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# Migration of nonylphenol from food-grade plastic is toxic to the coral reef fish species *Pseudochromis fridmani*

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## HIGHLIGHTS

- Nonylphenol (NP) migrates from FDA food-grade polyethylene bags to contained seawater.
- Seawater levels of NP from the bags in one treatment were similar to 96 h $LC_{50}$ values for *P. fridmani*.
- Dottybacks held in these bags accumulated high body concentrations of NP.
- Although labeled as food-grade polyethylene, the PE2 bags leached highly toxic levels of NP.
- NP could pose a greater risk than might be estimated from testing plastic from a single manufacturer.

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## ABSTRACT

Nonylphenol (NP) is a non-ionic surfactant used extensively in industrial applications, personal care products, and many plastics. We exposed marine orchid dottybacks (*Pseudochromis fridmani*) for 48 h to either glass, Teflon, or two bags labeled as FDA food-grade polyethylene (PE1 and PE2) from different manufacturers. The PE2 bags leached high levels of NP into the contact water, which were taken up by the fish, and decreased short and long-term survival. Concentrations of NP that leached from the bags were consistent with 96 h $LC_{50}$ values determined in this study, indicating NP is the likely toxic agent. Despite being similarly labeled, the NP concentrations that leached from the bags and the resultant toxicity to the fish varied dramatically between manufacturers. This study highlights that some plastics, labeled as food-safe, can be highly toxic to aquatic animals, and could pose a greater threat to humans than previously realized. This study also highlights risks for aquatic animals exposed to increasing quantities of plastic waste.



## 1. Introduction

Nonylphenol (NP) is a non-ionic surfactant used extensively in industrial applications as well as household and personal care products, including many plastics (Lorenc and Scheffer, 2003). The discharge of effluents from sewage treatment has led to considerable burdens of NP in aquatic environments, often found at concentrations above 10 μg $L^{-1}$ (Giger et al., 1984; Blackburn and Waldock, 1995; Langford and Lester, 2002; Fries and Puttmann, 2003; Vazquez-Duhalt et al., 2006; Chandrasekar et al., 2011; Maruya et al., 2012), although concentrations greater than 600 μg $L^{-1}$ have been reported (Sole et al., 2000).

Nonylphenol binds to estrogen receptors, and its estrogenicity has been well documented (Soto et al., 1991; White et al., 1994; Cakmak et al., 2006). In addition to a variety of estrogenic effects, other sublethal effects include altered immune function, metabolism, oxygen homeostasis, cell cycle regulation and DNA damage (Shelley et al., 2012). Radiolabeling studies in fish show NP to concentrate heavily in the bile and intestine, with moderate accumulation in the gills, skin, abdominal fat, eyes and brain (Arukwe et al., 2000). At elevated concentrations, NP is highly toxic to aquatic life causing blood cell lysis, exhaustion of the hemopoietic activity of the kidney and/or tissue damage (Kumaran et al., 2011).

Demand for NP was estimated to be more than 170,000 metric tons in 2010 (ICIS, 2007). Due to its widespread use and recalcitrant nature, NP is a ubiquitous contaminant in both wildlife and humans (Keith et al., 2001; Calafat et al., 2005; Lu et al., 2007; Ademollo et al., 2008; Diehl et al., 2012; Gyllenhammar et al., 2012). Human exposure to NP occurs through its use in pesticides, plastic food packaging, and its presence in household products such as detergents and cosmetics (Ying et al., 2002; Loyo-Rosales

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et al., 2004; Gyllenhammar et al., 2012). NPs enter the environment through wastewater discharge and other sources, and readily bioaccumulate in freshwater and marine organisms (McLeese et al., 1981; Ekelund et al., 1990; Uguz et al., 2003; Ishibashi et al., 2006; Spehar et al., 2010). NPs can persist for decades in anaerobic environments, such as the mudflats of estuaries, and therefore tend to be higher in sediments than in water (Ying et al., 2002; Soares et al., 2008). A survey of 95 organic wastewater contaminants in 139 US streams revealed that nonylphenol was one of the most commonly occurring contaminants and was measured at higher concentrations than most other aquatic pollutants (Kolpin et al., 2002).

The migration of NP from individual consumer products is generally thought to be low to moderate, with primary concern being cumulative exposures and intake that could lead to increased tissue concentrations (Guenther et al., 2002; Gyllenhammar et al., 2012). Although a number of studies have investigated the ability of NP to migrate from consumer packaging, relatively few of the thousands of NP-containing materials have been tested, limiting our understanding of the range of possible NP exposures (Guenther et al., 2002; Loyo-Rosales et al., 2004). Our growing awareness of the vast quantities of plastic debris accumulating in many ocean regions has led to concerns for resident organisms (Barnes et al., 2009; Law et al., 2010; Rios et al., 2010; Elliott and Elliott, 2013; Cozar et al., 2014; Law et al., 2014; Jambeck et al., 2015). The weathering of plastic waste causes particles to fragment, allowing ingestion by even small invertebrates (Goldstein and Goodwin, 2013). Estimates of contaminants leaching from plastic waste are based on the analysis of a variety of commonly encountered types of plastic, but no study has investigated the toxicity of NP of the same type of plastic produced by different manufacturers. Differential migration rates of NP from plastics could underestimate contamination risk; this is particularly concerning given that many ocean ecosystems, such as coral reefs, are already at risk due to environmental pollution (Downs et al., 2006; Brodie and Waterhouse, 2012; Kroon et al., 2014).

"Post-traumatic shipping disorder" (PTSD) refers to the often high-percentage of mortality and morbidity in the aquarium trade associated with shipping and is a significant problem for ecological conservationists, trade companies, and consumer satisfaction (Chow et al., 1994; Baquero, 1995; Lim et al., 2003; Rubec and Cruz, 2005). Shipping mortality from export facility to import-reception facility can range from 20% to 90%, (Vallejo, 1997). Once the aquarium-trade specimen makes it to the retail store, delayed mortality can be high as 90% in less than a year (Tullock, 1998; OATA, 1999). Recently, a number of aquarium-trade importers and distributors have seen high levels of PTSD of both newly imported specimens, as well as domestically cultured specimens. The common factor among these various companies was their use of plastic bags and Styrofoam coolers which came from a limited source of manufacturers.

Plastic bags have been used in shipping of aquarium trade specimens since the 1950s (Miller, 1956). The aquarium-trade industry will often use low-density polyethylene bags that are manufactured according to US FDA regulation 177.1520 (FDA-approved food-grade plastics) to "bag" fish and coral for shipping. There is an abundance of literature demonstrating chemical components of plastics leaching into consumer products and the environment, such as 4-methylbenzophenone, bisphenols, nonylphenols and phthalates (Soto et al., 1991; Teuten et al., 2009; Yang et al., 2011). Recent mortality in the commercial shipments led to concerns that contaminants migrating from plastic shipping-bags were the cause. The purpose of this study was to investigate the possibility that certain FDA approved plastics have the ability to leach NP at concentrations that could be toxic to aquatic life, and potentially pose a greater risk to humans than previously realized.

## 2. Materials and methods

### 2.1. Animals and exposure conditions

Captive bred orchid dottybacks (*Pseudochromis fridmani)* (Sea and Reef Aquaculture, Hancock, Maine) were shipped in PFA-Teflon bags and held communally in a 75 L glass aquaria for 14 d before initiation of the experiment. Synthetic seawater was created with Sigma sea salts (Sigma–Aldrich, Saint Louis, MO, USA) mixed with Scientific Environmental-Grade water (Thermo-Fisher Scientific, Waltham, MA, USA) to a salinity of 33 ppt (pH 8.17). Water chemistry was maintained with an activated carbon filter, as well as a zeolite silicate filter to remove ammonia. Both the carbon and zeolite were placed in stainless steel (316) tea strainer balls within the aquarium, and 80% water changes occurred every four days. Dissolved oxygen was measured using a YSI oxygen electrode calibrated with Na dithionite; pH was monitored using a Thermo-Orion 5 Star pH multimeter probe system. Day photoperiod was 10 h using a 5100 K LED white light. Temperature of the room was 23 °C.

Synthetic seawater (350 mL) was placed into one of four types of vessels which included a Pyrex glass bowl ($n = 10$), a Welchfluorocarbon PFA-Teflon bag ($n = 8$) or one of two bags from different manufacturers both labeled as FDA food-grade polyethylene (PE1, $n = 9$; PE2, $n = 10$). An initial survey of detectable contaminants identified with LC/MS leaching from PE2 bags is described in Table 1. A single fish was added to the seawater in each vessel, and each vessel was then flushed with medical-grade oxygen to fill the bags, and then the ends of the bags were tied to seal them. This process of flushing the bags with medical-grade oxygen simulates the procedure for shipping live, biological specimens in the aquarium trade. For the Pyrex® bowl, a 5 mm PFA-Teflon sheet was wrapped around the bowl and sealed with a rubber band as it was being flushed with oxygen. Both the glass bowl and Teflon sheet were cleaned according to EPA specifications for contaminant-free sample containers (EPA A540/R-94/051). At 12 and 24 h, the specimen in each vessel was examined for mortality. At the end of 48 h, each specimen was removed from the vessel and placed in an acetone-washed Teflon bag and frozen (−80 C).

Three of the Teflon bags, four of the PE1 bags and eight of the PE2 bags that were used in the experiments were resealed, flash frozen in dry ice, and sent frozen to an independent laboratory for NP compounds contaminant chemistry analysis of the contained synthetic seawater [Environmental Chemistry Consulting Services (ECCS), Inc., Madison, WI, USA]. Concurrent with the fish exposures, three PE2 bags were filled with 350 mL of synthetic sea water, and were sealed with medical-grade oxygen. No fish were added to these bags. Bags were kept in the dark, and incubated for 48 h. These bags were never opened, yet were otherwise treated identically to bags that held fish, and were sent in the same shipment with the other samples for analysis to ECCS.

**Table 1**
A survey of compounds migrating from the PE2 bags into seawater (Environmental Chemistry Consulting Services, Inc.).

| Library ID | Molecular formula |
|---|---|
| Nonylphenol | C15H240 |
| Indole | C8H7N |
| 9-octadeceneamide | C18H35NO |
| 7-heptadecene | C17H33Cl |
| 9-octadecenamide | C18H35NO |
| Erucylamide | C22H43NO |

### 2.2. LC/MS/MS analysis of fish body burdens of NP

Fish from the PE2 and glass (reference) treatments were sent to an independent laboratory (Jupiter Analytix, Jupiter, FL, USA) for concentration of NP compounds in the whole fish using LC/MS/MS.

Whole fish were pulverized into a frozen powder, placed into stainless steel extraction cells for a Dionex Accelerated Solvent Extractor 200, and the total amount of tissue in each cell was weighed against the tared weight of the empty cell. The frozen tissue in each cell was mixed with $Na_2SO_4$. Extraction solvent was acetone-n-hexane (1:1, v/v). Recovery of spiked samples using dimethyl heptylphenol, 4-tert-butylphenol and P-tert-amylphenol were all above 90%.

Samples were extracted using C-18E cartridges (500 mg, 6 mL Phenomenex Inc.) on a vacuum manifold (Phenomenex Inc.). Cartridges were conditioned with 5 mL of methanol, then 5 mL of water, after which the samples were added to the column. Following extraction, the cartridges were dried for 10 min, capped and frozen until processed. The cartridges were eluted with 2 mL acetone followed by 2 × 5 mL dichloromethane. The extracts were evaporated to dryness under a gentle stream of nitrogen. Then, 50 μL of MSTFA (N-Methyl-N-(trimethylsilyl) trifluoroacetamide, Sigma–Aldrich) was added, capped, vortexed for 30 s, and heated at 80 °C for 30 min. Extracts were transferred to gas chromatography vials with a rinse step to a final volume of 1 mL and the internal standard was added. Percentage recovery for all 8 target analytes using this method with seawater was above 95%.

For LC–MS analysis, samples were run on an AB_SCIEX 5500 QTRAP Triple Quadrupole Hybrid Linear Ion Trap Mass Spectrometer with a Spark Holland Symbiosis HPLC for analytical separation. The analytes were measured with MRM (multiple reaction monitoring) followed by switching to ion trap functionality (Q3-LIT) to confirm the fragmentation pattern of the MRMs. The source was set at 700 °C and the gasses were set to 60 arbitrary units of nitrogen. The curtain gas was set at 45 arbitrary units and all MRMs were optimized using infusion based introduction of analytical standards. Analytical separation was performed using a Phenomenex Hydro RP 4.6 × 50 2.6 μm particle size stationary phase, with the mobile phase composed of methanol and water with the addition of 0.1% formic acid and 5 mM ammonium acetate in both phases. The flow rate was set at 0.9 mL per minute and a ballistic gradient and re-equilibration was run over 5 min. Percentage recovery for target analytes was above 85%, limit of detection was 100 pptrillion, and quantitative limit of measurement was 5 ppbillion. Quantitative standards for nonylphenol, 4-ter-butyl phenol and p-tert-amyl phenol were obtained from Accustandard (New Haven, CT).

### 2.3. GC/MS analysis of water samples

In order to optimize extraction for these compounds, a deviation from ECCS standard operating procedure (SOP) PRE-001, Separatory Funnel Extraction occurred. The samples were extracted with two 30 mL aliquots of methylene chloride under neutral conditions (pH 7–9) followed by 2 additional 30 mL aliquots of methylene chloride under acidic conditions ($pH \leqslant 2$), exchanged with iso-octane, concentrated to a 2 mL final volume, and analyzed by GC/MS techniques in accordance with specifications in ECCS SOP LAM-006. The samples were extracted in a single batch along with the following quality control samples: method blank, laboratory control sample, and standard reference material (SRM). The laboratory control sample and SRM were spiked with the three phenols listed above. All samples and quality control samples were also spiked with surrogates in accordance with specifications in ECCS SOP LAM-006.

Using the NIST98 library, select ions were chosen for identification and quantitation for each of the phenols. 4-Tert-butylphenol and p-tert-amylphenol are single peak analytes. The nonylphenol standard is a mixture of isomers that elute primarily in a narrow 1 m retention time window. Quantitation for nonylphenol was based on the total of the selected quantitation ion found in the established one minute retention time window.

Five point calibration curves were used to quantify the phenol compounds using the internal standard technique. Continuing calibration verification (CCV) injections occurred after every 10 samples. All CCV and quality control sample results met method criteria. Each sample was also analyzed for tentatively identified compounds with Agilent automated library search software.

### 2.4. Long-term survival following exposure to PE2 bags

Using the same conditions as described for section 2.1., 26 fish were placed into PE2 bags with oxygen for 48 h. For controls, 12 fish were placed in individual glass beakers, flushed with oxygen, and capped with Pyrex® glass petri lids for 48 h. Following the 48 h exposure, surviving fish from the PE2 bags ($n = 12$) and all fish from the glass beakers ($n = 12$) were then transferred to individual Pyrex glass bowls that contained 400 mL of synthetic sea water. Each bowl contained an aeration bubbler to maintain oxygen concentrations. Fish were maintained in these bowls for 8 d, and mortality was assessed at least every 24 h. Fish were fed a feed formulation of ventral muscle of tilapia (Blue Ridge Aquaculture. Martinsville, VA) and Spirulina (Sunny Green, Park City, UT).

### 2.5. Acute toxicity of nonylphenol

Orchid dottybacks (*P. fridmani*) (Sea and Reef Aquaculture, Hancock, Maine) were acclimated for a minimum of 7 d prior to the toxicity trials and were held communally during the acclimation in a glass 150 L tank system free of plastics containing NP. Seawater was created with reverse osmosis water mixed with a commercial synthetic sea salt mixture (Marine Enterprises, Crystal Sea Marinemix) to a salinity of 32 ppt. Aeration manifolds were made of stainless steel, and plastics used in the aeration system, including tubing and air pumps, were free of NP. The exposure room was maintained at 26.0 ± 0.9 °C. The fish were not fed 48 h prior to and during the 96 h exposure treatments. Standard grade NP was purchased from Accustandard (New Haven, CT) at a concentration of 2500 μg $mL^{-1}$ in MeOH:AcCN (50:50).

The experiments consisted of a control group, a vehicle control group that received a 50:50 mixture of MeOH:AcCN at a concentration matching that of the highest NP treatment, and 5 NP exposure treatments. Preliminary tests were performed to determine an appropriate toxicity range. On the basis of these tests, seven fish per group were exposed to the NP concentrations (10, 27, 73, 197 and 531 μg $L^{-1}$) in aerated glass beakers containing 800 mL synthetic sea water. Following this trial, a similar trial was conducted but used eight fish per treatment group and six concentrations with a more narrow NP concentration range (70, 100, 130, 160, 190 and 220 μg $L^{-1}$). During the 96 h treatments, water solutions appropriate to the treatment group were replaced every 12 h. Mortalities were recorded every 24 h.

### 2.6. Statistical analyses

Statistical analyses for water NP concentrations, as well as mortality data, were performed using Statview (SAS Institute, Cary, NC, USA). For NP concentrations in water, F-tests were conducted to ensure homogeneity. Analysis of variance (ANOVA) was used to test differences among treatment groups; if significance was determined ($p \leqslant 0.05$), Fisher's protected least-significant difference

was used to determine differences among treatment means. Mortality data were ARCSIN transformed prior to analysis, and a Chi-square analysis was used to determine significant differences from the control ($p \leqslant 0.05$). Lethal concentration estimates ($LC_{50}$) were calculated using SPSS (IBM SPSS Statistics 22.0) by the Probit method (±95% confidence limits) and used the Pearson goodness of fit test to ensure the model adequately fit the data. All experiments followed Institutional Animal Care and Use Committee guidelines.

## 3. Results

### 3.1. Vessel leachate and fish body burdens of NP compounds

Mortality of *P. fridmani* exposed to either glass, Teflon or one of two bags from different manufacturers labeled as FDA food grade polyethylene (PE1 and PE2) for 12, 24 and 48 h is shown in Table 2. No mortality was experienced in the glass or Teflon treatments for any time period. There was no mortality following the PE1 exposure during 12 and 24 h, and a slight but insignificant mortality after 48 h. Mortality of *P. fridmani* following the PE2 bag exposure was significantly elevated by 24 h ($p < 0.001$) and resulted in 60% total mortality by 48 h ($p < 0.001$).

Following the 48 h contact period, mean concentrations (±SE) of NP in the synthetic seawater that contained a single fish held in either Teflon, PE1 or PE2 bags were 5.9 ± 0.2, 2.5 ± 0.2 or 41.0 ± 5.5 μg $L^{-1}$ respectively (Fig. 1). Synthetic seawater NP concentrations in PE2 bags held for 48 h without a fish were 163.3 ± 5.7 μg $L^{-1}$. Aquatic NP concentrations in the Teflon and PE1 bags that contained a single fish were not statistically different, and were significantly lower than the PE2 bag containing a fish ($p < 0.001$). Aquatic NP concentrations in the PE2 bag that did not contain a fish were significantly higher than all other treatment groups.

Body burdens of NP contaminants (μg $kg^{-1}$ ± SD) in *P. fridmani* exposed to either glass or PE2 bags for 48 h is shown in Table 3. Concentrations of 4-tert-butyl phenol and p-tert-amyl phenol were non-detectable in fish from either treatment. Nonylphenol was below detection limits in fish exposed to glass, but was 368 ± 39 μg $kg^{-1}$ in fish exposed to the PE2 bags.

### 3.2. Long-term survival following exposure to PE2 bags

Mortality of *P. fridmani* that were placed into fresh synthetic seawater following a 48 h containment in PE2 bags is shown in Fig. 2. By day eight, 100% of the fish had died ($n = 12$). All fish in the control treatment ($n = 12$) survived.

**Table 2**
Mortality of *P. fridmani* following a 12, 24 or 48 h exposure to either glass ($n = 10$), Teflon ($n = 8$) or one of two plastic bags from different manufacturers labeled as FDA food-grade polyethylene (PE1, $n = 9$ and PE2, $n = 10$).

| Exposure treatment | Exposure duration (h) | Mortality (%) |
|---|---|---|
| Glass | 12 | 0 |
| | 24 | 0 |
| | 48 | 0 |
| Teflon | 12 | 0 |
| | 24 | 0 |
| | 48 | 0 |
| PE1 | 12 | 0 |
| | 24 | 0 |
| | 48 | 11 |
| PE2 | 12 | 0 |
| | 24 | 40* |
| | 48 | 60* |

* Significant mortality ($p < 0.05$) based on Chi Square analysis.

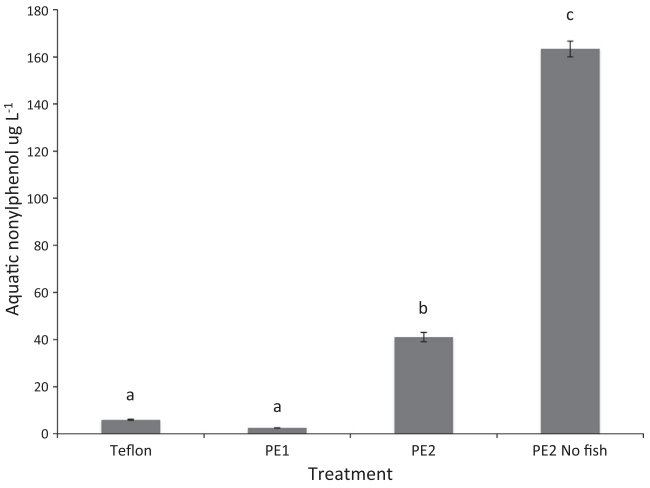


**Fig. 1.** Concentrations of nonylphenol (μg/L) in synthetic sea water in which a single fish was contained in a Teflon bag, one of two bags from different manufacturers both labeled as FDA food-grade polyethylene (PE1 and PE2), or a PE2 bag that did not contain a fish during the 48 h exposure period (PE2 No fish). Treatments with different superscripts are significantly different ($p < 0.05$).

**Table 3**
Concentrations of nonylphenol contaminants (±SD) in orchid dottybacks (*P. fridmani*) exposed to either glass ($n = 5$) or a commercial FDA food-grade polyethylene plastic bag (PE2) ($n = 8$) for 48 h.

| Exposure treatment | Contaminant | Concentration (μg/kg) |
|---|---|---|
| Glass | Nonylphenol | ND |
| | 4-tert-butyl phenol | ND |
| | p-tert-amyl phenol | ND |
| PE2 | Nonylphenol | 368 ± 39 |
| | 4-tert-butyl phenol | ND |
| | p-tert-amyl phenol | ND |

ND = Non-detectable.

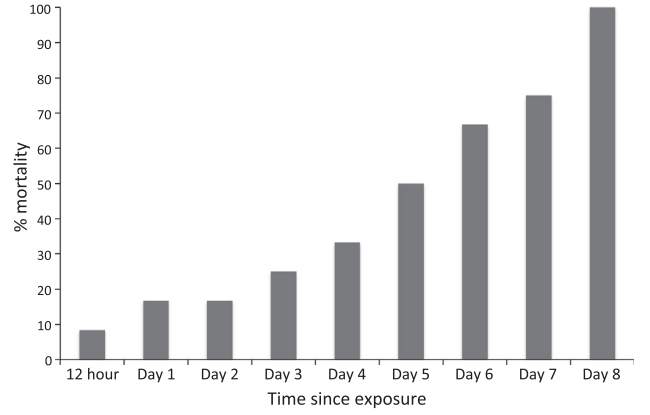


**Fig. 2.** Mortality (%) of *P. fridmani* following a 48 h exposure to an FDA food-grade polyethylene bag (referred to as PE2 in the text) ($n = 12$).

### 3.3. Acute toxicity of nonylphenol

No fish died in either the synthetic sea water or vehicle (MeOH:AcCN) control for either $LC_{50}$ trial. In the first trial, no fish died in the 10, 27 and 73 μg $L^{-1}$ NP concentrations, and all fish died in the 197 and 531 μg $L^{-1}$ concentrations. Because there were no partial mortalities, a Probit analysis could not be conducted for this trial. Mortality for each NP concentration in the second trial is shown in Fig. 3, and the 96 h $LC_{50}$ was estimated to be 175 ± 14 μg $L^{-1}$.

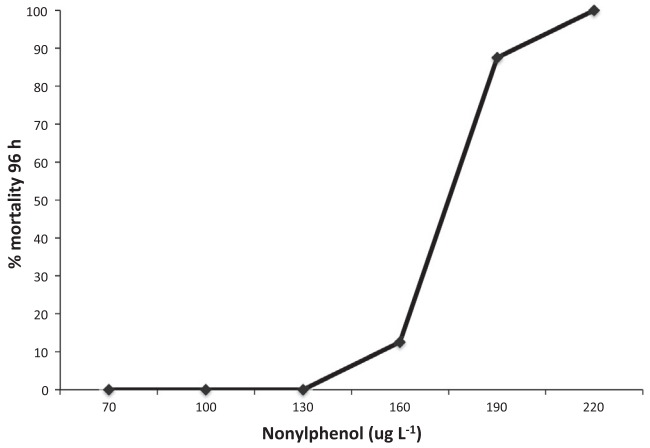


**Fig. 3.** Mortality data (96 h) versus nonylphenol concentration (μg/L) of *P. fridmani* ($n = 8$).

## 4. Discussion

This is the first study to show that certain FDA food-grade plastics leach concentrations of NP that are highly toxic to fish. The mean concentration of NP that migrated from the PE2 plastic bags (163 μg $L^{-1}$) was similar to that shown to be toxic to dottybacks using the $LC_{50}$ criterion (175 μg $L^{-1}$) (Figs. 1 and 3). Removing the fish from the PE2 bags after a 48 h exposure and placing them in fresh seawater free of NP resulted in 100% mortality after eight days, showing that the effects of the NP exposure caused irreversible damage to this species (Fig. 2). Teflon bags, as well as another FDA food-grade polyethylene bag from a different manufacturer than the PE2 bags, showed no significant mortality and significantly less aquatic NP than the PE2 bags. Although 96 h $LC_{50}$ estimates for NP can vary considerably, most fish species tested to date fall in the range of 100–400 μg $L^{-1}$ (Staples et al., 1998; Kelly and Di Giulio, 2000; TenEyck and Markee, 2007; Bhattacharya et al., 2008; Lu et al., 2012). Therefore, the 96 h $LC_{50}$ estimate for NP in this study (175 μg $L^{-1}$) aligns with other species, and supports that orchid dottybacks are not especially sensitive to NP, and results observed in this study are likely applicable to many other fish species.

Nonylphenols are a ubiquitous environmental contaminant, and enter aquatic systems through a number of anthropogenic sources (Ying et al., 2002; Soares et al., 2008). Incomplete breakdown of NPs in wastewater treatment (WWT) can result in high effluent concentrations, and WWT effluent is considered a significant contributor to environmental burdens. Wastewater discharge into the river Aire in Europe contained 330 μg $L^{-1}$ NPs, and concentrations up to 180 μg $L^{-1}$ were measured at sites along the river (Blackburn and Waldock, 1995). The US EPA water quality criteria (WQC) for acute and chronic exposure to NP is 7 and 1.7 μg $L^{-1}$ respectively in seawater, and approximately four times higher for freshwater (EPA, 2005). That surface water concentrations of NP exceed the EPA's WQC in a number of locales, and exceed concentrations shown to be toxic in this study, raises concern for the health of aquatic species living in these regions. Additionally, NP concentrations of estuarine seawater in Morro Bay, CA in the US, were shown to be four times lower than the chronic WQC set by the EPA, yet resident arrow gobies (*Clevelandia ios*) showed NP body burdens of 237 ng $g^{-1}$ (Diehl et al., 2012). The orchid dottybacks in this study readily absorbed the NP, either through ingestion or contact with skin and gills, and body burdens of 368 ng $g^{-1}$ NP were shown to result in 100% mortality; it is therefore likely the aquatic concentrations of NP in some natural environments could pose a considerable threat to aquatic life, even when aquatic concentrations are below the WQC.

A recent study estimated that up to 12.7 million metric tons of plastic waste entered the ocean in 2010 alone, and the quantity of plastic waste available to enter the ocean is predicted to increase by an order of magnitude by 2025 (Jambeck et al., 2015). Contaminants from plastic waste leach into the surrounding water, and the plastics themselves can be ingested by marine organisms (Derraik, 2002; Andrady, 2011; Gassel et al., 2013; Goldstein and Goodwin, 2013; Koelmans et al., 2014). The North Pacific Central Gyre is a site of considerable plastic contamination. Studies have shown that yellowtail (*Seriola lalandi*) from this region had whole body concentrations of 53 ng $g^{-1}$ NP, and the mean whole body concentration was more than three times that if researchers excluded fish from which NP was not detected (Gassel et al., 2013). Researchers concluded that plastic mediated exposure best explained their findings of NP in the yellowtail. It has been argued that low diffusivities of NP from several plastics suppose an assumption of low risk (Berens, 1997; Koelmans et al., 2014). Our study supports that differences in the manufacture of some plastics can underscore disparities in the migration of contaminants. In this way, estimates of contaminant exposures from plastics could be significantly underestimated.

With rare exception, fish and invertebrates for ornamental trade are transported in plastic bags. Both polyethylene bags evaluated in this study (PE1 and PE2) are marketed and used for ornamental fish trade, as well as food packaging. This study shows that although the product may have an identical label (i.e. FDA food-grade polyethylene), the toxicity of these products can vary considerably between manufacturers. Although this study evaluated the ability of NP to migrate into seawater, it is likely food held in the PE2 bags could receive increased levels of NP as well. Guenther et al. (2002) showed that the content of NP in food varied between 0.1 and 19.4 ng $g^{-1}$, regardless of the food's fat content. This is interesting given that NP is lipophilic and could be expected to readily migrate into fatty foodstuffs over foods with lower lipid content. This indicates that the variability of NP content in foods could be driven largely by the resistance of the packaging material to NP migration. Our study supports a high variability of NP migration from plastic, including plastics that are considered safe for food packaging.

## 5. Conclusion

Our study revealed that NP can migrate from food-safe plastic at levels shown to be toxic to fish. This study highlights that NP could pose a greater risk to humans and wildlife than might be estimated from testing the properties of plastic from a single manufacturer, which is the current paradigm in plastic toxicity testing.

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