Phosphoprotein (f)	10.2 (2.07) ^a	6.58 (1.23) ^{abc}	7.23 (1.78) ^{abc}	5.30 (0.42) ^{bcd}	7.23 (1.54) ^{abc}
Phosphoprotein (m)	4.85 (0.41) ^{bcd}	4.43 (1.13) ^{bcd}	4.76 (1.18) ^{bcd}	8.53 (1.97) ^{ad}	5.97 (0.76) ^{ac}
GTP (m)	2770 (1120) ^a	2610 (1630) ^a	3060 (1620) ^a	5490 (2300) ^b	5870 (1930) ^b
Day 14					
Estradiol (f)	423 (73.9) ^a	334 (61.4) ^{ad}	197 (39.1) ^{bc}	189 (24.9) ^{bc}	203 (38.0) ^{bc}
Testosterone (m)	221 (7.49) ^{ab}	283 (41.8) ^a	239 (46.2) ^{ab}	149 (79.8) ^{ab}	449 (21.4) ^c
Phosphoprotein (f)	9.12 (0.72) ^{abc}	11.5 (1.02) ^a	5.78 (1.54) ^{ce}	7.58 (1.11) ^{abc}	8.83 (0.64) ^a
Phosphoprotein (m)	3.31 (0.63) ^{bc}	5.24 (0.69) ^{bcd}	3.46 (0.64) ^{bef}	7.92 (2.79) ^{abc}	3.94 (0.15) ^{cf}
γ -GTP (m)	3090 (1610) ^a	3310 (1300) ^{ab}	4420 (2280) ^{ab}	3930 (2590) ^{ab}	3910 (2130) ^{ab}
Day 14 (continued)					
EROD (f)	66.2 (5.11) ^a	91.4 (6.18) ^a	38.7 (8.12) ^a	25.7 (4.61) ^b	6.24 (1.55) ^b
EROD (m)	321 (66.4) ^a	224 (25.9) ^a	110 (41.8) ^b	94.1 (23.7) ^b	38.6 (9.62) ^b

Mean ($\pm$ S.E.M.) reproductive and biochemical parameters of adult rainbowfish following 3 and 14 days of exposure to increasing concentrations of naphthalene. Values within rows sharing the same superscript are not significantly different ($p > 0.05$). Units: Testosterone and estradiol (pg/mL), phosphoprotein ($\mu\text{g}/\text{mg}$ protein), GTP (units/ mg protein), EROD (pmol/min/ mg protein), f = female, m = male.

Fig. 2. One-day-old rainbowfish showing (A) a normal larvae and (B) larvae with a heart edema and spinal deformity.

Table 2

Percentage of hatched larvae, showing the contribution of larvae with deformities in brackets.

	Naphthalene concentration ($\mu\text{g/L}$)				
	0	CC	130	200	400
1	54 (0)	15 (0)	36 (0)	44 (0)	69 (0)
2	38 (0)	24 (2)	59 (7)	39 (0)	50 (3)
3	43 (0)	34 (0)	41 (2)	62 (24)	40 (7)
4	24 (0)	20 (0)	30 (5)	70 (35)	43 (4)
5	94 (0)	83 (0)	49 (3)	83 (35)	74 (20)
6	80 (0)	82 (0)	72 (9)	36 (4)	67 (23)
7	85 (0)	75 (0)	74 (7)	44 (0)	30 (10)
8	88 (0)	59 (0)	71 (7)	43 (17)	67 (29)
9	91 (0)	77 (0)	83 (4)	60 (5)	28 (18)
10	99 (0)	51 (0)	53 (17)	83 (27)	75 (31)
11	55 (0)	69 (0)	50 (7)	40 (10)	43 (14)
12	57 (0)	76 (0)	54 (18)	37 (12)	44 (31)
13	20 (0)	24 (0)	44 (10)	71 (24)	59 (56)
14	34 (0)	21 (0)	65 (11)	81 (20)	100 (51)

Mean percentage hatchability of eggs produced over the exposure period. The figures in brackets represent the mean percentage of abnormal larvae of the percentage hatched eggs.

concentrations were decreased following 14 days of exposure to all test solutions. Similarly, no changes in testosterone concentrations were measured after 3 days of exposure. By contrast, after 14 days of exposure plasma testosterone was increased at 400 $\mu\text{g/L}$. There was little variation in control estradiol and testosterone concentrations over the exposure period.

No significant differences in plasma vitellogenin concentrations for male or female fish were observed at either of the sample periods. Female concentrations were generally higher than males (Table 2).

γ -GTP concentrations in male fish were significantly higher at 200 and 400 $\mu\text{g/L}$ after 3 days of exposure to naphthalene (Table 2). By contrast, there were no significant differences between exposure groups at day 14.

3.3. EROD

After 3 days of naphthalene exposure, no significant changes in EROD activities were observed in female or male fish (Table 2). However, following 14 days naphthalene exposure females had lower EROD activities at the two highest exposure concentrations, 200 and 400 $\mu\text{g/L}$, and males had lower activities at all test concentrations.

4. Discussion

If gametes and early life stage fish are exposed to toxicants, general developmental mechanisms may be disrupted. This can lead to abnormal cell or tissue differentiation, excessive or inadequate cell death during development, inadequate cell movement, improper cell communication and/or disrupted metabolism (Weis and Weis, 1989). In our study, an increase in larval spinal flexures and pericardial edema occurred. Pericardial edema may result from ionic disturbances and a toxic response from metabolic activation of PAHs in the embryo (Carls et al., 1999). Exposures of 0.85 mg/L naphthalene to fathead minnows (*Pimephales promelas*) have been shown to cause a reduction in hatchability and depressed growth of fry (DeGraeve et al., 1982). Although naphthalene was found to be non-lethal to inland silverside (*Menidia beryllina*) embryos, teratogenic effects were observed (Middaugh et al., 1988).

Maternal transfer of toxicants has been shown to cause malformations and edemas post-hatch as well as to increase the sensitivity of offspring to stress (Billsson et al., 1998). Although early life stage of rainbowfish were exposed to waterborne naphthalene for up to 24 h prior to collection, parental transfer of toxicants was likely to be causing part of the observed toxicity to larvae, as the incidence of larval deformities increased over the 2-week exposure period. Naphthalene has been shown to accumulate in the ovaries of English sole (*P. vetulus*) (Reichert and Varanasi, 1982). As ovarian tissues can bioaccumulate naphthalene, and yolk reserves are highly lipophilic, the potential for the maternal transfer exists.

In mature fish, PAH exposure has been shown to alter in steroid levels. Tintos et al. (2007) observed in rainbow trout where plasma levels of both cortisol and estradiol were reduced following injections and implants of 10 and 50 mg/kg naphthalene. Exposure of Atlantic croaker (*M. undulatus*) to benzo(a)pyrene also caused a decline in plasma estradiol (Thomas, 1990). Declines in vitellogenin levels, impaired steroidogenesis and decreased ovarian growth were also observed (Thomas, 1990). Female sole (*P. vetulus*) captured at sites contaminated with naphthalene also had depressed plasma levels of estradiol, which coincided with induced aryl hydrocarbon hydroxylase activities, as a measure of cytochrome P4501A activity (Johnson et al., 1988). Likewise, in rainbowfish naphthalene caused a decline in estradiol; however, EROD activities were reduced and vitellogenin was unchanged in both sexes.

Given the role of the liver in both sexual maturation and xenobiotic metabolism (Arukwe et al., 2000), changes in reproductive parameters are likely to be associated with changes with hepatic detoxification processes, including the cytochrome P450 system. Many studies have shown that alterations in P450 enzyme activities coincide with changes to the reproductive success of fish, having both anti-estrogenic and estrogenic outcomes (Thomas, 1990; Meucci and Arukwe, 2006; Arukwe et al., 2008). As male rainbowfish did not have any changes in vitellogenin production, naphthalene is unlikely to be acting as a xenoestrogen. Given the measured hormonal changes, being reductions in estradiol and increases testosterone, naphthalene is more likely to

be acting as an anti-estrogenic compound. Although EROD activities were unchanged in rainbowfish after 3 days of exposure to naphthalene, following 14 days of exposure EROD activity was inhibited at all exposure concentrations. Inhibition of EROD activity could have been the result of cytotoxic effects, where the synthesis of new enzymes is disrupted (Steadman et al., 1991).

The anti-estrogenic action of P450-inducing compounds could also disrupt vitellogenin synthesis and egg production (Anderson et al., 1996). With a decline in estradiol, decreased liver synthesis of vitellogenin during the pre-vitellogenic and vitellogenic reproductive period in female fish would result in reduced plasma vitellogenin and subsequently regressed ovaries and reduced egg production. Following the exposure of rainbowfish to naphthalene in this study, no changes in egg production, vitellogenin or structural organisation of the ovaries were found. Further studies are required to investigate the effects of a longer exposure duration.

Unlike the decline in estradiol observed in female fish, an increase in plasma testosterone in rainbowfish males was measured at 400 µg/L. Alterations in the concentrations of plasma sex steroids may be related to disturbances in the cytochrome P450 enzyme, aromatase, which catalyses the conversion of androgens to estrogen. Males convert testosterone to estradiol in the testis. As discussed, cytochrome P450 enzymes can be disrupted by naphthalene, disturbing steroid homeostasis. Inhibition of aromatase could explain the lack of conversion of testosterone to estradiol, with reduced estradiol and increased testosterone being observed in female and male rainbowfish, respectively. Monteiro et al. (2000) found that the aromatase enzyme is inhibited by PAHs resulting in a decrease in estradiol synthesis. Further study is required to definitively decide whether naphthalene is inhibiting aromatase. GTP activities suggest that spermatogenesis was not disrupted by short-term exposures to naphthalene.

Whether the link between cytochrome P450 enzyme activities and reproductive parameters (in particular estradiol) is causal or correlative requires further investigation. The results of our study show that naphthalene is likely to be acting as an anti-estrogenic compound to rainbowfish, although further studies are required to definitively identify the mechanism and to determine whether the fitness of offspring from exposed parents is likely to decrease.

5. Conclusion

Naphthalene compounds are major components of PAHs in crude oil. Naphthalene is also found in produced water, the by-product of oil and gas production. The measured concentration of naphthalene in produced water was 133 µg/L (Middaugh et al., 1988), which is within the concentration range in this study causing toxic effects. The environmental hazard report by Gavin et al. (1996) concluded that under normal situations naphthalene was unlikely to cause adverse environmental effects. However, measured levels in heavily contaminated sites may have adverse effects on the aquatic environment. Although the naphthalene concentrations examined in this study are likely to be found only at chronically polluted sites or those following an oil spill, the results confirm that naphthalene can have a negative impacts on fish reproduction at heavily contaminated sites.

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