Impairment of the reproductive potential of male fathead minnows by environmentally relevant exposures to 4-nonylphenol

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Abstract

The synthetic organic compound 4-nonylphenol (NP) has been detected in many human-impacted surface waters in North America. In this study, we examined the ability of NP to alter reproductive competence in male fathead minnows after a 28 day flow-through exposure in a range of environmentally relevant concentrations bracketing the U.S. Environmental Protection Agency toxicity-based NP chronic exposure criterion of 6.1 μg NP/L. Exposure to NP at and above the EPA chronic exposure criterion resulted in an induction of plasma vitellogenin (VTG) within 14 days. However, 7 days after the cessation of exposure, VTG concentrations had dropped more than 50% and few males expressed VTG above the detection threshold. All of the morphological endpoints, including gonadosomatic index, hepatosomatic index, secondary sexual characters, and histopathology, were unaltered by all NP treatments. However, when NP-exposed male fish were allowed to compete with control males for access to nest sites and females, most treatments altered the reproductive competence of exposed males. At lower NP concentrations, exposed males out-competed control males, possibly by being primed through the estrogenic NP exposure in a fashion similar to priming by pheromones released from female fathead minnows. At higher NP exposure concentrations, this priming effect was negated by the adverse effects of the exposure and control males out-competed treated males. Results of this study indicate the complexity of endocrine disrupting effects and the need for multiple analysis levels to assess the effects of these compounds on aquatic organisms.

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1. Introduction

Endocrine disrupting compounds (EDCs) have been found in many anthropogenically altered surface waters (Desbrow et al., 1998; Barber et al., 2000; Kolpin et al., 2002) and have been correlated with changes in anatomy (Jobling et al., 1996, 1998), physiology (Kwak et al., 2001; Pawlowski et al., 2004), and behavior (Bayley et al., 1999; Bjerselius et al., 2001; Schoenfuss et al., 2002) of exposed organisms. Alkylphenolpolyethoxylates (APEs) are nonionic surfactants used in large quantities in commercial and household cleaning products (Naylor et al., 1992). A range of APE parent surfactants and degradation products, such as nonylphenol (NP), primarily enter the aquatic environment through treated municipal wastewater effluents, where they are commonly found in surface waters (Naylor et al., 1992; Ahel et al., 1994b; Barber et al., 2000). Although steroidal hormones such as 17β-estradiol, estrone, and the synthetic estrogen 17α-ethynylestradiol are as much as 100,000 times more estrogenic than APEs (Routledge and Sumpter, 1996; Thorpe et al., 2001), the widespread occurrence of APEs in surface waters at concentrations orders of magnitude higher than steroidal hormones (Kolpin et al., 2002) suggests that they play a potential role in environmental endocrine disruption. The U.S. Environmental Protection Agency (USEPA) has developed a toxicity-based, freshwater chronic exposure criterion of 6.1 μg NP/L (USEPA, 2005). Concentrations of NP in treated wastewater effluent are usually measured in low μg NP/L concentrations (Ahel et al., 1994a; Barber et al., 2000) and vary widely between effluents.
and over time. In receiving waters, NP concentrations are usually below 1 μg NP/L (Naylor et al., 1992; Barber et al., 2000; Kolpin et al., 2002) but have been reported as high as 4 μg NP/L (Ahel et al., 1994b).

A principal indication for the estrogenic effects of APEs has been the presence of the egg yolk pre-cursor protein vitellogenin (VTG) in male and juvenile oviparous organisms. This protein has become a widely used biomarker for exposure to estrogenic compounds (Hemmer et al., 2001, 2002).

In addition to this physiological endpoint, morphological endpoints, such as histopathology of testis, organosomatic index, and secondary sexual characteristics, also are used in EDC exposure experiments. It remains unclear, however, whether environmentally relevant concentrations of EDCs adversely affect the exposed fish’s reproductive competence (defined as the ability to successfully compete for reproductive resources, excluding the mate’s contribution to reproductive fitness). Fathead minnows, like many other small stream fishes, are nest spawners and male fish occupy a nest cavity to attract females for spawning. After eggs have been laid by the female and fertilized by the male, the male fish defends the nest site for up to 5 days to protect the developing larvae from predation. This aggressive behavior is likely androgen-mediated and exposure to estrogens may result in decreased aggressiveness in nest holding males. A male unable to defend a nest site is generally unable to achieve reproductive success (Sargent, 1989).

The series of experiments reported here tested the hypothesis that prolonged exposure to NP would adversely affect the reproductive potential of male fathead minnows by reducing their ability to acquire and defend a nest site in direct competition with a control male. In addition, we attempted to correlate observed reproductive effects with physiological (VTG concentrations) and morphological (histopathological) changes in exposed males to assess how these endpoints serve as indicators not only of exposure but also of effect.

2. Materials and methods

2.1. Experimental design

Exposure experiments were conducted at the St. Cloud State University Aquatic Toxicology Laboratory in St. Cloud, MN. The facility is supplied by non-chlorinated ground water. The experiments utilized a continuous flow-through system, designed to maintain constant chemical exposure conditions throughout the experiment. Two 28-day experiments were conducted in succession utilizing 16 aquaria. Eight aquaria received ground water and eight aquaria received ground water spiked with NP.

In both experiments, mature male fathead minnows were randomly assigned to control or exposure aquaria at a concentration of 6 fish (first experiment) or 5 fish (second experiment) per 16 L aquarium. Two aquaria were assigned to each of the eight exposure concentrations (12 or 10 fish/treatment for the first and second set of experiments) and 16 aquaria were assigned to control fish to provide males for the competitive spawning assay. The continuous flow-through exposures lasted 28 days and were followed by a 7-day competitive spawning period (conducted in unamended ground water) during which exposed males were individually paired with control males to compete for reproductive opportunities (see Section 2.4.2.5). Following the 7-day competitive spawning trials, all male fish were analyzed for plasma VTG concentrations, secondary sexual characteristics, and organosomatic indices. In the second experiment, an additional 20 males per concentration were exposed in separate aquaria and used for a time series analysis of plasma VTG concentrations. Subsamples of five fish were collected at 24 h, 4, 7, and 14 days after onset of the exposure.

2.2. Exposure chemicals

Thirty identical 1-mL aliquots of NP stock solutions (Schenectady International, Schenectady, NY) were prepared in 100% ethanol for each of the eight exposure concentrations and stored at 4°C until use. The technical NP standard that was used is a complex isomeric mixture of 4-NP with minor (<10%) amounts of 2-NP, 4-octylphenol, and dodecylphenol (Wheeler et al., 1997; Bhatt et al., 1992). The stock solutions were prepared to achieve nominal aqueous concentrations in the aquaria of 0.061, 0.61, 6.1, and 61 μg NP/L for experiment 1, and 1.0, 6.0, 12, and 24 μg NP/L for experiment 2. For all treatments, fresh aqueous dosing solutions were prepared daily by adding 1-mL aliquots of the appropriate concentration stock solution to 4 L (experiment 1) or 10 L (experiment 2) of distilled water in an amber glass bottle containing a Teflon stir bar. The solvent concentration did not exceed 1.8 μL ethanol/L, well below solvent concentrations used in previous experiments (Schoenfuss et al., 2002; Bistodeau et al., 2006; Barber et al., 2007) and 2 orders of magnitude lower than the no-effect concentrations for ethanol reported by Yokota et al. (2001) for medaka. After aliquot addition, each amber bottle was gently agitated for 10 s, the neck of the bottle was covered with aluminum foil, and the bottle was placed on a magnetic stir plate and stirred continuously. A stainless steel tube was used to draw the stock solution into the mixing chamber at a nominal rate of 0.0025 L/min (experiment 1) or 0.0069 L/min (experiment 2—to adjust for the greater stock solution volume) using a Cole-Palmer Masterflex 7523-40 peristaltic pump (Vernon Hills, IL), where it mixed with the ground water inflow (0.2 L/min). The water from the mixing chamber was split to the separate aquaria and delivered approximately 14 water exchanges each day.

2.3. Exposure organisms

For both experiments, 3-month-old fathead minnows were obtained from a fish hatchery (Kurtz’s Fish Hatchery, PA) and raised to maturity in the St. Cloud State University Aquatic Toxicology Laboratory. Fish were maintained throughout their development and during the experiments at constant environmental conditions (16:8 h light:dark, 25°C water temperature) and fed frozen brine shrimp (Artemia franciscana, San Francisco Bay Brand, Inc., Newark, CA) twice daily ad libitum. Once the fish reached maturity (approximately 5–6 months old), they were separated by sex and maintained at densities near
2.5 L per fish until the experiments commenced. Fish in the first experiment were approximately 8 months old at the onset of the experiment, while fish in the second experiment were approximately 9 months old. Animal use and care in all experiments was approved by the St. Cloud State University Institutional Animal Care and Use Committee (IACUC).

2.4. Analysis

2.4.1. Water quality

Weekly water samples for each treatment were collected from the outflow of the stainless steel mixing chambers where ground water and the treatment-specific NP concentrations were mixed prior to delivery to the aquaria, and monitored for dissolved oxygen, pH, and temperature (USGS, 2003). Concentrations prior to delivery to the aquaria, and monitored for dissolved water and the treatment-specific NP concentrations were mixed the outflow of the stainless steel mixing chambers where ground water and the treatment-specific NP concentrations were mixed.

2.4.2. Biological endpoints

A series of biological endpoints encompassing physiological, morphological, and behavioral parameters were measured to determine whether male fathead minnows exhibited reduced reproductive competence as a result of prior exposure to NP. By assessing complex behaviors in the context of simple physiological changes, we evaluate the suitability of VTG induction as an indicator of reduced reproductive potential in addition to being an indicator of exposure to environmental estrogens.

2.4.2.1. Vitellogenin analysis. After fish were deeply anaesthetized in 1% 2-phenoxethanol (Sigma, St. Louis, MO), the male fish tails were severed to harvest blood using a capillary tube. The blood was immediately centrifuged to isolate plasma. Whenever possible, two aliquots of plasma were collected, placed on ice and transferred to a −80°C freezer until analysis. A commercially available indirect sandwich enzyme-linked immunosorbant assay (ELISA) specific to the VTG protein molecule synthesized by carp (Cyprinus carpio) was used in the VTG analysis (Biosense Laboratories, Bergen, Norway). Substantial cross-reactivity exists between carp and fathead minnow VTG and resulting antibodies. Using this assay, VTG content of exposed male fish was compared to the VTG content of control male fish within each experiment. All samples with plasma VTG concentrations below the 0.001 mg/mL detection limit were recorded as 0.0005 mg/mL (one-half the detection limit) for statistical analysis (Blazevic et al., 2001).

2.4.2.2. Organosomatic index. Whole body weights were measured for each male fish at the time of analysis (0.01 g precision, Acculab Vicon). Gonads and livers from each male were excised and immediately weighed (0.00001 g precision, Mettler Toledo AG245) and weights were recorded to the nearest 0.001 g. Liver and whole body weights were used to calculate the hepatosomatic index (HSI = liver weight/whole body weight). Conversely, gonad and whole body weights were used to calculate the gonadosomatic index (GSI = gonad weight/whole body weight).

2.4.2.3. Histopathology. Following removal, gonads were fixed in Bouin’s solution for 24 h (Gabe, 1976). After fixation, tissues were dehydrated in a series of ethanol and toluene baths before being embedded in paraffin. Embedded tissues were sectioned at approximately 1/3 and 2/3 of the length of the testis using a Reichert–Jung cassette microtome (4–6 μm sections). Sectioned tissues were stained using a standard haematoxylin and eosin counter stain protocol modified after Gabe (1976). Histological slides were visually inspected by an experienced histologist (HLS) for the simultaneous occurrence of ovarian and testicular tissues and other pathological alterations to gonadal tissues.

2.4.2.4. Secondary sexual characteristics. After the gonads were excised from each fish, the remaining carcass was stored in Bouin’s solution until the secondary sexual characteristics analysis could be completed. This analysis used a simple, blind scoring system similar to that described by Smith (1978). The prominence of the tubercles was scored on a scale of 0–3. The dorsal pad was evaluated by a similar method and scored on a scale of 0–3.

2.4.2.5. Competitive spawning assay. Once the 28-day NP exposure period was completed, each exposed male was paired with a control male for the competitive spawning assay (Bistodeau et al., 2006; Barber et al., 2007; Martinović et al., 2007). Both the exposed and treated males received small caudal fin clips (a corner of either the superior or inferior portion of the caudal fin was removed) so that observations could distinguish fish from the two treatments. These fin clips alternated between the top and bottom of the caudal fin and between treatments to create “blind observations,” where the observer was unaware of the fish’s exposure history. In addition, alternating fin clips avoided bias between control and exposed males as both groups received similar numbers of upper and lower fin clips. One treated male and one control male of comparable size (judged only visually, as it was important for the experimental integrity to avoid extended periods of stress for the fish) were then simultaneously placed into a 7-L all-glass aquarium. Based on the number of available males, 10–12 competitive spawning assays were setup for each treatment. Each of these spawning aquaria contained two mature females (raised in ground water) and a nest site, made of a short section of 8 cm diameter stainless steel pipe cut in half. Twice daily (between 8 and 10 a.m. and 2 and 4 p.m.), for the following 7 days, the nest holding male was identified by its respective fin clip. The times of observation coincide with the highest reproductive activity (usually in the morning) and after most reproductive activity for the day was completed (afternoon). A nest holding male was defined as one that exhibited aggressive behavior towards other fish in the aquarium, while clearly protecting the nest site. This behavior typically includes butting, using the newly formed tubercles, and
chasing away other fish from the nest site (Unger, 1983). Each observation of a male fish defending a nest site was scored as a nest holding event.

2.5. Statistical analysis

The assumption of normality for all data sets was tested with the Kolmogorov–Smirnov test for normality prior to any additional analysis (Prism 4.01 statistical package, GraphPad Software, Inc., Oxnard, CA). Data that passed the normality test were analyzed using a one-way ANOVA followed by a Tukey post-test. Non-parametric data were analyzed using Kruskal–Wallis followed by Dunn’s post-test. Nest holding abilities of the exposed and control males in the competitive spawning assays were assessed using a Fisher’s exact test (contingency table). A probability of \( p < 0.05 \) was set as level of significance for all comparisons.

3. Results

3.1. Survival rates and aqueous nonylphenol concentrations

Survival rates exceeded 90% in most NP treatments in both experiments, but were at 58% for 0.15 \( \mu \)g NP/L, 84% for 3.2 \( \mu \)g NP/L, and 80% for 0.3 \( \mu \)g NP/L. Environmental conditions were stable throughout the experiments (dissolved oxygen = 6.4 ± 0.3 mg/L, pH = 7.2 ± 0.1) although the temperature differed slightly between experiment 1 (24 ± 0.4°C) and experiment 2 (27 ± 0.6°C). These conditions are reflective of rearing conditions described by Denny (1987) and environmental conditions for fathead minnows during the reproductive season.

During the first experiment, the average measured aqueous NP concentrations in the first experiment (Fig. 1a) were 0.15, 0.25, 0.63, and 3.2 \( \mu \)g NP/L for the four exposed groups. The measured concentrations were less than the nominal concentrations, particular at the higher dosings, as the result of solubility limitations in the stock solution. As a result, the volume of the stock solution and mixing ratios were adjusted in the second experiment to avoid reaching the aqueous solubility limit of NP (5.4 ± 0.2 mg NP/L at 25°C, Ahel and Giger, 1993). Measured NP concentrations in the second experiment (0.3, 5, 11, and 15 \( \mu \)g NP/L; Fig. 1b) were in better agreement with the nominal values. In all subsequent discussion and figures, the average measured concentrations will be used. No NP was detected in the ground water control treatments during either experiment.

3.2. Vitellogenin concentrations

Plasma VTG concentrations exhibited a non-significant increase in samples collected on day 4 at all but the lowest concentrations (Fig. 2). On sub-sampling days 7 and 14 plasma vitellogenin concentrations differed significantly from the control for the 15 \( \mu \)g NPL treatment (Fig. 2, Kruskal–Wallis followed by Dunn’s post-test, \( p < 0.05 \)). However, during both experiments (Fig. 3a and b) plasma VTG concentrations in fish analyzed 7 days after the end of the NP exposure (day 35—following the competitive spawning scenario) did not vary significantly among treatments and controls (experiment 1, \( p = 0.325 \); experiment 2, \( p = 0.116 \)). The seemingly higher VTG concentrations in the 15 \( \mu \)g NPL treatment are the result of just two males expressing high VTG concentrations (2.5 and 1.8 \( \mu \)g VTG/mL) while the remaining nine males did not express VTG above the detection limit (0.001 mg VTG/mL).


3.3. Morphological endpoints

The HSI values did not vary significantly between treated and control fish in the first experiment (p = 0.833). In the second experiment HIS values were significantly elevated from the control only in the 0.3 μg NP/L treatment (p = 0.035). Similarly, the GSI did not vary significantly between treatment groups in either experiment (p = 0.46; p = 0.66). Expression of secondary sexual characteristics did not vary significantly between treatments in either experiment (p = 0.794; p = 0.714). However, the secondary sexual characteristics score was on average 0.5 points greater in all treatments in the second experiment compared to the first experiment. Histological analysis did not identify any pathological findings of ovarian tissues in testis, extensive apoptosis or inflammation, or proliferation of connective tissues in either testis or liver during either experiment.

3.4. Behavioral endpoint

Male fish in all of the competitive spawning scenarios behaved in an expected competitive manner, and nest holding ability showed significant differences between treatments in both experiments. In the first experiment (Fig. 4a), male fathead minnows from the 0.15 μg NP/L exposure out-competed control males for access to nest sites, holding about 75% of all nest sites. However, at the three higher concentrations in the first experiment, control males out-competed exposed males by 6–8%. A similar trend was observed in the second experiment (Fig. 4b) with NP-treated males at the lower concentrations out-competing control males, while at the higher concentrations, control males out-competed exposed males by 5–10%.

4. Discussion

In this study we conducted two experiments to investigate the physiological, morphological, and behavioral effects of NP at a series of environmentally relevant concentrations that bracket the USEPA chronic exposure criterion of 6.1 μg NP/L. Although we found strong VTG induction (>20 mg VTG/mL) in several treatments early in the exposures, final plasma VTG concentrations at the end of the entire experiment, including the 7-day competitive spawning assay without exposure, were

Fig. 3. Vitellogenin concentrations in plasma of male fathead minnows after 28 days of nonylphenol exposure and 7-day competitive spawning assay during (a) experiment 1 and (b) experiment 2 (number in parentheses indicates sample size; solid line indicates mean; to calculate mean values, sample concentrations below detection limit (as shown by the dotted line) were scored at 0.0005 mg VTG/mL (one-half detection limit)).

Fig. 4. Nest holding ability of nonylphenol exposed male fathead minnows in direct competition with control males in (a) experiment 1 and (b) experiment 2 (number in parentheses indicates total observations of nest holding; p values are presented for each treatment, Fisher’s exact test).
below 1 mg VTG/mL. Induction of VTG did not correlate with morphological endpoints, and GSI, HSI, and secondary sexual characteristics were unchanged despite increasing plasma VTG concentrations (Table 1). Exposure to NP resulted in measurable increases in plasma VTG concentrations only in the second experiment with males being exposed to concentrations >5 μg NPL. This is consistent with previous studies in which similar NP concentrations resulted in measurable increases in plasma VTG after 5–16 days (Hemmer et al., 2001, 2002). Investigation of VTG clearance rates in male sheepshead minnows (Hemmer et al., 2002) found rapid declines in plasma VTG levels with a 13-day half-life after exposure to 56 μg NPL. Although they were unable to calculate similar half-life values with a 5.6 μg NPL exposure, a reduction of plasma VTG within a week of exposure termination was evident, and was comparable to the reduction in plasma VTG concentrations observed in our second experiment at the 5.0 and 15 μg NPL concentrations. Other studies have reported variability in plasma VTG concentrations in fishes exposed to concentrations of NP <6 μg NPL (Villeneuve et al., 2002; Pickford et al., 2003). The variability of plasma VTG concentrations in fishes exposed to NP at environmentally relevant concentrations and the relatively expedient clearance rates suggest the plasma VTG concentrations may not serve as a reliable biomarker for NP exposure in wild fishes.

Fish exposed to NP in both experiments did not exhibit significant changes to their liver or testis weights. This finding is consistent with published literature where environmentally relevant NP concentrations (0.1–10 μg NPL) are reported to have little effect on the relative weight of either organ (Villeneuve et al., 2002). Smith (1978) correlated expression of secondary sexual characteristics with testis development and GSI. Because we did not find changes in GSI, it also is consistent with Smith’s findings, that secondary sexual characteristics in NP-exposed fish in our study also remained unaffected.

In contrast to results from physiological and morphological endpoints, during both experiments the behavioral endpoint of the ability of male fathead minnows to obtain and defend a nest site was affected by most NP exposures (Table 1). However, the effects were complex with apparent beneficial effects for male fathead minnows exposed to lower NP concentrations, while males exposed to the highest NP concentrations suffered adverse consequences to their nest holding ability. These seemingly contradictory results from the competitive spawning assay demonstrate the complexity of responses to EDCs and the potential biases introduced through experimental designs. In both experiments, male fish were separated from females upon maturation (when separation by external features was possible). After several more months, the 28-day exposures began. As a result, male fish were not in contact with female fish for 10–12 weeks prior to the start of the competitive spawning assay. The ichthyological literatures have demonstrated that male fish are “primed” for reproductive performance by the presence of female fish through pheromones secreted by the females immediately prior to and after ovulation (Sorensen and Stacey, 1987; Sorensen et al., 1988; Zheng and Stacey, 1997). It is possible that the estrogenic characteristics of NP act as an exogenous primer to stimulate male fathead minnows. These fish would be in an advantageous situation when paired with control males that have received no prior stimulation and immediately need to compete with the NP-exposed males for a nest site and access to females. As in most competitive reproductive scenarios, dominance is established quickly and once a fish has obtained a nest site it is unlikely to lose the site for the remainder of the competitive spawning assay.

It is more difficult to explain why the 0.3 and 5.0 μg NPL exposure treatments resulted in an excitatory response in the second experiment, while similar and lower concentrations in the first experiment resulted in inhibitory responses in the competitive spawning assay. Although our study was not designed to elucidate underlying mechanisms of behavioral interactions among male and female fathead minnows, the two experiments were conducted with subsequent year classes of fathead minnows. The overall greater values obtained for secondary sexual characters may indicate a greater robustness of fishes in the second experiment, and may have provided less sensitivity to the adverse effects of the treatments. In addition, average temperatures in the second experiment were three degrees higher than in the first experiment and may have influenced the physiology of the exposed fish. However, this is speculative and illustrates the complexity that live organisms introduce to EDC exposure experiments.

The competitive spawning assay utilized in this study is noteworthy as it introduces reproductive competition, a hallmark of natural selection, into the experimental design. Reproductive selection is often excluded in experiments designed to assess the reproductive ability of an organism exposed to a pollutant. However, for male fathead minnows, the ability to compete for access to spawning sites is crucial to ensure reproductive success and should, therefore, be included as a design component when possible. The competitive spawning assay also acknowledges the

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<th>Table 1</th>
<th>Distribution of statistically significant differences (p &lt; 0.05) among various biological endpoints measured</th>
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<tr>
<td>Endpoint</td>
<td>Experiment 1, measured NP (μg/L)</td>
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<td>0.15</td>
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<td>Plasma vitellogenin</td>
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<td>Hepatosomatic index</td>
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+ indicates exposure exceeded control; − indicates control exceeded exposure.
differential exposure history that fish interacting in a natural setting will likely have experienced. Many fish species are known to move frequently, creating the potential that unexposed, or less exposed fish will compete with fish of differing exposure history. If pollutants affect the males’ ability to occupy and defend a nest site, the pollutant will become an artificial selective factor with unknown consequences for the health of the population. The use of other relative biomarkers of the effects of EDC exposure, such as secondary sexual characters, also inherently acknowledges the fact that not all fish in a population will be exposed to the same compounds at the same concentrations for the same length of time in a natural aquatic environment. Otherwise one would expect the endpoints to be equally depressed in all exposed fish and there would be no reproductive consequences.

This study indicates that single physiological and anatomical endpoints are insufficient to assess the reproductive effects of NP at environmentally relevant concentrations (Table 1). It is noteworthy that several studies have exposed male fathead minnows to NP at concentrations between 5 and 10 μg NPL and have reported either non-significant results for plasma VTG concentrations and/or great variability among fish in the same treatment. These studies also have failed to find a correlation between NP exposure and GSI or HSI. Previous results are corroborated by our seemingly contradictory nest holding results and the lack of correlation between plasma VTG induction and testis or liver size. It may be necessary to focus attention on the variability of effects among fishes exposed to environmentally relevant concentrations of suspected endocrine disrupting compounds rather than merely accepting its occurrence. The reproductive consequences of NP exposure presented in this study also indicate that the effects of exposure are likely complex and may defy any singular assessment tool. Instead, an index of biological health of fish exposed to contaminants such as EDCs may be needed to adequately assess the consequences of exposure by each fish and ultimately the fish population.

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