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EXPOSURE OF FEMALE JUVENILE RAINBOW TROUT TO ALKYLPHENOLIC COMPOUNDS RESULTS IN MODIFICATIONS TO GROWTH AND OVOSOMATIC INDEX.

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Abstract-- The alkylphenol ethoxylates (APEO's) are a major group of non-ionic surfactants. Biodegradation of these compounds is incomplete during sewage treatment, thus they are ubiquitous aquatic pollutants. All the main degradation products of APEOs have recently been demonstrated to have estrogenic properties *in vitro*, but their effects *in vivo* remain to be established. In this study, female juvenile rainbow trout (*Oncorhynchus mykiss* Walbaum) were exposed to octylphenol (OP), nonylphenol (NP), nonylphenol diethoxylate (NP2EO) and nonylphenol mono-carboxylic acid (NP1EC) at environmentally relevant concentrations (Experiment 1: 1, 10 and 50 μg/L; Experiment 2: 1, 10 and 30 μg/L). Exposure to APEO's commenced at hatch (day 0) and was terminated on day 22 (Expt. 1) or day 35 (Expt. 2). Body weight and fork length of representative samples of fish from each treatment group were recorded at intervals up to 108 days (Expt. 1) or 466 days (Expt. 2). In Experiment 1, significant differences in size of the exposed fish, related to treatment, were still apparent on day 108, 86 days after withdrawal of the treatments. These observations were confirmed during Experiment 2, in which significant changes in body weight and fork length as a consequence of exposure to the compounds were observed approximately 15 days after exposure was terminated. These differences were sustained for at least 466 days in the case of NP and NP1EC. In addition, the ovosomatic index (OSI) of fish exposed to NP and NP1EC was significantly affected by the treatment. Survival of fish in the natural environment is strongly influenced by body size, and an appropriate OSI is a crucial factor in successful reproduction. Therefore, exposure of natural populations of fish to these chemicals at concentrations currently measurable in the aquatic environment may have an impact on the performance of those populations.

Keywords—Alkylphenols, Growth, Gonadosomatic index, Rainbow Trout, Endocrine disrupters.

INTRODUCTION

During the last decade, an increasing number of anthropogenic pollutants capable of disrupting the endocrine system of vertebrates has been identified in the aquatic environment [1]. The major estrogen in vertebrates is 17β-estradiol, which in female fish regulates the development and maintenance of the ovaries and somatic sexual characteristics and has a crucial role in vitellogenesis [2]. Estrogenic contaminants found in the aquatic environment include ethinylestradiol, phytoestrogens, organochlorine compounds such as dichloro,diphenyl-trichloroethane (DDT) and polychlorinated biphenyls (PCBs), and some alkylphenolic compounds [3]. Recently, the degradation products of one group of non-ionic surfactants, the alkylphenol polyethoxylates (APEOs), have been demonstrated to possess estrogenic activity [4].

The APEO's and their breakdown products, of which the alkylphenols (AP's) are a major group, are ubiquitous aquatic pollutants. The APEO's were first introduced during the 1940's and are now the second largest group of non-ionic surfactants in commercial use. Nonylphenol ethoxylates comprise 80% of the market, with 14500-18500 tonnes being used in the U.K. during 1992 [5]. The remaining 20% consists primarily of octylphenol ethoxylates. Up to 60% of APEO's find their way into sewage treatment works after use. Levels of APEO's in sewage effluent in Switzerland have been reported to be between 36 and 202 μg/l [6]. Once in the environment, the fate of these compounds is determined by microbial transformation and physicochemical processes such as autoxidation [7]. The overall biodegradation of APEOs is

limited, due to the formation of relatively stable metabolites; these being nonylphenol, octylphenol and their mono- and di-ethoxylates and mono-carboxylic acids in particular. Due to the incomplete biodegradation of these compounds, breakdown products of APEOs can be detected not only in sewage effluent but also in river systems and estuaries. For example, levels as high as 330 μg/l NP have been reported to be present in wastewater entering the River Aire in England and levels of up to 180 μg/l NP have been detected in the river itself at sites receiving high inputs of surfactants from textile plants [8]. Levels of NP in the outer Tees estuary have been reported to be as high as 5.2 μg/l where the mouth of the estuary is heavily industralised [8]. Much lower levels of NP, between 0.2 and 12 μg/l, have been reported for six other U.K. rivers [8] , and also for many rivers in the U.S.A., and this concentration range seems to be more typical [7].

Recent studies have strongly indicated that alkylphenolic compounds possess estrogenic activity. For example, Soto et al. [9] reported that nonylphenol released from plastic centrifuge tubes during cell culture procedures induced proliferation of human breast tumour cells, an estrogen-dependent phenomenon. Studies on fish and mammals have confirmed the estrogenic nature of these chemicals. Cultured fish hepatocytes synthesize vitellogenin in the presence of a number of different alkylphenolic chemicals [4], and exposure of sexually mature male fish to APEO's caused a dose-dependent elevation of plasma vitellogenin levels, accompanied by a reduction in testicular weight [10]. Rats exposed during gestation and neonatally to octylphenol (OP) and an octylphenol ethoxylate (OP5EO) for a period of 3-8 weeks showed a significant reduction in mean testicular weight [11]. The recognition that many ubiquitious environmental contaminants may act as 'endocrine disrupters' has coincided with concerns that the prevalence of developmental disorders of the reproductive tract may be increasing and sperm counts may

be declining in human males [12]. Among the possible causes suggested to be responsible for the increased incidence of these disorders over the last fifty years are changes in diet, the presence of phytoestrogens in the diet, and the proliferation of estrogenic chemicals in the environment [12].

The aim of the present study was to determine the physiological effects on juvenile female rainbow trout of exposure to environmentally-relevant concentrations of a variety of alkylphenolic chemicals. These compounds have been shown to influence the reproductive system in male rainbow trout, causing vitellogenin synthesis and inhibition of testicular growth [10]. However, the effects of these compounds on other aspects of the physiology and behaviour of fish have yet to be addressed. This report concerns the long-term effects on growth and gonado(ovo)somatic index arising from the exposure of female rainbow trout to these compounds at an early stage of development.

MATERIALS AND METHODS

Experimental design

The impact of four estrogenic alkylphenolic compounds on the growth of female rainbow trout from hatch to early sexual maturation was assessed by exposing groups of 200 fish, in duplicate, to three concentrations of each alkylphenol. Solvent control groups were included. Exposure to the test compounds was limited to the first month following hatching. Weight and length of the fish were measured at regular intervals during development and relative ovary size was measured at termination of the study.

Experimental procedure

Two experiments were conducted. In the first study, exposure to the test compounds was terminated after 22 days and the fish were monitored for a further 86 days. In the second experiment, the fish were exposed to the test compounds for 35 days and were monitored for a further 431 days.

No data are available concerning the effects of these compounds on juvenile trout. Therefore, to eliminate any variation arising from sex of the fish an all-female population was used. All-female rainbow trout (*Oncorhynchus mykiss*) ova (that is, ova fertilized by "all X" sperm from sex-reversed females, such that all eggs were XX, and hence female) were obtained from Glen Wyllin trout farm, Isle of Man. Ova were reared in egg incubator trays until hatching and were then transferred immediately to indoor test tanks. Two hundred eggs were stocked per tank, and no compensation was made for subsequent mortalities. Each tank was of 80 l capacity and supplied with a constant flow (40 ml/min) of Windermere lake water, sand filtered to remove detritus and algae, at ambient temperature (range 7-13^{o}C during the experiment). Water hardness was the equivalent of 12.5 mg/l as calcium carbonate and pH was 6.5. Tanks were continually aerated using a pump and diffusers. Tanks were kept in darkness until the alevins reabsorbed their yolk sac, and began to swim-up as fry, at which point a 12 hour light : 12 hour dark photoperiod was implemented.

Four compounds were used: 4-tertiary-nonylphenol (NP), 4-tertiary-octylphenol (OP), nonylphenol diethoxylate (NP2EO) (all obtained from Aldrich) and nonylphenol mono-carboxylic acid (NP1EC) (ICN Flow). All four chemicals were mixtures of different isomers and oligomers. The fish were exposed to environmentally relevant nominal concentrations of 1, 10 and 30 μg/l (50 μg/l in Experiment 1) of the test compounds. The actual concentrations achieved in the tanks during the study may have differed from the nominal concentration due to

adsorption of the compounds onto pipework or tank surfaces. The two lower concentrations employed represent levels typical of a 'clean' U.K. river while the higher concentration is more typical of an industrialised river site. Stock solutions of the test chemical were dissolved in methanol because of the hydrophobic nature of the compounds (0.4, 4, and 12 mg/ml in 0.1 %, 0.1 % and 0.5 % methanol, respectively; GPR 99.5 %, BDH). These stock solutions were delivered to the test tanks at 0.1 ml/min which, after dilution with the incoming water supply at 40 ml/min, resulted in the desired concentrations within the test tank (Fig. 1). Control tanks received 0.1 % and 0.5 % methanol. Toxicity studies on the fathead minnow (*Pimephales promelas*) indicate a 96 hour LC50 of 29.4 g/l for methanol (2.94 %; [13]). The concentrations of methanol used in this study (maximum 0.0005% in the test tanks) are well below this limit and unlikely to be toxic. During the experiment, mortality of experimental fish was recorded daily, and flow rates were checked weekly to ensure that the desired concentrations were maintained in the treatment tanks.

Exposure of the fish to the test compounds was initiated at hatch and continued for 22 days in Experiment 1 and for 35 days in Experiment 2. Swim-up and the onset of exogenous feeding occurred on day 23. From this time onward, fry were fed twice daily with a commercial feed (BP Mainstream). The amount of food offered was calculated as recommended by the manufacturer taking into consideration the size of the fish, the number of fish in the tank, and the water temperature. The initial feed was a starter diet (granule size 0.3 - 0.6 mm), the size of feed was increased as fish grew, following the manufacturers recommendations. Food was spread over the surface of the tanks and fish were fed to satiation. Excess food was allowed to settle to the base of the tanks and was removed daily. The amount of food offered was recalculated on a monthly basis throughout the experiment. On day 22 in Expt. 1 and day 35 in Expt. 2, all treatments were terminated and the fish were maintained in lake water only.

Sampling

In Experiment 1, fish were sampled once, on day 108. During Experiment 2, fish were sampled 24, 55, 84, 108, 144, 220, 300, and 466 days after the start of exposure. Five fish were removed from each test tank (10 fish per treatment) using a dip net and placed in anaesthetic (2-phenoxyethanol; 1:2000). Surface water was removed from each fish by blotting and they were then weighed to the nearest 0.1 mg and measured (fork length) to the nearest mm before being killed by spinal section. The condition factor of each fish was calculated [(100 x weight)/length^3]. At the terminal sample (466 days), the ovaries were dissected from the fish, weighed, and the ovosomatic index (OSI) was calculated [100 x gonad weight/(body weight-gonad weight)].

Statistics

The data from each experiment was analysed as a two strata analysis (ANOVA) using the program GENSTAT. The design was a two way factorial with the main effects of treatment, time and their interaction. The two error strata were at the whole tank level and the fish within whole tanks. The differences between treatments were applied to whole tanks. The differences between times, times by treatment interaction, and the natural variability between tanks, was assessed by comparison with the the residual variability between fish within tanks. All weight data were transformed (data$^{0.333}$) prior to analysis to improve the homogeniety of variance. Length data were analysed without transformation. Means of the transformed weight data, and untransformed length data, have been plotted with 95% confidence limits.

RESULTS

All compounds to which the fish were exposed produced a significant modification in the growth of the fish and for ease of interpretation the results for each compound will be considered separately. There was no significant difference in total mortality between the controls and the treated fish (mean mortality rate for duration of experiment 2 = 14.89 %, SEM = 1.25).

The body weight data for fish from the final sample (day 108) of Experiment 1 are presented in Fig. 2. Fork length and body weight data from all time points from Experiment 2 are presented in Figs. 3 - 7. OSI for fish on the final sample (day 466) are displayed in Fig. 8.

Octylphenol

Experiment 1: All fish exposed to OP (1, 10 and 50 μg/l) displayed a significant reduction in body weight, relative to the controls, at the terminal sample (1 and 10 μg/l: $P<0.001$; 50 μg/l: $P<0.01$; Fig. 2b).

Experiment 2: A significant modification in growth was not observed until day 84 (Fig. 3). The most marked effect was on the weight of the fish, with the two highest concentrations (10 and 30 μg/l) causing a significant reduction in weight ($P<0.05$) relative to the control fish. Fish exposed to 1 μg/l OP displayed body weights significantly higher than the controls at this time ($P<0.05$). Fish exposed to 10 μg/l OP continued to show significantly lower weight and length than control fish at 144 days. At day 466 (Fig. 7b) the body weight of fish exposed to the two highest concentrations of OP was no longer significantly different from control values; however, the body weight of fish exposed to the lowest concentration of OP was still

significantly lower than that of the controls ($P<0.01$). Length was significantly reduced in fish exposed to 10 μg/l OP at day 144 ($P<0.01$) but at no other time. At no point during the study was the condition factor or OSI (Fig. 8b) in any group of fish exposed to OP significantly different from that of the controls.

Nonylphenol

Experiment 1: At the terminal sample, fish exposed to 1 and 50 μg/l NP displayed a significantly lower body weight relative to the controls ($P<0.001$ and $P< 0.01$, respectively), whereas the weight of fish exposed to 10 μg/l NP was not significantly different from that of the control fish (Fig. 2a).

Experiment 2: On day 55, the weight and length of fish exposed to 30 μg/l NP was significantly lower than that of control fish ($P<0.05$ and $P<0.01$, respectively; Fig. 4). Although there was no effect on weight, the mean length of fish exposed to 10 μg/l NP at this point was significantly lower than that of the controls ($P<0.05$). The differences became more pronounced at day 84, with significantly lower weight in 10 and 30 μg/l treatments ($P<0.001$ and $P<0.01$, respectively) than controls. The body weight of fish exposed to 1 μg/l NP was not significantly different from that of the controls at any time. Exposure to the highest dose of NP (30 μg/l) continued to cause a significant reduction in weight up to and including the final sample, on day 466 ($P<0.01$). Fish exposed to 10 μg/l NP displayed a reversal of the effect observed in the other treatment groups, with an increase in body weight compared to the controls ($P<0.05$) becoming apparent between day 300 and the final sample (Fig. 7a). At no time was condition factor found to vary between the NP-exposed fish and the controls. OSI was significantly elevated in fish treated with 30 μg/l ($P<0.05$; Fig. 8a).

Nonylphenol diethoxylate

Experiment 1: At the terminal sample, all fish exposed to NP2EO (1, 10 and 50 μg/l) displayed a reduction in body weight relative to the control fish ($P<0.01$; Fig. 2c).

Experiment 2: A significant reduction in length and weight was observed from day 55 in fish exposed to 1μg/l ($P<0.01$ and $P<0.05$, respectively) and 10 μg/l ($P<0.05$) NP2EO (Fig 5). This was sustained in fish exposed to both 1 and 10 μg/l NP2EO through today 84 ($P<0.01$ and $P<0.001$, respectively). However, at that time length was suppressed only in the fish exposed to 10 μg/l ($P<0.05$). No effect was observed after this point (Fig. 7c). The highest dose of NP2EO (30 μg/l) caused no effects on either weight or length at any time. No effect of NP2EO on condition factor or OSI was observed (Fig. 8c).

Nonylphenol mono-carboxylic acid

Experiment 1: No effect on growth was observed with the lowest concentration (1 μg/l) of NP1EC relative to the controls. However, a significant reduction in growth was displayed by fish exposed to 10 and 50 μg/l NP1EC ($P<0.001$) in comparison to the control fish (Fig. 2d).

Experiment 2: This compound produced the most variable results both within and between treatments (Fig. 6). Fish exposed to NP1EC at 10 μg/l showed a significantly reduced length on day 55, relative to the control fish ($P<0.05$). This effect was reversed at day 144, with both length and weight being significantly higher in the 10 μg/l NP1EC-exposed fish compared to the controls ($P<0.05$ and $P<0.01$, respectively.) This effect was still apparent at day 466 ($P<0.001$; Fig. 7d). At the lowest concentration (1 μg/l), a similar enhancement of growth

relative to the control fish was seen at day 84 ($P<0.05$) and from day 220 until the final sample ($P<0.001$). No effects on weight or length were observed for the highest dose (30 μg/l), and no effect of any treatment on condition factor was noted. OSI was reduced in fish exposed to 1 and 10 μg/l compared to the controls ($P<0.01$ and $P<0.05$, respectively). No effect was apparent at 30 μg/l (Fig. 8d).

DISCUSSION

From the results of both experiments, it is apparent that all the test compounds modified the growth of exposed fish. In Experiment 2, significant changes in body weight and fork length were not observed until approximately 15 days after exposure was terminated. This apparent delay in effect may be due to the very small size of the fish at this time (they were weighed to the nearest 0.1 mg and measured to the nearest mm), hence small differences in weight or length may have been obscured by imprecision in the measuring techniques. Compensatory growth was observed in most of the treated fish, resulting in no significant variation in size of these fish compared to controls by the time the final sample was taken, 431 days after exposure to the chemical ceased. However, it is apparent that in some treatments (particularly NP and NP1EC) the influence on growth is of a more permanent nature, with the effects on body weight continuing to persist for at least one year after exposure ceased.

Compounds with estrogenic activity have previously been shown to modify growth in fish. Growth reduction was reported by Bulkley [14] in channel catfish *Ictalurus punctatus* exposed to the synthetic estrogen diethylstilbestrol (DES). Johnstone et al. [15] also described a significant suppression of both length and weight in rainbow trout during a period of dietary administration of 17β-estradiol. Alevins and fry have been reported to be particularly sensitive to the uptake of estrogen [16]. In the present study, exposure occurred during part of this 'critical

window' of estrogen sensitivity. In rainbow trout, this sensitivity is reported to occur at the time of sexual differentiation, which coincides with yolk sac reabsorption and first exogenous feeding [17]. Thus, the effects observed may be in response to the estrogenic activity of the administered compounds. Growth was affected by all the compounds used in this study. However, no clear pattern of effects was observed. In most cases, growth was suppressed (OP, NP, NP2EO), but in some cases growth was accelerated (NP1EC). Different doses of a single chemical in some instances had opposite effects on growth rate. In the case of NP1EC, a single concentration had different effects at different times. The possibility must be considered that these results arise as a consequence of random or systematic deviations from the control groups. Because of inevitable constraints imposed on experimental design by considerations of tank availability and time, fish were serially sampled from within treatment groups, albeit from duplicate tanks within each group, and therefore exposure time was not wholly independent of other factors. However, very similar, rather variable, effects on growth have been observed in juvenile rats exposed to alkylphenolic chemicals [11]. In that study, female rats were given either OP or an octylphenol polyethoxylate (OP5EO) via their drinking water during pregnancy and lactation. In all cases, effects on the growth of the progeny were observed. Just as our data show for fish, these effects were small (±10%), variable between experiments, and variable in direction, although growth was more often suppressed than stimulated. Furthermore, a clear dose-related effect was not always apparent. The similarity between the data of Sharpe et al. [11] and our results suggest APs are capable of influencing growth in both juvenile fish and in mammals.

Growth may be affected by APs and APEOs in a number of ways. These chemicals have been shown to bind to rainbow trout estradiol receptors [18], to induce vitellogenin mRNA *in vivo* [19] and to induce vitellogenin production by trout hepatocytes [4]. The synthesis of the

yolk protein vitellogenin requires energy, thus diverting resources from growth, which might account for the depression of length and weight observed in some groups during the present study. Blazquez et al. [20] report on the depression of growth in sea bass (*Dicentrarchus labrax*) when exposed to 17β-estradiol and 17α-ethynylestradiol incorporated into food. In their study, it was observed that exposure to estrogens between 60 to 260 days post-fertilization produced the most significant effects. If exposure was earlier or later, the effects were greatly reduced. They suggest that these sex steroids are acting as anabolic hormones and exerting a lipolytic action, thereby mobilizing fat reserves from viscera to muscle, leading to reduction of growth. AP's may also be acting via this mechanism, and hence causing the suppression of growth observed in the present study.

It is, however, equally possible that the compounds may not be influencing growth via a direct estrogenic effect, but instead by other mechanisms. For example, sex steroid hormones (and therefore possibly their mimics) can modify the synthesis and secretion of growth hormone (GH) in fish. Growth hormone plays a major role in the control of growth [21]. Trudeau et al. [22] report that implantation of capsules containing estradiol (25 to 100 mg/kg; 5 days) elevated serum GH levels in female goldfish. Conversely, in trout, a single injection of estradiol (10 mg/kg) suppressed GH levels [23]. GH is also known to enhance appetite and improve food conversion. Markert et al. [24] report that the administration of bovine GH to coho salmon resulted in a significantly improved food and protein conversion, leading to growth being enhanced. Thus an effect of APEO treatment on plasma GH concentrations would be expected to influence the growth rate. Alternatively, the effects seen may have a toxicological basis. Nonylphenol is known to have a greater bioconcentration factor than its diethoxylate [25] and hence different toxicokinetics. Greater bioaccumulation, slower depuration and hence a longer narcotic effect could lead to modified food consumption by the fish and result in the effects on

growth we have observed. Guillette et al. [26] suggest that endocrine disrupting chemicals such as alkyphenols may be exerting an organisational effect on the genetic programming of embryos, this effect being of a permanent nature, occurring early in life during a critical or sensitive period of development. Hence, the persistent effects on growth seen here may be due to a permanent organisational effect arising from exposure at an early developmental stage. At this point in time, the exact mechanism(s) of action of alkylphenolic chemicals on growth rate of fish and mammals remains unknown.

Exposure to NP and NP1EC also seem to affect the OSI of fish in the present study. In fish exposed to NP at 30 μg/l, the OSI was significantly increased above that of the controls. Conversely, NP1EC treatment caused a significant reduction of the OSI at 1 and 10 μg/l, whereas no effect was observed with the highest dose. Van den Hurk and Slof [17] present data indicating a linear relationship between body weight and gonad weight in female rainbow trout, i.e. the ovary size increases in proportion to the size of the fish. Our data, therefore, indicate that ovarian development in NP and NP1EC-treated fish is accelerated or suppressed and that this is independent of the fish size. The size of the ovary can be affected by both the number of oocytes present or by the size of these oocytes. However, data on the composition of the ovaries in treated fish are not available at this time, and hence we cannot speculate on the mechanism (s) underlying the observed effect on OSI.

Although the mechanism(s) by which these compounds modify growth is unclear at present, these data highlight a potentially adverse effect on fish exposed to environmentally-relevant concentrations of alkylphenolic chemicals. Our data suggest that these chemicals may not display the same potency of estrogenic effect *in vivo* as has been observed *in vitro*. For example, octylphenol is most potent in the hepatocyte assay [4] whereas the results of the present study indicate that nonylphenol exerts a greater effect, in terms of growth, than

octylphenol. The potential impact of these chemicals on biological processes should therefore be assessed at both cellular and whole animal levels. In terms of the wider significance of these results, survival of fish in the natural environment is closely linked to growth rate, primarily because smaller fish compete less successfully for resources, such as food and territory, than do larger fish [27]. Therefore, if the growth of fish is retarded, they are at a disadvantage. It is clearly not possible to assess whether the extent to which growth was influenced in the present study is likely to impact on the performance of fish in the natural environment. However, these data demonstrate that compounds exerting an "endocrine disruptive" effect may do so more widely within the animal than the current focus on the reproductive system would suggest. As the chemicals used in this experiment are ubiquitous aquatic pollutants, the question of decreased survival in natural populations due to modified growth alone, or in combination with other adverse effects, is an obvious concern.

CONCLUSION

This study has demonstrated that exposure to some alkylphenolic chemicals at environmentally relevant concentrations modifies growth and OSI in female juvenile rainbow trout. How the chemicals instigate these changes is not yet known. Further research into the metabolism, behavioural effects, toxicity and possible effects on the endocrine system of these compounds in fish is necessary.

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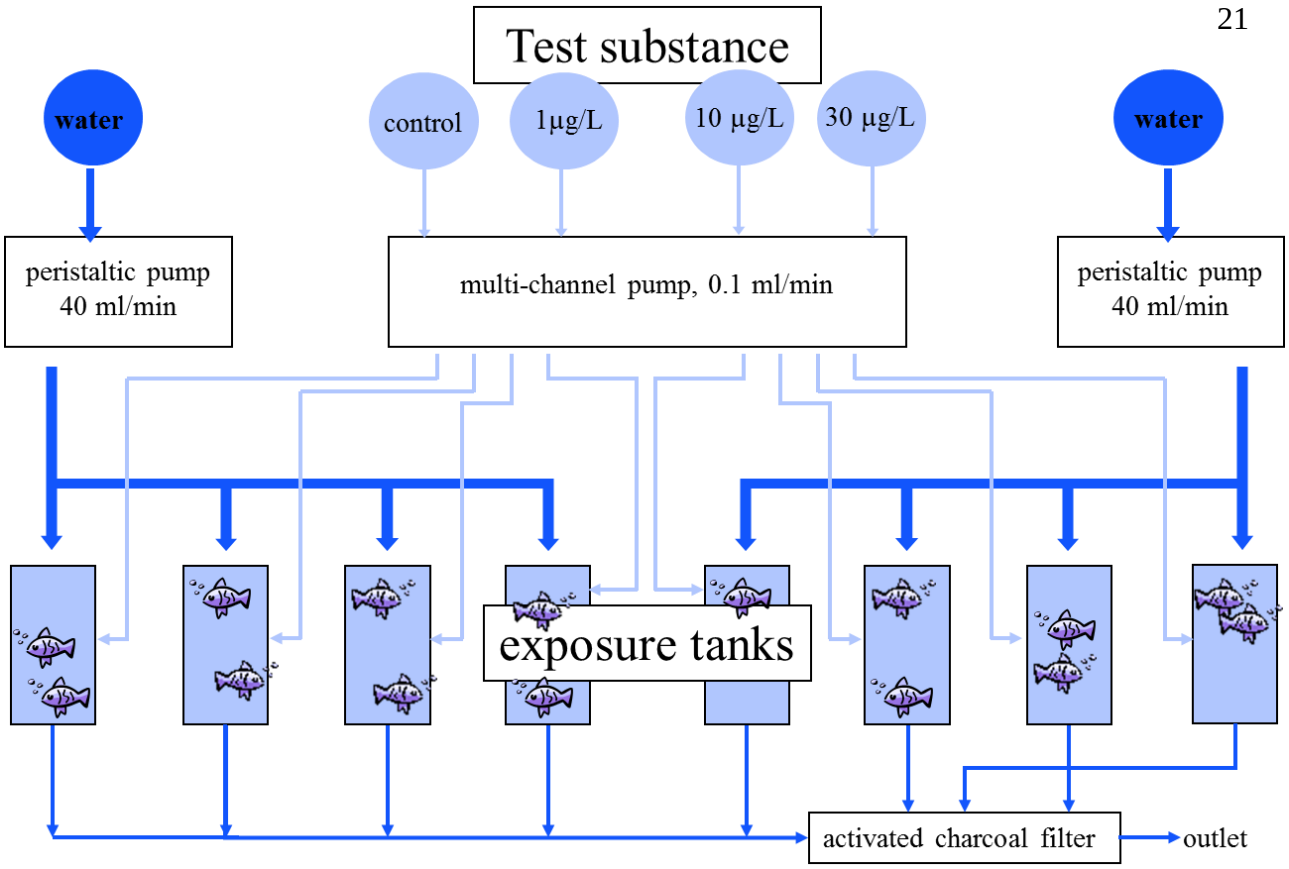

Figure 1. A schematic diagram of the experimental apparatus. The test compound stock solution was pumped into the exposure tanks where it was mixed with inflowing lake water to produce the desired nominal concentration. The effluent was passed through an activated charcoal filter before discharge.

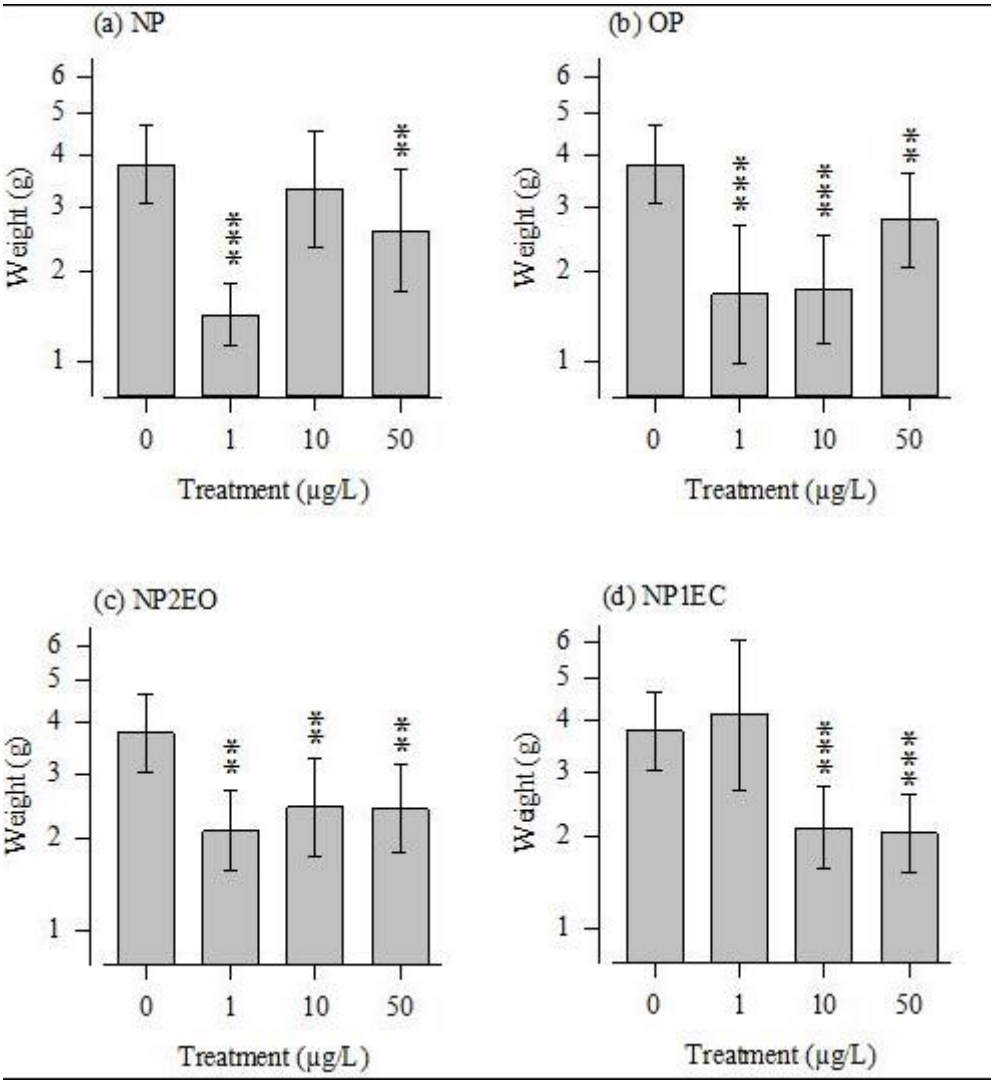

Figure 2. Experiment 1. Body weight data for fish at the terminal sample (day 108). Fish were exposed to three concentrations (1, 10, and 50 µg/L) of (a) nonylphenol, (b) octylphenol, (c) nonylphenol diethoxylate and (d) nonylphenol mono-carboxylic acid. Each column represents the mean of transformed data (weight$^{0.333}$). The vertical lines indicate the 95% confidence limits about each mean. Each mean represents a total of 10 observations, 5 from each of two test tanks. Significant differences from control values are denoted ** P<0.01, *** P<0.001.

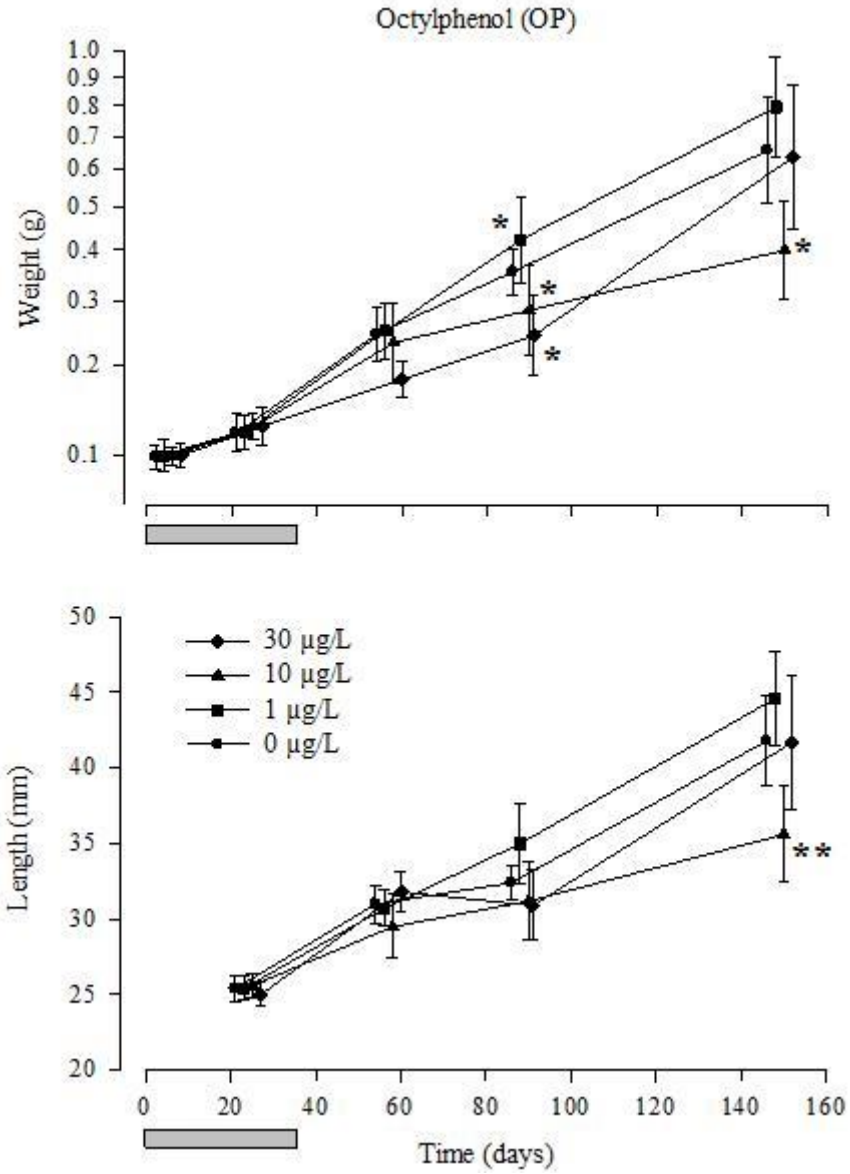

Figure 3. Experiment 2. Body weight and fork length of fish exposed to octylphenol at concentrations of 0, 1, 10, or 30 µg/L for 35 days from hatch. The horizontal bar denotes the period of exposure to the test compound. Each point is the mean of transformed (weight$^{0.333}$) or untransformed data (length). The vertical lines indicate the 95% confidence intervals about each mean. Each mean represents a total of 10 observations, 5 from each of two test tanks. Significant differences from control values are denoted * $P<0.05$, ** $P<0.01$.

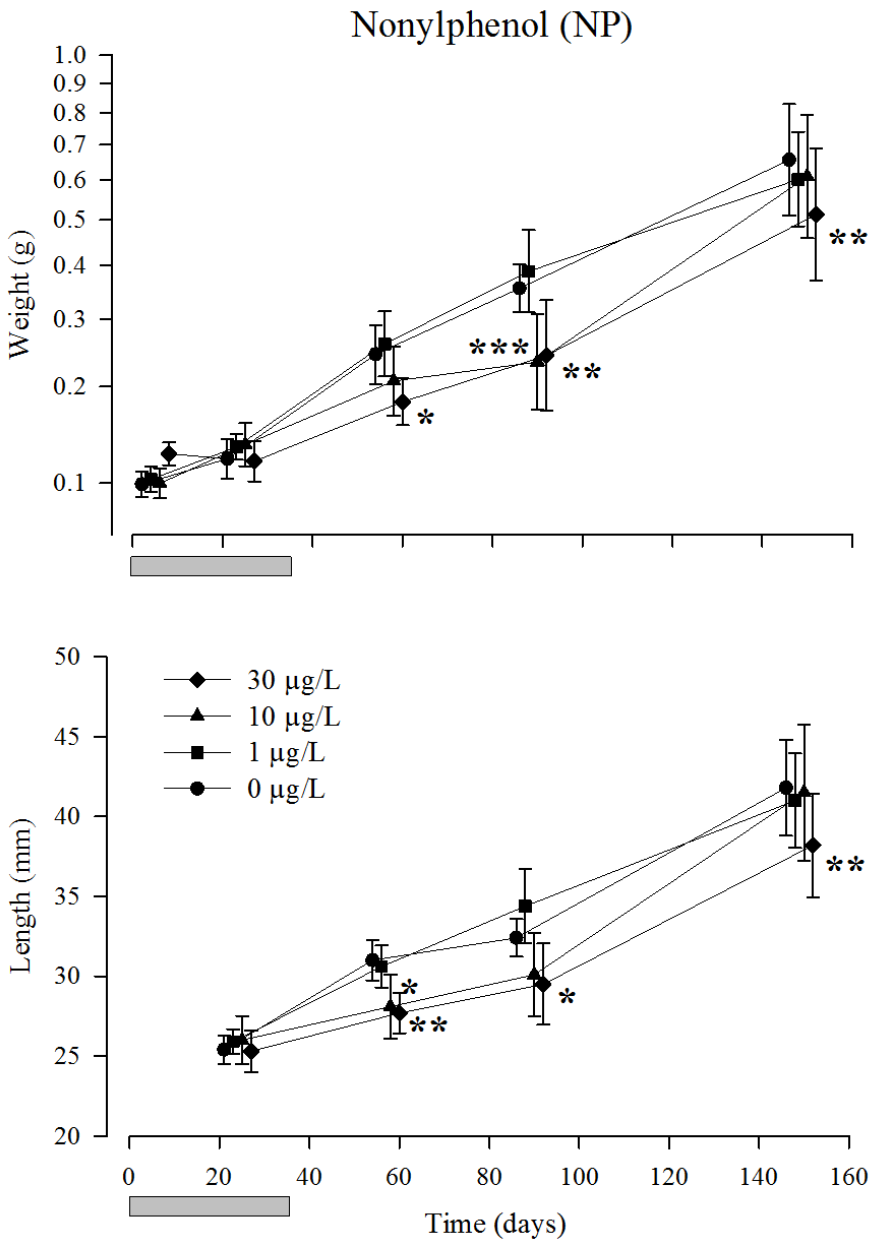

Figure 4. Experiment 2. Body weight and fork length of fish exposed to nonylphenol at concentrations of 0, 1, 10, or 30 µg/L for 35 days from hatch. The horizontal bar denotes the period of exposure to the test compound. Each point is the mean of transformed (weight$^{0.333}$) or untransformed data (length). The vertical lines indicate the 95% confidence intervals about each mean. Each mean represents a total of 10 observations, 5 from each of two test tanks. Significant differences from control values are denoted * P<0.05, ** P<0.01, *** P<0.001.

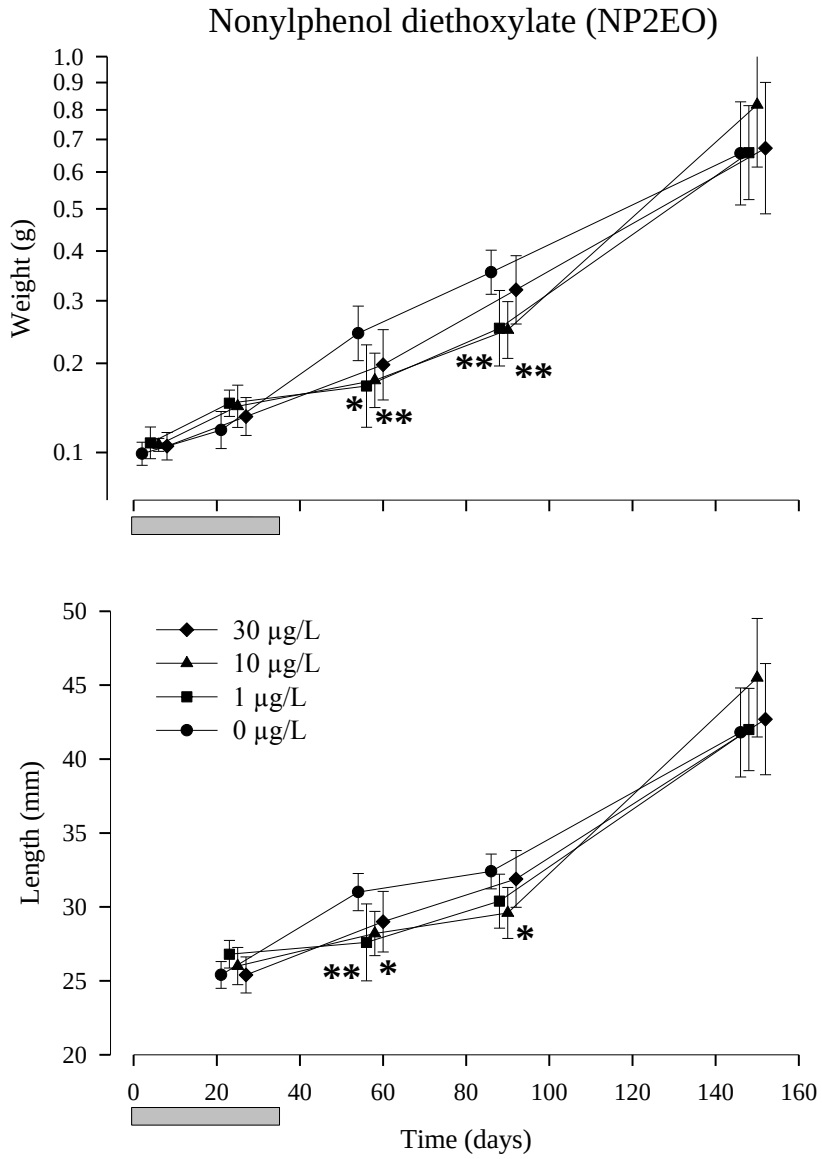

Figure 5. Experiment 2. Body weight and fork length of fish exposed to nonylphenol diethoxylate at concentrations of 0, 1, 10, or 30 µg/L for 35 days from hatch. The horizontal bar denotes the period of exposure to the test compound. Each point is the mean of transformed ($weight^{0.333}$) or untransformed data (length). The vertical lines indicate the 95% confidence intervals about each mean. Each mean represents a total of 10 observations, 5 from each of two test tanks. Significant differences from control values are denoted * $P<0.05$, ** $P<0.01$.

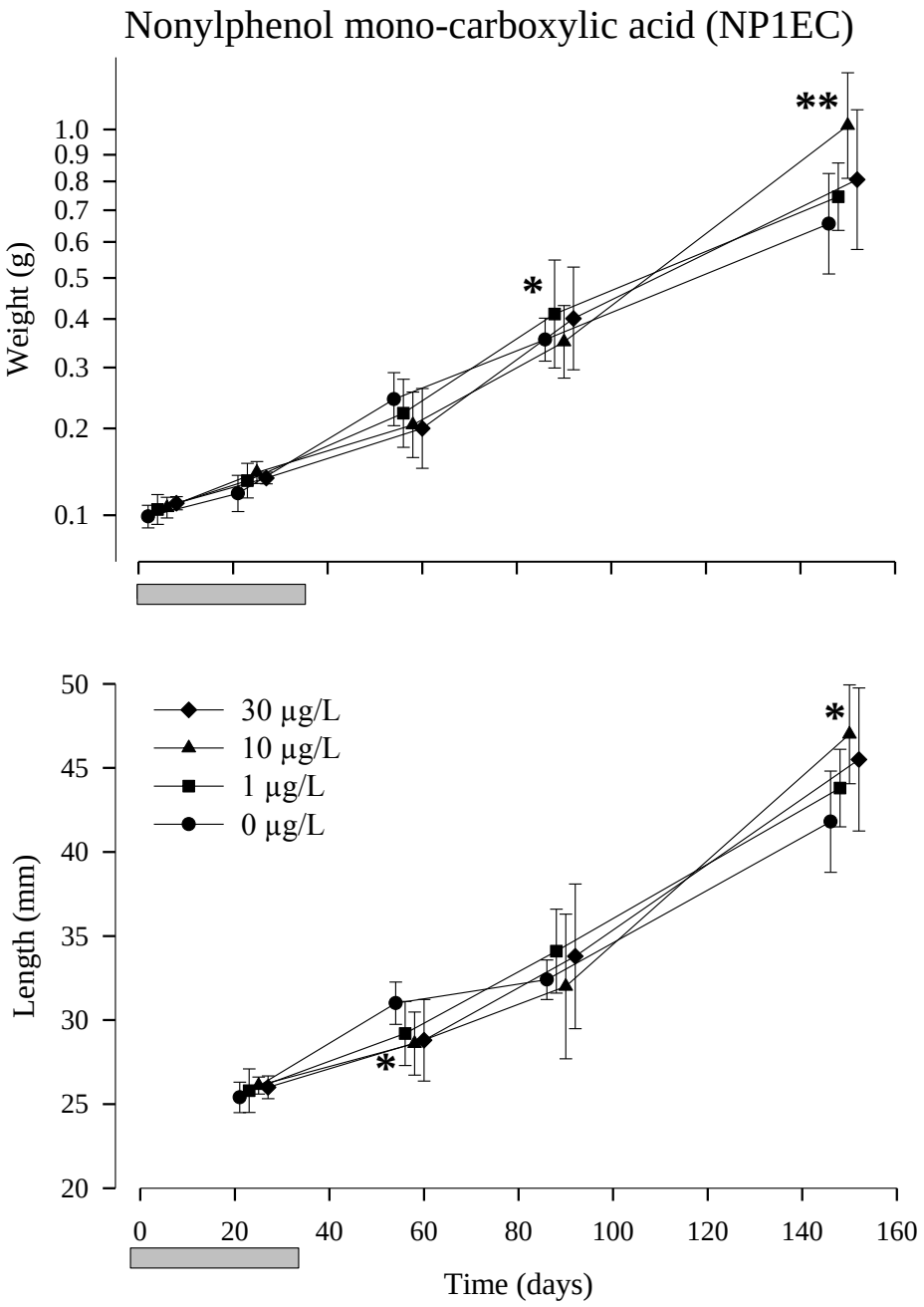

Figure 6. Experiment 2. Body weight and fork length of fish exposed to nonylphenol mono-carboxylic acid at concentrations of 0, 1, 10, or 30 µg/L for 35 days from hatch. The horizontal bar denotes the period of exposure to the test compound. Each point is the mean of transformed ($weight^{0.333}$) or untransformed data (length). The vertical lines indicate the 95% confidence intervals about each mean. Each mean represents a total of 10 observations, 5 from each of two test tanks. Significant differences from control values are denoted * $P<0.05$, ** $P<0.01$.

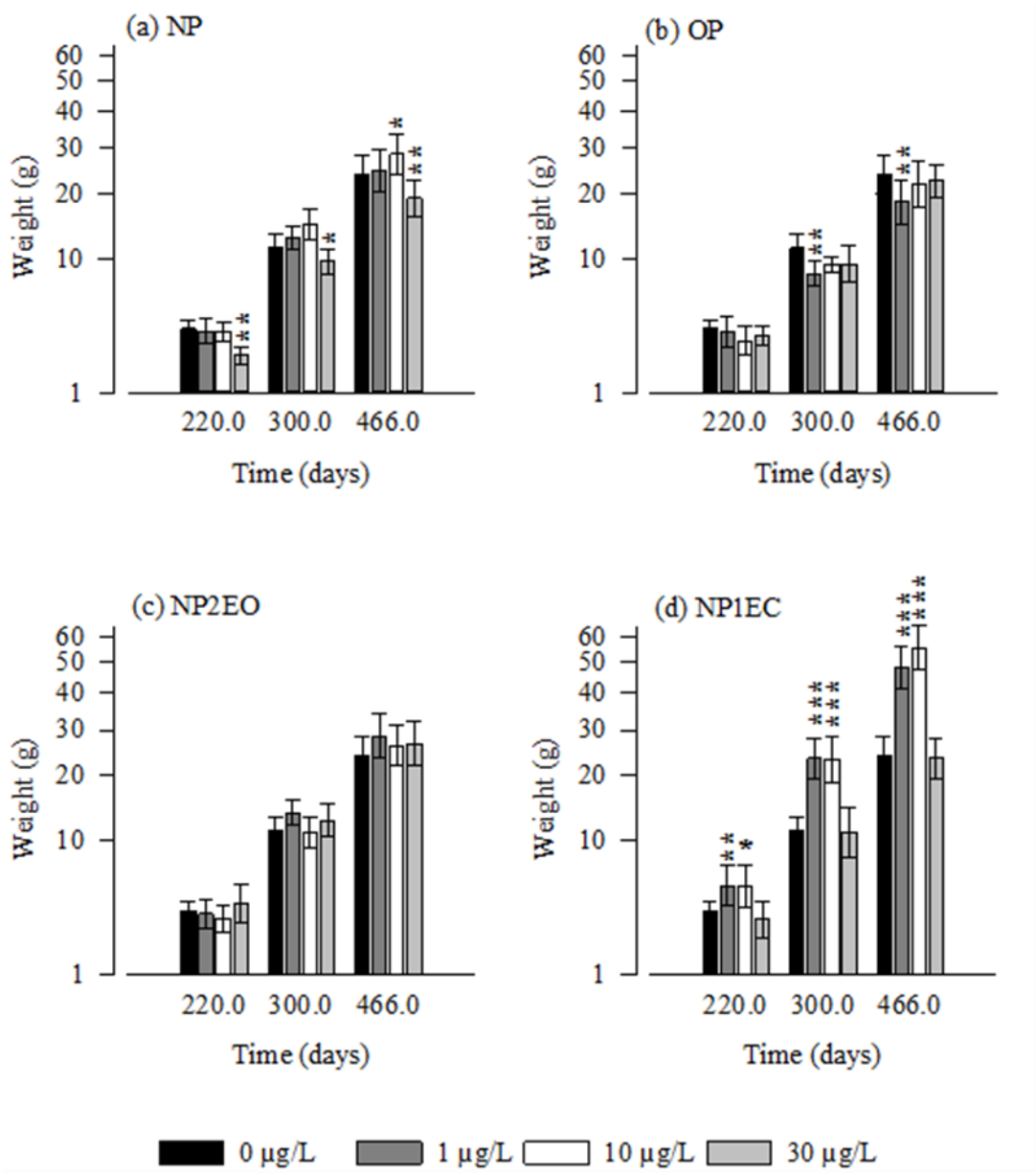

Figure 7. Experiment 2. Body weight data for fish sampled on days 220, 300, and 466. Fish were exposed to three concentrations (1, 10, and 30 µg/L) of (a) nonylphenol, (b) octylphenol, (c) nonylphenol diethoxylate and (d) nonylphenol mono-carboxylic acid. Each column represents the mean of transformed data (weight$^{0.333}$). The vertical lines indicate the 95% confidence limits about each mean. Each mean represents a total of 10 observations, 5 from each of two test tanks. Significant differences from control values are denoted * P<0.05, ** P<0.01, *** P<0.001.

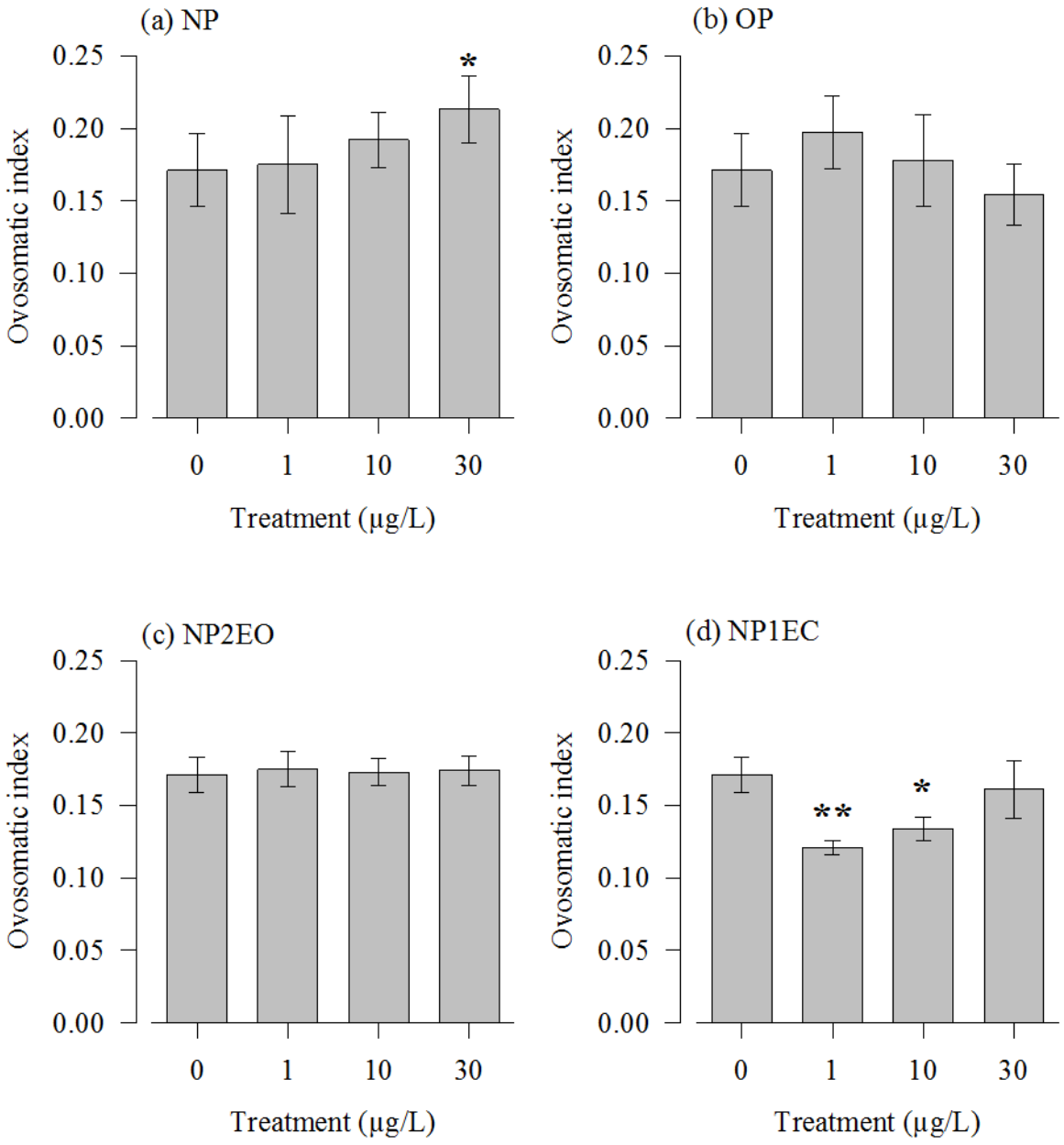

Figure 8. Experiment 2. Ovosomatic indices for fish exposed to three concentrations (1, 10, and 30 µg/L) of (a) nonylphenol, (b) octylphenol, (c) nonylphenol diethoxylate and (d) nonylphenol mono-carboxylic acid, determined on day 466. Each column is the mean and associated 95% confidence interval of 10 observations, 5 from each of two test tanks. Significant differences from control values are denoted * $P<0.05$, ** $P<0.01$.