Final Progress Report (2016-2017)

MOU between Environmental Protection Operations (Contaminated Sites) and Water Science and Technology (Aquatic Contaminants Research)

Assessing the lethal and sublethal toxicity of perfluorooctanoic acid (PFOA) to *Hyalella azteca* and *Pimephales promelas*

Report prepared by:

Adrienne Bartlett, Amila De Silva, Daniel Schissler, Amanda Hedges, Lisa Brown, Kallie Shires, Jason Miller, Cheryl Sullivan, Christine Spencer, and Joanne Parrott

Environment and Climate Change Canada

Aquatic Contaminants Research Division, Burlington, Ontario. 35 pp.
October 27, 2017

Environment and Climate Change Canada | Environnement et Changement climatique Canada

RESEARCH OBJECTIVE

To conduct chronic, aqueous, laboratory exposures with PFOA to assess the lethal and sublethal toxicity to *Hyalella azteca* (amphipod) and *Pimephales promelas* (fathead minnow).

ABSTRACT

Perfluoroalkyl substances (PFASs) are used in a variety of industrial and commercial products, including surfactants, polymers, lubricants, adhesives, paints, household cleaners, pesticides, and fire-fighting foams. Significant environmental concerns are associated with PFASs due to their persistence, potential for bioaccumulation, toxicity, and capacity for long-range transport. PFASs, including perfluorooctanoic acid (PFOA), are widely present in the Canadian environment, particularly at some contaminated sites due to historic firefighting training operations. Environmental concentrations of PFOA can be 10s of µg/L in streams close to industrial spills. Despite a large body of research on environmental exposure, the toxicity of PFOA is an emerging field of study, and insufficient aquatic toxicity data exist to develop water quality guidelines. Thus, our objective was to conduct chronic, aqueous exposures with PFOA to assess the lethal and sublethal toxicity to *Hyalella azteca* (amphipod) and *Pimephales promelas* (fathead minnow). Amphipod exposures were 6 weeks (1-100 mg/L nominal) and examined survival, growth, and reproduction. Fathead minnow exposures were 21 days (0.01 to 100 mg/L nominal) and covered the period of hatching (5 days) and larval stages (until 16 days post-hatch, dph); endpoints included hatching success, deformities at hatch, larval survival, and growth. Measured PFOA concentrations in exposures were 80-90% nominal; therefore, toxicity data were expressed as nominal. Amphipod survival was significantly reduced at 100 mg/L, with a 6-week LC50 of 53 mg/L. Growth and reproduction of amphipods were more sensitive endpoints than survival, with 6-week EC50s of 2.3-2.4 mg/L. Fathead minnows were less sensitive than *Hyalella*, with only a 10% decrease in larval survival at 100 mg/L. There were some indications of increased deformities in larval fish at 100 mg/L, but these were not statistically significant. Hatching success and growth of larval fish were not affected by PFOA exposure up to 100 mg/L; however, PFOA concentrations of 500 mg/L did cause 40% mortality in fathead minnow at the hatching stage. Maximum concentrations of PFOA in the surface waters of the Great Lakes are generally < 50 ng/L, and as the toxicity of PFOA to amphipods and fathead minnows occurred at concentrations > 1 mg/L, it is likely that most environmental concentrations are far below those that cause toxicity to these species. However, localized areas could be highly contaminated due to historical activities or recent spills (where concentrations as high as 11 µg/L have been found). Our data will provide valuable information with which to assess the risk of PFOA at contaminated sites, and to set a target for site remediation.

METHODS

PFOA solutions

PFOA was purchased from Sigma Aldrich (96% purity, $CF_3(CF_2)_6COOH$, Molecular Weight: 414.07, CAS Number 335-67-1), and stock solutions of 100 mg/L (amphipod tests) and 1000 mg/L (fathead minnow tests) were made in culture water without use of a solvent, as the solubility of PFOA is reported to be 20,000 mg/L (US EPA 2002). Waste PFOA solutions were saved in 200-L barrels and disposed of as fluorinated waste via a professional hazardous waste disposal company.

Hyalella azteca

Detailed culturing methods for *H. azteca* are described by Borgmann et al. (1989). Cultures and experiments were maintained in dechlorinated municipal tap water (Burlington, Ontario, Canada, originating from Lake Ontario; hardness 120-140 mg/L, alkalinity 90-110 mg/L, pH 8.2-8.6) at 25 °C with a photoperiod of 16 h light:8 h dark. Amphipods were fed finely ground Tetra-Min fish food flakes (Tetra GMBH, Melle, Germany), and juveniles were removed from breeding containers weekly for use in toxicity tests (i.e., age of amphipods was 2-10 d at test initiation).

Six-week aqueous, static-renewal toxicity tests were conducted to assess the effects of PFOA on amphipod survival, growth, and reproduction. The nominal concentration range was based on the results of a one-week static range-finding test (LC50 = 99 mg/L; Table 1): 1, 3, 10, 30, and 100 mg/L. Each test also included a negative control (culture water). Tests were conducted in 2-L HDPE containers, and were initiated by adding 2.5 mg Tetra-Min, 1 piece of 5 cm x 5 cm cotton gauze, 1 L of exposure solution, and 20 juvenile amphipods to each container. Amphipods were removed from containers weekly, counted, and transferred to clean containers with fresh test solution and fresh cotton gauze to minimize accumulation of uneaten food and waste products (e.g., ammonia) and to allow for weekly monitoring of survival and reproduction during the test. Amphipods were fed Tetra-Min as follows: 2.5 mg three times during weeks 1 and 2, 5 mg three times during weeks 3 and 4, and 5 mg five times during weeks 5 and 6. At the end of the six-week exposure, the number of adults, mating pairs, and juveniles from each beaker was recorded. Adult *Hyalella* were examined under a dissecting microscope to identify males and females, and the number of each was recorded. Male amphipods were identified by the presence of enlarged second gnathopods, and females were identified by the absence of enlarged second gnathopods and/or the presence of eggs/juveniles under the carapace. Amphipods which were too small to distinguish if they were male or female were classified as indeterminate. Adult amphipods were then rinsed three times in clean water, blotted dry, weighed as a group to determine wet weights, and transferred to a small beaker containing 50 mL clean water for 24-h to clear their guts. Following gut clearance, amphipods were blotted dry, transferred to cryovials, and frozen at – 20 °C. Two tests were conducted, with each test consisting of five replicates per treatment.

Water quality parameters (pH, conductivity, DO, and total ammonia) were measured at the beginning (one replicate per treatment per test) and end (all replicates) of each renewal period, and

are summarized in Table 2.

Water samples (10 mL) were collected from each treatment in 15-mL HDPE Falcon tubes at the beginning and end of each renewal period for chemical analysis of exposure concentrations. Equivalent volumes were collected from each replicate and then pooled to obtain an average exposure concentration for each treatment (i.e., 2 mL/replicate from 5 replicates to obtain each 10 mL sample). A subset of these samples was submitted for chemical analysis as follows: all samples from the beginning and end of week 1 and week 6 renewals from one test to determine the full range of exposure concentrations and confirm that it did not differ between the first and last renewal period, and all samples from both tests at 10 mg/L nominal to confirm that exposure concentrations remained consistent between weeks and between tests. This was done in order to reduce the number of samples analyzed, but still provide a thorough characterization of exposure during the tests.

Pimephales promelas

Animal Care

All fathead minnow exposures to PFOA and sampling and euthanasia methods were conducted under an approved animal use protocol (# 1640 and # 1740) from the Department of Fisheries and Oceans / Environment and Climate Change Canada Joint Animal Care Committee for the Canada Centre for Inland Waters (Burlington, ON, Canada), operated under the approval of the Canadian Council of Animal Care.

Fathead minnow early-life stage assays were performed in accordance with OECD TG 210 (OECD 2013) guidelines, but typically ended 16 days post-hatch (dph). Replicates were started with eggs from 5-10 egg batches (from different fathead minnow breeding groups) to maximize genetic diversity and variability. There were 20 eggs per beaker, with 8 replicates of controls and 4 replicates of each PFOA exposure concentration in each test. Thus, the group of fish tested in this experiment would represent responses of larval fish from over 20 different fathead minnow breeding groups. All water quality parameters were within acceptable limits and there were no issues with water quality over the test periods.

Special considerations for PFOA fish exposures

Fathead minnow embryos and larvae were held in glass, Nitex mesh-bottomed (mesh size 500 µM) egg cups within 800-mL HDPE beakers filled to 700 mL. For this experiment HDPE beakers were used rather than glass beakers, as PFOA is reported to bind to glass and we wanted to minimize the glass surfaces in the exposure. Use of the mesh-bottomed egg cup allows for circulation of the exposure solution, and facilitates daily changes of the PFOA exposure solutions as fish can be transferred quickly to new beakers containing new PFOA exposure solutions (Figure 1). We used 700 mL of exposure solution to reduce the volume of PFOA solutions and thus the amount of waste solution generated by our tests. The loading rate of fish in 700 mL was still lower than that recommended in

aquatic bioassay guidelines. The fish were 5 mg at 9 dph and 14 mg at 16 dph, so the maximum loading was 100 mg at 9 dph or 140 mg at 16 dph (the end of the test). In 700 mL of PFOA solution, the maximum loading was 0.2 mg/mL/d or 0.2 g/L/d, which is lower than the guideline of 0.3-0.5 g/L/d (Sprague 1969; 1973).

PFOA fathead minnow exposure conditions

Water used for the PFOA fish exposures and exposure solutions was charcoal filtered UV-sterilized Burlington City Water sourced from Lake Ontario (hardness 120-130 mg/L, alkalinity 89-93 mg/L, pH 7.4-7.8). Fathead minnows were exposed to nine nominal PFOA concentrations of 0.01, 0.032, 0.1, 0.32, 1, 3.2, 10, 32, and 100 mg/L. PFOA exposures of fathead minnow embryos/larvae were 21 days in length (5 days in the egg stage, and 16 days post-hatch). Exposures were divided into a low concentration exposure (0.01-10 mg/L) and a high concentration exposure (32-100 mg/L). Each exposure had 4 replicates of each PFOA concentration and 8 replicates of controls (lab water). Each replicate beaker contained 20 newly-fertilized fathead minnow eggs at the start.

Newly-fertilized fathead minnow eggs were purchased from a supplier (Aquatox Labs, Guelph, ON). Eggs were (< 18 h post fertilization). Briefly, beakers containing 20 fathead minnow eggs/larvae were aerated, loosely covered with a parafilm lid, and held in a 25 °C incubator with 16 h light and 8 h dark (with dawn and dusk dimming). Incubators were checked daily for temperature, and solutions were measured weekly for temperature, dissolved oxygen (DO), conductivity, and pH. Feeding of the fathead minnow fry began on the day of hatch. Larvae were fed twice per day, 10 µL/fish (0 to 9 dph) and 20 µL/fish (9-16 dph) of a newly-hatched brine shrimp slurry (mean density of 6 nauplii/µL, brine shrimp < 24 h old). The first feeding (of half the daily aliquot) was 2 hours prior to the daily solution changeover (to remove excess food and waste), and the second feeding (the other half of the daily food aliquot) was after solution changeover so that food was available at all times during the exposures.

Embryos and larvae were inspected each day for mortalities, which were recorded and removed. Severely deformed and/or immobile larvae with necrosis, but still with heartbeats, were described, removed, and euthanized via immersion in tricane methane sulfonate solution (250 mg/L). The number of larvae in the beaker was randomly culled at day 14 of the test (9 dph) to a maximum of 10 larvae. The culled individuals were assessed for total length to 0.01 mm (at 6.3x magnification), and mass (to 0.01 mg). At day 21 of the test (16 dph), all remaining surviving larvae were euthanized and similarly assessed (length and weight measured, and condition factor (CF = weight/length^3) was calculated.

Water quality parameters, including temperature, pH, conductivity, DO, and total ammonia were measured in each test container at three stages: egg, first larval week, and second larval week (Table 3).

Endpoints in fathead minnow assay

Survival was calculated at several time points during each test. Survival from 'egg to hatch' was the # larvae hatching on day 4-5/# eggs in the replicate beaker at the start. Survival from 'egg to 9 dph' was the # larvae alive on day 9 post-hatch (prior to the cull)/# eggs in that replicate beaker at the start. Survival from '9 dph to 16 dph' was the # larvae alive on day 16 post-hatch/# larvae left after the cull on day 9 post-hatch (usually 10 larvae). Survival from 'egg to 16 dph' was calculated as the product of 'egg to 9 dph' and '9 dph to 16 dph' survival rates.

Endpoints were % survival to hatch, time to hatch, hatching success, deformities at hatch, survival from the egg until 9 and 16 dph, and weight, length, tail length, and condition factor (CF) of larvae at 9 and 16 dph.

Chemical analysis of exposure water

All exposure water samples were diluted with HPLC-grade water (Fisher Scientific, Ottawa) to ensure quantification was within the linear dynamic range of the instrument. The diluted exposure water was subsampled (1 mL) and spiked with isotopically labeled PFOA which served as an internal standard. The final concentration of the internal standard was 1.3 ng/mL $^{13}C_{1,2,3,4}$-PFOA (Wellington Labs, Guelph) prior to analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Dilution factors were specific to target concentrations and are shown in Table 4. Quantification of PFOA was by relative response to the internal standard using a 15-level calibration curve: 0.05, 0.07, 0.10, 0.16, 0.26, 0.36, 0.55, 0.86, 1.4, 2.1, 3.0, 4.1, 6.3, 9.5, 14 ng/mL PFOA ($R^2 > 0.99$). Method blanks consisted of HPLC-grade water and internal standard and were free of PFOA. Instrumental parameters are presented in Table 5. The instrument detection limit for PFOA was 0.03 ng/mL based on a signal-to-noise ratio of 3.

For amphipods, each treatment was analyzed at the beginning (t = 0 d) and end (t = 7 d) of the static renewal period in duplicate. To better ascertain variability in analysis, the 10 mg/L treatment was analyzed in duplicate from 6 different exposures at two time points (t = 0 d, t = 7 d) for a total of 24 samples. The mean ± standard deviation concentration for the nominal 10 mg/L treatment was 8.9 ± 0.73 mg/L, corresponding to a relative standard deviation of 8.2%.

Statistical analysis

Statistical analyses for all toxicity tests were performed using SYSTAT 12 for Windows (SYSTAT Software). Methods described in Bartlett et al. (2004) were used to determine lethal concentrations of PFOA resulting in 10, 25, and 50% mortality (LC10s, LC25s, and LC50s), and effect concentrations of PFOA causing a 10, 25, and 50% reduction in growth or reproduction of *H. azteca* (EC10s, EC25s, and EC50s).

Mortality rates (m) were calculated using Equation 1

$$m = -\ln(N_{final}/N_{initial})/t \quad (1)$$

where t is time (in weeks), N_{final} is the number of animals surviving at t, and $N_{initial}$ is the number of

animals at t = 0. If mortality was 100% in one of the replicates at the end of the exposure, 0.5 animal was assumed to have survived for the purpose of calculating m. This was only done for the lowest concentration resulting in 100% mortality. Mortality rates were fourth-root transformed and then fitted to the nonlinear regression model described by Equation 2

$$m = m' + a(C_{PFOA})^n$$

$$= m' + \ln(2)/t \times (C_{PFOA}/LC50)^n \quad (2)$$

where m' is control mortality, C_{PFOA} is the nominal concentration of PFOA in water (mg/L), and a and n are constants. The LC25s and LC10s for each week were calculated using Equations 3 and 4

$$LC25 = (\ln[4/3]/\ln[2])^{1/n} \times LC50 \quad (3)$$

$$LC10 = (\ln[10/9]/\ln[2]^{1/n} \times LC50 \quad (4)$$

Model parameter estimates, 95% CIs, and r^2 values were provided by SYSTAT. Control mortality rates and values of n are summarized in Table 6.

Growth of amphipods was measured as average growth per replicate at the end of week 6 (wet weight/N_{final}). Growth EC50s were determined on log-transformed data using the nonlinear regression model described by Equation 5

$$G = max/(1 + a[C_{PFOA}]^n)$$

$$= max/(1 + [C_{PFOA}/EC50]^n) \quad (5)$$

where G is growth, max is the maximum G at C_{PFOA} = 0, and EC50 is the concentration at G = 0.5max. The EC25s and EC10s for growth were calculated using Equations 6 and 7

$$EC25 = EC50/3^{1/n} \quad (6)$$

$$EC10 = EC50/9^{1/n} \quad (7)$$

Model parameter estimates, 95% CIs, and r^2 values were provided by SYSTAT. Values for max and n are reported in Table 6.

Amphipod reproduction was defined in this study as the total number of live juveniles produced per female per test container. Reproduction data were log-transformed, and a value of 1 was added to allow the transformation of data from test containers that produced no juveniles. Reproduction EC50s were estimated using the nonlinear regression model described by Equation 8

$$J + 1 = (max + 1)/(1 + a[C_{PFOA}]^n)$$

$$= (max + 1)/(1 + [C_{PFOA}/EC50]^n) \quad (8)$$

where J is total number of juveniles produced per female in each replicate, max is the maximum J at

C_{PFOA} = 0, and EC50 is the concentration at J + 1 = 0.5(max + 1). The EC25s and EC10s for were calculated as described for growth above (Equations 6 and 7).

Model parameter estimates, 95% CIs, and r^2 values were provided by SYSTAT. Values for max and n are reported in Table 6.

Data from individual amphipod tests were analyzed first, and then the duplicate tests were pooled for statistical analysis, as the differences between LC50s (or EC50s) were two-fold or less, and data between experiments were visually indistinguishable.

Amphipod and fathead minnow toxicity data were analyzed using a one-way analysis of variance (ANOVA) to determine if observed effects were significantly different from controls. If ANOVAs were significant ($p < 0.05$), Tukey's honestly significant difference post hoc test was used for pairwise comparisons. These methods were used to assess the following amphipod endpoints: transformed (as described above) mortality rate, growth, and reproduction data, as well as untransformed data on percent male, percent female, and percent indeterminate amphipods. Untransformed data for fathead minnow hatch success, survival from egg to 16 dph, deformities at 16 dph, and growth at 16 dph (both mass and length of fry) were also evaluated.

RESULTS

Measured PFOA concentrations in exposure waters were close to nominal and remained stable during the renewal period. Average measured concentrations at the beginning and end of renewals were 93% (standard deviation [SD] = 9.5%) and 92% (SD = 17%) of nominal for *Hyalella*, and 82% (SD = 26%) and 90% (SD = 23%) for fathead minnow, respectively (Figure 2). As measured concentrations were a close approximation of nominal, the statistical analysis of toxicity data was conducted based on nominal concentrations. The raw data for measured PFOA concentrations in amphipod and fathead minnow exposures are summarized in Tables 7 and 8.

Survival of amphipods was reduced by PFOA exposure, and the dose-response relationship was described well by the nonlinear regression models (r^2 = 0.87-0.91; Figure 3, Table 9). Toxicity increased approximately 2-fold over the duration of exposure, with LC50s of 120 mg/L after 1 week and 53 mg/L after 6 weeks (Table 9). Survival decreased significantly compared to controls at 30 and 100 mg/L, dropping from 95 to 74% of controls at 30 mg/L and 60 to 3% of controls at 100 mg/L during the 6-week exposure (Figure 4).

Amphipod growth also decreased in response to PFOA exposure, but at concentrations much lower than survival. The EC50 for growth was 2.4 mg/L, 20-fold lower than the 6-week LC50, and the nonlinear regression model fit the data well (r^2 = 0.91; Figure 5A, Table 9). Amphipods were significantly smaller than controls at 3 mg/L and higher (Figure 6A), with sizes ranging from 50% of controls (3 mg/L) down to 7% of controls (100 mg/L).

As a result of the decreased growth of amphipods exposed to PFOA, reproduction and development of amphipods were also reduced. The EC50 for reproduction was 2.3 mg/L, similar to that for growth, although the data were more variable which resulted in a poorer fit of the nonlinear regression (r^2 = 0.47; Figure 5A, Table 9). Reproduction was significantly lower than controls at 3 mg/L (7% of controls) and 10 mg/L (no juveniles produced at 10 mg/L; Figure 6B). The proportion of males to females was approximately equal in controls, at 42% and 47% respectively, with 11% of amphipods classified as indeterminate (Figure 7). A similar proportion occurred at 1 mg/L, with 45% males, 40% females, and 15% indeterminate. However, the reduced size of amphipods at 3 mg/L and 10 mg/L made it increasingly difficult to differentiate between males and females, with 55% and 85% of amphipods classified as indeterminate, respectively), and at 30 mg/L and 100 mg/L, 100% of amphipods were classified as indeterminate (Figure 7). Amphipods that were classified as indeterminate were small, undeveloped, and resembled juveniles.

The raw data for all endpoints measured in amphipod exposures are summarized in Table 10.

Fathead minnows were less sensitive than *Hyalella*. There were no effects on hatch success, and there was only a 10% decrease in larval survival at 100 mg/L, which was not statistically significant (p = 0.7; Figure 8). There were some indications of increased deformities in larval fish at 100 mg/L, but these were not statistically significant and there was no dose-response relationship (p = 0.5; Figure 8). Growth of larval fish was not affected by PFOA exposure up to 100 mg/L (Figure 9). In range-finding tests, where fathead minnows were exposed from egg to hatch, hatch success at 500 mg/L was reduced to 60% (controls = 100%) and the incidence of deformities in hatched fry was 25% at 500 mg/L and 20% at 250 mg/L (controls = 0%; Table 11).

The raw data for all endpoints measured in fathead minnow exposures are summarized in Tables 12 and 13.

KEY FINDINGS

1. Amphipods were more sensitive than larval fathead minnows to aqueous PFOA exposures
2. Amphipods
    a. Sublethal endpoints were more sensitive than survival: survival was significantly reduced at 30 mg/L and higher, growth was significantly reduced at 3 mg/L and higher
    b. Strong growth effects likely caused the decrease in reproduction, as amphipods were too small and underdeveloped to reproduce
3. Fathead minnows
    a. There were no significant effects for any endpoint measured in embryo-larval tests
    b. Results from range-finding tests from egg to hatch: hatch success was 60% at 500 mg/L (controls = 100% hatch success), incidence of deformities in hatched fry was 25% at 500 mg/L and 20% at 250 mg/L (controls = 0% deformities)
4. PFOA toxicity in amphipods was observed at concentrations well below the reported water

solubility of PFOA of 3.5 g/L (Barton et al. 2007)

ACKNOWLEDGEMENT

The study was funded by Environment and Climate Change Canada's Environmental Protection Division. We are grateful for the technical assistance of Lisa Brown, Amanda Hedges, Jason Miller, Daniel Schissler, Kallie Shires, Christine Spencer, and Cheryl Sullivan for assisting in amphipod and fish exposures and in chemical analyses.

REFERENCES

Bartlett AJ, Borgmann U, Dixon DG, Batchelor SP, Maguire RJ. 2004. Accumulation of tributyltin in *Hyalella azteca* as an indicator of chronic toxicity: Survival, growth, and reproduction. Environ Toxicol Chem 23:2878-2888.

Barton CA, Kaiser MA, Russell MH. 2007. Partitioning and removal of perfluorooctanoate during rain events: The importance of physical-chemical properties. J Environ Monit 9:839-846.

Borgmann U, Ralph KM, Norwood WP. 1989. Toxicity test procedures for *Hyalella azteca*, and chronic toxicity of cadmium and pentachlorophenol to *H. azteca*, *Gammarus fasciatus*, and *Daphnia magna*. Arch Environ Contam Toxicol 18:756-764.

OECD. 2013. Test Guideline no. 210: Fish, early-life stage toxicity test. OECD Publishing. Available from http://www.oecd.org/chemicalsafety/risk-assessment/1948269.pdf

Sprague JB. 1969. Measurement of pollutant toxicity to fish i. Bioassay methods for acute toxicity. Water Research. 3(11):793-821.

Sprague JB. 1973. The ABC's of pollutant bioassay using fish. American Society for Testing and Materials. (STP 528):6-30.

USEPA. 2002. Revised draft hazard assessment of perfluorooctanoic acid and its salts. OPPT, Risk Assessment Division. Washington, DC. Available from http://www.fluoridealert.org/wp-content/pesticides/pfoa.epa.nov.4.2002.pdf

TABLES

Table 1. Raw data from one-week aqueous static range-finding PFOA exposures with *Hyalella azteca*. TRT = treatment (nominal PFOA [mg/L], C = control), REP = replicate, N0 = number of amphipods added at the start of the test, N1 = number of amphipods surviving at the end of the test.

TRT	REP	N0	N1
C	1	15	15
C	2	15	15
C	3	15	15
0.001	1	15	15
0.001	2	15	15
0.001	3	15	15
0.01	1	15	15
0.01	2	15	14
0.01	3	15	14
0.1	1	15	15
0.1	2	15	15
0.1	3	15	15
1	1	15	14
1	2	15	15
1	3	15	15
10	1	15	14
10	2	15	15
10	3	15	15
100	1	15	8
100	2	15	9
100	3	15	9
500	1	15	0
500	2	15	0
500	3	15	0

Table 2. Water quality parameters measured in 6-week aqueous static-renewal PFOA tests with *Hyalella azteca* at the beginning (t = 0 d) and end (t = 7 d) of weekly renewals.

Renewal Sampling Time		pH	Conductivity (mS/cm)	Dissolved Oxygen (mg/L)	Total Ammonia (mg/L)
Beginning (t = 0 d)	Mean	8.1	0.41	8.0	ND[a]
	SD[b]	0.18	0.016	0.35	ND
	Min	7.7	0.38	7.1	ND
	Max	8.4	0.46	8.6	ND
	N	72	72	72	72
End (t = 7 d)	Mean	8.1	0.41	7.1	0.20
	SD	0.29	0.021	0.70	0.24
	Min	7.5	0.37	5.2	ND
	Max	9.4	0.52	8.8	1.0
	N	354	354	354	354

[a] ND = not detected

[b] SD = standard deviation

Table 3. Water quality parameters measured in 21-d aqueous static-renewal PFOA tests with *Pimephales promelas* at the beginning (t = 0 d) and end (t = 7 d) of weekly renewals.

Stage		Temperature (°C)	pH	Conductivity (mS/cm)	Dissolved Oxygen (mg/L)	Total Ammonia (mg/L)
Egg	Mean	23	8.5	350	8.0	ND[a]
	SD[b]	0.40	0.042	9.4	0.13	ND
	Min	22	8.3	340	7.8	ND
	Max	24	8.6	380	8.2	ND
	N	52	52	52	52	52
First larval week	Mean	23	8.4	370	7.9	0.10
	SD	0.74	0.054	12	0.092	0.12
	Min	22	8.2	350	7.7	ND
	Max	25	8.5	400	8.1	0.30
	N	52	52	52	52	52
Second larval week	Mean	24	8.3	360	7.9	0.020
	SD	0.58	0.055	8.0	0.081	0.035
	Min	22	8.2	350	7.8	ND
	Max	25	8.4	380	8.2	0.30
	N	52	52	52	52	52

[a] ND = not detected

[b] SD = standard deviation

Table 4. Dilution of PFOA exposure water in amphipod and fish experiments for chemical analysis. PFOA exposure solutions were very concentrated and required dilution prior to chemical analysis.

Treatment (nominal)	Dilution factor[a]
Amphipods	
Control	2
1 mg/L	200
3 mg/L	600
10 mg/L	1000
30 mg/L	2000
100 mg/L	50000
Fish	
Control	2
0.01 mg/L	2
0.032 mg/L	4
0.1 mg/L	20
0.32 mg/L	80
1.0 mg/L	200
3.2 mg/L	500
10 mg/L	1500
32 mg/L	2000
100 mg/L	50000

[a] Exposure water was diluted with HPLC-grade water (Fisher Scientific, Ottawa, ON)

Table 5. Instrumental parameters for PFOA analysis by LC-MS/MS.

Liquid Chromatograph	
Instrument	Waters Acquity
Mobile phase	A: 0.1 mM ammonium acetate in water, B: Methanol
Gradient elution	Initial conditions 25% B held for 0.5 min Ramp to 85%B at 5 min Increase to 100% B at 5.1 min and hold for 2 min Revert to equilibrium conditions at 9 min and hold 4 min
Injection volume	9 µL
Stationary phase	Acquity C18 column BEH, (2.1 x 100 mm, 1.7 µm)
Column temperature	50 °C
Triple Quadrupole Mass spectrometer	
Instrument	Waters Xevo TQS
Ionization mode	Electrospray negative ionization
Source temperature	150 °C
Desolvation temp.	450°C
Capillary	0.60 kV
Precursor to product ion transitions	413 → 169 m/z (cone 8, collision 18) 413 → 369 m/z (cone 16, collision 10)

Table 6. Estimated parameters for nonlinear regression models used to calculate effects of PFOA on survival, growth, and reproduction of *Hyalella azteca* during 6-week aqueous static-renewal exposures.

Week	Effect	Parameter	Test 1	Test 2	Combined Tests
1	Survival	m’	0.028	0.041	0.034
		n	2.2	3.0	2.6
2	Survival	m’	0.016	0.029	0.022
		n	2.1	1.9	2.0
3	Survival	m’	0.015	0.026	0.020
		n	2.5	1.9	2.2
4	Survival	m’	0.014	0.023	0.018
		n	2.7	1.9	2.2
5	Survival	m’	0.015	0.021	0.018
		n	2.8	2.0	2.4
6	Survival	m’	0.016	0.022	0.19
		n	2.6	2.1	2.4
	Growth	max	3.3	2.4	2.9
		n	0.72	1.1	0.8
	Reproduction	max	1.7	1.5	1.6
		n	1.2	0.37	0.50

Table 7. Raw data for measured exposure concentrations in aqueous, static-renewal PFOA exposures with *Hyalella azteca*. TRT = treatment (nominal PFOA [mg/L], C = control), TEST = test number, WEEK = sampling week, DAY = sampling day (0 = beginning and 7 = end of static renewal period), MEAS = measured PFOA (mg/L).

TRT	TEST	WEEK	DAY	MEAS	TRT	TEST	WEEK	DAY	MEAS
C	1	1	0	9.70×10^{-5}	10	2	1	0	10.84
C	1	6	0	4.51×10^{-5}	10	2	1	7	7.33
C	1	1	7	4.16×10^{-4}	10	2	2	0	8.93
1	1	1	0	0.89	10	2	2	7	9.08
1	1	1	7	0.85	10	2	3	0	8.50
1	1	6	0	0.77	10	2	3	7	8.51
1	1	6	7	0.84	10	2	4	0	9.20
3	1	1	0	2.75	10	2	4	7	8.68
3	1	1	7	4.78	10	2	5	0	8.48
3	1	6	0	3.20	10	2	5	7	8.15
3	1	6	7	2.65	10	2	6	0	8.80
10	1	1	0	9.36	10	2	6	7	7.85
10	1	1	7	8.89	30	1	1	0	26.25
10	1	2	0	9.13	30	1	1	7	30.15
10	1	2	7	9.23	30	1	6	0	33.76
10	1	3	0	8.94	30	1	6	7	26.92
10	1	3	7	10.53	100	1	1	0	111.32
10	1	4	0	8.67	100	1	1	7	93.77
10	1	4	7	9.53	100	1	6	0	92.27
10	1	5	0	8.78	100	1	6	7	92.39
10	1	5	7	8.42					
10	1	6	0	8.94					
10	1	6	7	8.78					

Table 8. Raw data for measured exposure concentrations in aqueous, static-renewal PFOA exposures with *Pimephales promelas*. TRT = treatment (nominal PFOA [mg/L], B = HPLC water blank, C = control), STAGE = sampling time (egg, first larval week, or second larval week, and pre- or post-test solution change), MEAS = measured PFOA (mg/L).

TRT	STAGE	MEAS
B		0.00
B		0.00
C	2nd larval post	0.13
C	2nd larval post	0.00
C	2nd larval post	0.001
C	2nd larval post	0.00
0.01	2nd larval post	0.010
0.032	2nd larval post	0.046
0.1	2nd larval post	0.091
0.32	2nd larval post	0.28
1	2nd larval post	0.88
3.2	2nd larval post	2.7
10	2nd larval post	12.0
32	Eggs pre	17
32	Eggs post	18
32	1st larval pre	38
32	1st larval post	27
32	2nd larval pre	30
32	2nd larval post	26
100	Eggs pre	53
100	Eggs post	57
100	1st larval pre	81
100	1st larval post	78
100	2nd larval pre	90
100	2nd larval post	97

Table 9. Chronic toxicity of PFOA to *Hyalella azteca* in 6-week aqueous static-renewal exposures. Data are based on nominal exposure concentrations. Numbers in parentheses are 95% confidence intervals.

Week	Effect	LC/EC10[a]	LC/EC25[b]	LC/EC50[c]	r^{2d}
1	Survival	56 (47-66)	82 (76-89)	120 (110-130)	0.88
2	Survival	35 (28-43)	58 (51-66)	90 (82-99)	0.87
3	Survival	30 (23-39)	48 (41-56)	72 (65-79)	0.88
4	Survival	25 (19-33)	40 (33-47)	59 (52-66)	0.89
5	Survival	25 (20-33)	39 (32-47)	56 (50-63)	0.90
6	Survival	24 (18-31)	36 (30-44)	53 (47-60)	0.91
	Growth	0.16 (0.066-0.38)	0.62 (0.30-1.2)	2.4 (1.4-4.0)	0.91
	Reproduction	0.028 (0.00027-3.0)	0.26 (0.017-3.9)	2.3 (0.65-8.3)	0.47

[a] LC10 = lethal concentration associated with 10% mortality; EC10 = effect concentration associated with 10% reduction in growth or reproduction

[b] LC25 = lethal concentration associated with 25% mortality; EC25 = effect concentration associated with 25% reduction in growth or reproduction

[c] LC50 = lethal concentration associated with 50% mortality; EC50 = effect concentration associated with 50% reduction in growth or reproduction

[d] r^2 = Goodness of fit for nonlinear regression models

Table 10. Raw data from 6-week aqueous static-renewal PFOA exposures with *Hyalella azteca*. TRT = treatment (nominal PFOA [mg/L], C = control), EXP = experiment, REP = replicate, N0 = number of amphipods added at the start of the test, N1-N6 = number of adult amphipods surviving at weeks 1-6, TWW6 = total adult wet weight (mg) per replicate at week 6, J5-J6 = number of juveniles at weeks 5-6, MP5-MP6 = number of mating pairs at weeks 5-6, M5-M6 = number of male amphipods at weeks 5-6, F5-F6 = number of female amphipods at weeks 5-6, I5-I6 = number of indeterminate amphipods at weeks 5-6.

TRT	EXP	REP	N0	N1	N2	N3	N4	N5	N6	TWW6	J5	J6	MP5	MP6	M5	M6	F5	F6	I5	I6
C	1	1	20	20	20	19	19	18	18	48.8	0	13	2	2	7	7	11	7	0	4
C	1	2	20	20	20	19	19	17	17	53.9	0	30	5	3	9	9	8	8	0	0
C	1	3	20	20	19	18	18	18	17	59.28	0	8	4	4	9	10	9	7	0	0
C	1	4	20	20	20	20	18	17	17	44.97	0	19	4	2	6	8	11	8	0	1
C	1	5	20	21	21	21	21	21	21	55.03	0	7	3	0	3	3	18	16	0	2
1	1	1	20	20	20	20	20	19	19	57.76	0	17	2	5	8	8	11	10	0	1
1	1	2	20	19	19	18	17	17	17	47.85	0	0	1	3	7	7	10	8	0	2
1	1	3	20	20	19	19	19	19	19	53.4	0	4	3	4	11	11	8	6	0	2
1	1	4	20	20	20	19	19	18	17	48.59	15	10	2	2	9	9	9	5	0	3
1	1	5	20	20	20	20	20	19	19	51.94	0	0	3	2	6	8	13	10	0	1
3	1	1	20	20	20	19	20	20	19	27	0	0	0	1	4	4	2	1	14	14
3	1	2	20	20	19	19	17	17	17	21.7	0	0	1	0	1	3	1	6	15	8
3	1	3	20	19	19	19	19	19	17	20.86	0	0	0	1	4	4	1	3	15	10
3	1	4	20	20	19	19	19	19	18	32.16	0	0	1	0	4	7	4	4	11	7
3	1	5	20	20	20	20	20	20	20	23.3	0	0	0	0	1	2	3	6	16	12
10	1	1	20	20	19	19	19	19	17	10.48	0	0	0	0	0	0	0	1	19	16
10	1	2	20	19	19	19	18	18	18	11.08	0	0	0	0	1	1	0	1	17	16
10	1	3	20	20	20	18	18	17	16	10.46	0	0	0	0	0	0	0	0	17	16
10	1	4	20	19	19	19	19	19	19	13.53	0	0	0	0	1	3	0	0	18	16
10	1	5	20	20	20	20	18	17	17	10.97	0	0	0	0	0	1	0	0	17	16

Table 10 (cont'd). Raw data from 6-week aqueous static-renewal PFOA exposures with *Hyalella azteca*. TRT = treatment (nominal PFOA [mg/L], C = control), EXP = experiment, REP = replicate, N0 = number of amphipods added at the start of the test, N1-N6 = number of adult amphipods surviving at weeks 1-6, TWW6 = total adult wet weight (mg) per replicate at week 6, J5-J6 = number of juveniles at weeks 5-6, MP5-MP6 = number of mating pairs at weeks 5-6, M5-M6 = number of male amphipods at weeks 5-6, F5-F6 = number of female amphipods at weeks 5-6, I5-I6 = number of indeterminate amphipods at weeks 5-6.

TRT	EXP	REP	N0	N1	N2	N3	N4	N5	N6	TWW6	J5	J6	MP5	MP6	M5	M6	F5	F6	I5	I6
30	1	1	20	19	19	19	19	18	16	4.26	0	0	0	0	0	0	0	0	18	16
30	1	2	20	20	19	18	17	17	17	5.4	0	0	0	0	0	0	0	0	17	17
30	1	3	20	20	18	17	16	16	16	8.4	0	0	0	0	0	0	0	0	16	16
30	1	4	20	18	18	18	18	18	17	7.11	0	0	0	0	0	0	0	0	18	17
30	1	5	20	18	18	18	17	17	15	6.43	0	0	0	0	0	0	0	0	17	15
100	1	1	20	12	10	6	3	2	2	0.47	0	0	0	0	0	0	0	0	2	2
100	1	2	20	14	10	8	2	2	1	0.19	0	0	0	0	0	0	0	0	2	1
100	1	3	20	14	11	4	2	2	1	0.22	0	0	0	0	0	0	0	0	2	1
100	1	4	20	14	12	8	6	4	1	0.27	0	0	0	0	0	0	0	0	4	1
100	1	5	20	10	8	3	1	0	0											
C	2	1	20	20	19	19	17	17	17	43.94	0	11	2	2	8	8	8	8	1	1
C	2	2	20	20	20	19	19	18	18	41.44	0	5	1	2	8	9	7	6	3	3
C	2	3	20	19	19	17	17	16	15	36.17	6	7	0	2	5	6	4	6	7	3
C	2	4	20	20	19	19	18	18	18	41.73	0	10	2	4	6	6	8	10	4	2
C	2	5	20	20	20	19	19	19	19	39.61	10	6	1	5	7	7	8	9	4	3
1	2	1	20	20	20	20	20	19	19	39.76	0	15	1	3	5	8	11	9	3	2
1	2	2	20	19	18	17	17	17	17	38.17	0	5	0	3	9	9	3	5	5	3
1	2	3	20	19	18	18	18	18	16	29.83	0	3	2	4	5	5	5	6	8	5
1	2	4	20	18	17	17	16	16	15	36.49	0	0	1	5	6	7	4	6	6	2
1	2	5	20	19	19	18	18	18	18	37.59	0	0	1	2	7	8	5	5	6	5

Table 10 (cont'd). Raw data from 6-week aqueous static-renewal PFOA exposures with *Hyalella azteca*. TRT = treatment (nominal PFOA [mg/L], C = control), EXP = experiment, REP = replicate, N0 = number of amphipods added at the start of the test, N1-N6 = number of adult amphipods surviving at weeks 1-6, TWW6 = total adult wet weight (mg) per replicate at week 6, J5-J6 = number of juveniles at weeks 5-6, MP5-MP6 = number of mating pairs at weeks 5-6, M5-M6 = number of male amphipods at weeks 5-6, F5-F6 = number of female amphipods at weeks 5-6, I5-I6 = number of indeterminate amphipods at weeks 5-6.

TRT	EXP	REP	N0	N1	N2	N3	N4	N5	N6	TWW6	J5	J6	MP5	MP6	M5	M6	F5	F6	I5	I6
3	2	1	20	20	20	19	19	20	19	21.29	0	0	0	0	4	6	2	0	14	13
3	2	2	20	19	19	19	19	17	16	22.43	0	3	0	2	3	3	4	7	10	6
3	2	3	20	19	18	18	18	19	19	25.63	0	0	0	2	3	4	4	5	12	10
3	2	4	20	20	19	19	19	19	17	26.58	0	5	1	2	3	3	7	7	9	7
3	2	5	20	19	18	18	18	17	15	14.26	0	0	1	0	2	2	3	3	12	10
10	2	1	20	19	18	17	18	17	16	10.51	0	0	0	0	0	0	1	2	16	14
10	2	2	20	18	19	19	17	17	17	9.01	0	0	0	0	0	0	0	6	17	11
10	2	3	20	20	19	19	18	19	19	10.49	0	0	0	0	0	0	1	1	18	18
10	2	4	20	19	18	18	17	17	16	9.46	0	0	0	0	0	1	0	6	17	9
10	2	5	20	20	18	16	16	16	16	7.75	0	0	0	0	0	0	0	2	16	14
30	2	1	20	20	18	15	13	12	11	2.27	0	0	0	0	0	0	0	0	12	11
30	2	2	20	19	16	15	14	13	13	3.29	0	0	0	0	0	0	0	0	13	13
30	2	3	20	18	17	16	15	15	15	3.47	0	0	0	0	0	0	0	0	15	15
30	2	4	20	20	17	17	14	14	14	3.69	0	0	0	0	0	0	0	0	14	14
30	2	5	20	18	16	15	15	15	14	3.28	0	0	0	0	0	0	0	0	15	14
100	2	1	20	10	4	1	0	0	0											
100	2	2	20	11	7	6	0	0	0											
100	2	3	20	10	7	6	2	0	0											
100	2	4	20	13	8	3	2	1	1	0.05	0		0		0		0		1	1
100	2	5	20	12	5	4	4	2	0		0		0		0		0		2	

Table 11. Raw data from aqueous static-renewal range-finding PFOA exposures with *Pimephales promelas*. Exposures were conducted from egg to hatch (5 eggs per replicate).

Nominal PFOA (mg/L)	Hatch Success (%)	Deformed Larvae (%)
Control 1	100	0
Control 2	100	0
31	100	0
62	100	0
125	100	0
250	100	20
500	60	25

Table 12. Raw survival and growth data from low concentration, aqueous, static-renewal PFOA exposures with *Pimephales promelas*. TRT = treatment (nominal PFOA [mg/L], C = control), REP = replicate, DPH = days post-hatch, H = percent hatchability, DEF-H = percent deformities at hatch, TTH = time to hatch, EGG-H = percent survival egg to hatch, EGG-9 = percent survival egg to 9 dph, EGG-16 = percent survival egg to 16 dph, N = number of larvae assessed, MM = mean mass (mg), ML = mean total length (mm; nose to tail), MSL = mean standard length (mm; nose to caudal peduncle), TL = tail length (mm; difference between ML and MSL).

TRT	REP	HATCH			SURVIVAL			9 DPH					16 DPH				
		H	DEF-H	TTH	EGG-H	EGG-9	EGG-16	N	MM	ML	MSL	TL	N	MM	ML	MSL	TL
C	A	100.0	10.0	4.90	90.0	90.0	90.0	8	4.72	9.46	8.43	1.03	10	13.7	13.2	11.0	2.28
C	A	100.0	10.5	4.95	94.7	89.5	89.5	7	4.05	9.39	8.36	1.03	10	13.0	12.5	10.3	2.17
C	B	100.0	0.0	4.75	100.0	100.0	100.0	10	4.44	9.86	8.72	1.13	10	14.7	13.0	10.7	2.24
C	B	100.0	0.0	4.80	100.0	100.0	100.0	10	5.04	10.20	8.82	1.38	10	14.3	12.8	10.6	2.20
C	C	100.0	5.0	5.00	95.0	95.0	95.0	9	5.00	10.12	8.84	1.28	10	14.5	13.4	11.0	2.40
C	C	94.7	11.1	5.00	84.2	84.2	84.2	6	5.68	10.42	9.07	1.35	10	15.7	13.7	11.4	2.32
C	D	100.0	5.0	4.65	95.0	95.0	95.0	9	4.66	9.43	8.63	1.22	10	15.0	12.9	10.7	2.24
C	D	100.0	15.0	4.45	85.0	80.0	80.0	6	4.99	9.58	8.45	1.12	10	15.0	13.0	10.7	2.33
0.01	A	100.0	0.0	4.95	100.0	100.0	100.0	10	4.18	9.36	8.10	1.26	10	13.1	12.5	10.2	2.27
0.01	B	100.0	0.0	4.55	100.0	100.0	100.0	10	5.39	9.93	8.70	1.23	10	13.9	12.8	10.6	2.13
0.01	C	100.0	15.0	5.00	85.0	85.0	85.0	7	4.87	10.02	8.65	1.37	10	14.1	13.5	11.2	2.29
0.01	D	100.0	25.0	4.85	75.0	75.0	75.0	5	5.07	9.87	8.67	1.21	10	14.1	12.7	10.6	2.18
0.032	A	100.0	0.0	4.85	100.0	100.0	100.0	10	4.68	9.67	8.29	1.38	10	14.2	12.9	10.4	2.44
0.032	B	100.0	5.0	4.60	100.0	100.0	100.0	10	4.21	9.37	8.27	1.10	10	14.9	13.1	10.8	2.25
0.032	C	95.0	5.3	5.00	90.0	85.0	85.0	7	5.28	10.06	8.80	1.26	10	14.9	13.6	11.3	2.31
0.032	D	100.0	10.0	4.70	90.0	90.0	90.0	8	4.76	9.46	8.32	1.14	10	14.3	12.9	10.7	2.14
0.1	A	100.0	0.0	5.00	100.0	100.0	100.0	10	4.38	9.25	8.13	1.12	10	13.3	12.5	10.2	2.37
0.1	B	100.0	0.0	4.60	100.0	100.0	100.0	10	5.04	9.73	8.56	1.17	10	13.2	12.9	10.9	2.02
0.1	C	95.0	10.5	4.84	90.0	85.0	85.0	7	4.51	9.60	8.93	1.32	10	13.4	13.2	11.0	2.23
0.1	D	100.0	20.0	4.70	80.0	80.0	80.0	6	4.24	9.35	8.35	1.01	10	13.4	12.6	10.5	2.16

Table 12 (cont'd). Raw survival and growth data from low concentration, aqueous, static-renewal PFOA exposures with *Pimephales promelas*. TRT = treatment (nominal PFOA [mg/L], C = control), REP = replicate, DPH = days post-hatch, H = percent hatchability, DEF-H = percent deformities at hatch, TTH = time to hatch, EGG-H = percent survival egg to hatch, EGG-9 = percent survival egg to 9 dph, EGG-16 = percent survival egg to 16 dph, N = number of larvae assessed, MM = mean mass (mg), ML = mean total length (mm; nose to tail), MSL = mean standard length (mm; nose to caudal peduncle), TL = tail length (mm; difference between ML and MSL).

TRT	REP	HATCH			SURVIVAL			9 DPH					16 DPH				
		H	DEF-H	TTH	EGG-H	EGG-9	EGG-16	N	MM	ML	MSL	TL	N	MM	ML	MSL	TL
0.32	A	100.0	10.5	5.00	89.5	89.5	89.5	7	4.32	9.57	8.48	1.09	10	13.3	12.9	10.5	2.44
0.32	B	100.0	0.0	4.45	100.0	100.0	100.0	10	4.87	9.64	8.53	1.11	10	13.9	12.8	10.7	2.12
0.32	C	95.0	0.0	5.00	95.0	90.0	90.0	8	4.14	9.59	8.43	1.16	10	15.0	13.5	11.1	2.32
0.32	D	95.0	5.3	4.68	90.0	85.0	85.0	7	5.58	9.97	8.79	1.18	10	15.8	13.2	10.9	2.29
1	A	100.0	19.0	4.95	81.0	81.0	81.0	7	4.17	9.21	8.19	1.02	10	14.6	13.4	11.0	2.40
1	B	100.0	0.0	4.55	100.0	100.0	100.0	10	5.15	9.90	8.67	1.23	10	13.7	13.2	11.1	2.12
1	C	95.0	5.3	4.89	90.0	90.0	90.0	8	4.58	9.66	8.76	1.35	10	14.9	13.5	11.1	2.37
1	D	100.0	15.0	4.85	85.0	85.0	85.0	7	5.13	9.76	8.57	1.19	10	14.6	12.8	10.7	2.13
3.2	A	100.0	9.1	5.00	90.9	90.9	90.9	10	4.25	9.39	8.27	1.05	10	12.4	12.8	10.6	2.27
3.2	B	100.0	0.0	4.45	100.0	100.0	100.0	10	5.30	10.11	8.90	1.21	10	13.6	12.7	10.6	2.13
3.2	C	95.0	0.0	4.79	95.0	95.0	95.0	9	4.29	9.45	8.58	1.29	10	13.5	13.1	10.9	2.21
3.2	D	100.0	10.0	4.60	90.0	85.0	85.0	7	4.42	9.42	8.38	1.04	10	13.8	12.7	10.5	2.19
10	A	100.0	10.0	5.00	90.0	90.0	90.0	8	4.09	9.11	8.07	1.04	10	13.6	13.3	10.9	2.40
10	B	100.0	5.0	4.80	95.0	95.0	85.5	9	4.67	9.72	8.59	1.13	9	15.9	13.9	11.6	2.35
10	C	100.0	5.0	4.70	95.0	95.0	95.0	9	5.53	10.26	8.90	1.37	10	14.4	13.4	11.1	2.25
10	D	100.0	0.0	4.75	100.0	100.0	100.0	10	4.18	9.22	8.19	1.03	10	11.2	12.0	10.0	1.95

Table 13. Raw survival and growth data from high concentration, aqueous, static-renewal PFOA exposures with *Pimephales promelas*. TRT = treatment (nominal PFOA [mg/L], C = control), REP = replicate, DPH = days post-hatch, H = percent hatchability, DEF-H = percent deformities at hatch, TTH = time to hatch, EGG-H = percent survival egg to hatch, EGG-9 = percent survival egg to 9 dph, EGG-16 = percent survival egg to 16 dph, N = number of larvae assessed, MM = mean mass (mg), ML = mean total length (mm; nose to tail), MSL = mean standard length (mm; nose to caudal peduncle), TL = tail length (mm; difference between ML and MSL).

TRT	REP	HATCH			SURVIVAL			9 DPH					16 DPH				
		H	DEF-H	TTH	EGG-H	EGG-9	EGG-16	N	MM	ML	MSL	TL	N	MM	ML	MSL	TL
C	A	100.0	0.0	4.1	100.0	95.5	95.5	11	5.34	10.32	9.02	1.31	10	14.6	13.6	11.2	2.35
C	A	100.0	10.0	4.3	90.0	90.0	90.0	8	4.65	10.12	8.90	1.22	10	12.8	12.7	10.4	2.21
C	B	100.0	10.0	5.00	95.0	95.0	95.0	9	4.87	9.98	8.99	1.00	10	14.3	13.6	11.3	2.28
C	B	100.0	5.0	5.00	100.0	100.0	100.0	10	3.77	9.32	8.31	1.01	10	15.2	13.7	11.4	2.34
C	C	100.0	10.0	4.90	90.0	90.0	90.0	8	5.30	9.65	8.43	1.22	10	16.2	13.2	10.9	2.33
C	C	100.0	5.0	4.85	95.0	95.0	95.0	9	5.18	9.66	8.48	1.18	10	13.1	12.5	10.4	2.17
C	D	100.0	0.0	5.00	100.0	100.0	100.0	10	4.84	9.60	8.40	1.19	10	14.6	13.5	11.2	2.37
C	D	95.0	5.3	5.00	90.0	90.0	90.0	8	5.23	9.73	8.51	1.22	10	13.2	13.1	10.9	2.21
32	A	100.0	0.0	4.10	100.0	100.0	100.0	10	5.01	10.24	8.95	1.29	10	13.4	12.7	10.5	2.21
32	B	95.0	10.5	4.89	90.0	80.0	80.0	6	3.47	9.16	8.23	0.93	10	14.7	13.6	11.3	2.32
32	C	95.0	0.0	4.89	95.0	90.0	90.0	8	3.58	8.73	7.81	0.92	10	16.8	13.4	11.1	2.26
32	D	100.0	0.0	5.00	100.0	100.0	100.0	10	5.26	9.68	8.43	1.25	10	13.9	13.2	11.0	2.20
100	A	100.0	0.0	4.10	100.0	100.0	100.0	10	5.12	10.25	8.93	1.32	10	14.4	13.5	11.2	2.27
100	B	100.0	30.0	5.00	80.0	70.0	70.0	4	5.41	10.38	9.23	1.15	10	13.5	13.2	11.1	2.09
100	C	90.0	22.2	4.94	90.0	70.0	70.0	4	3.35	8.57	7.70	0.87	10	14.6	12.9	10.7	2.25
100	D	95.0	5.3	4.95	90.0	90.0	90.0	8	4.92	9.35	8.17	1.17	10	14.5	13.4	11.1	2.24

FIGURES

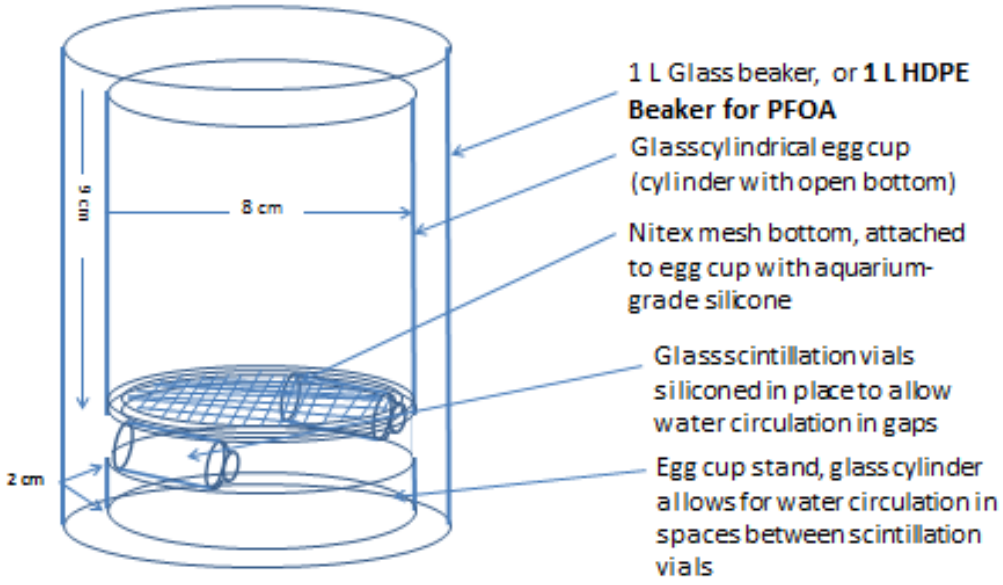

Figure 1. Egg cups constructed of two sections of glass cylinders, two small glass scintillation vials, and 500 µm-Nitex mesh (two sizes of egg cups; tall cups have a 6 cm bottom section, short cups have a 2 cm bottom section; top sections 9 cm, all sections 8 cm external diameter)

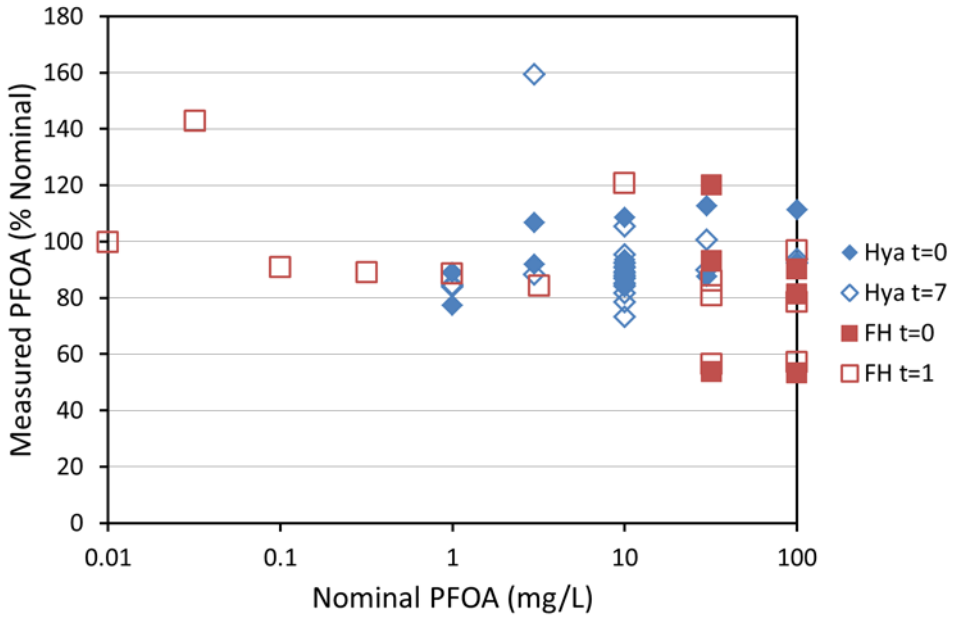

Figure 2. Measured PFOA concentrations at the beginning (filled symbols) and end (open symbols) of static-renewal periods for amphipod (Hya) and fathead minnow (FH) aqueous exposures.

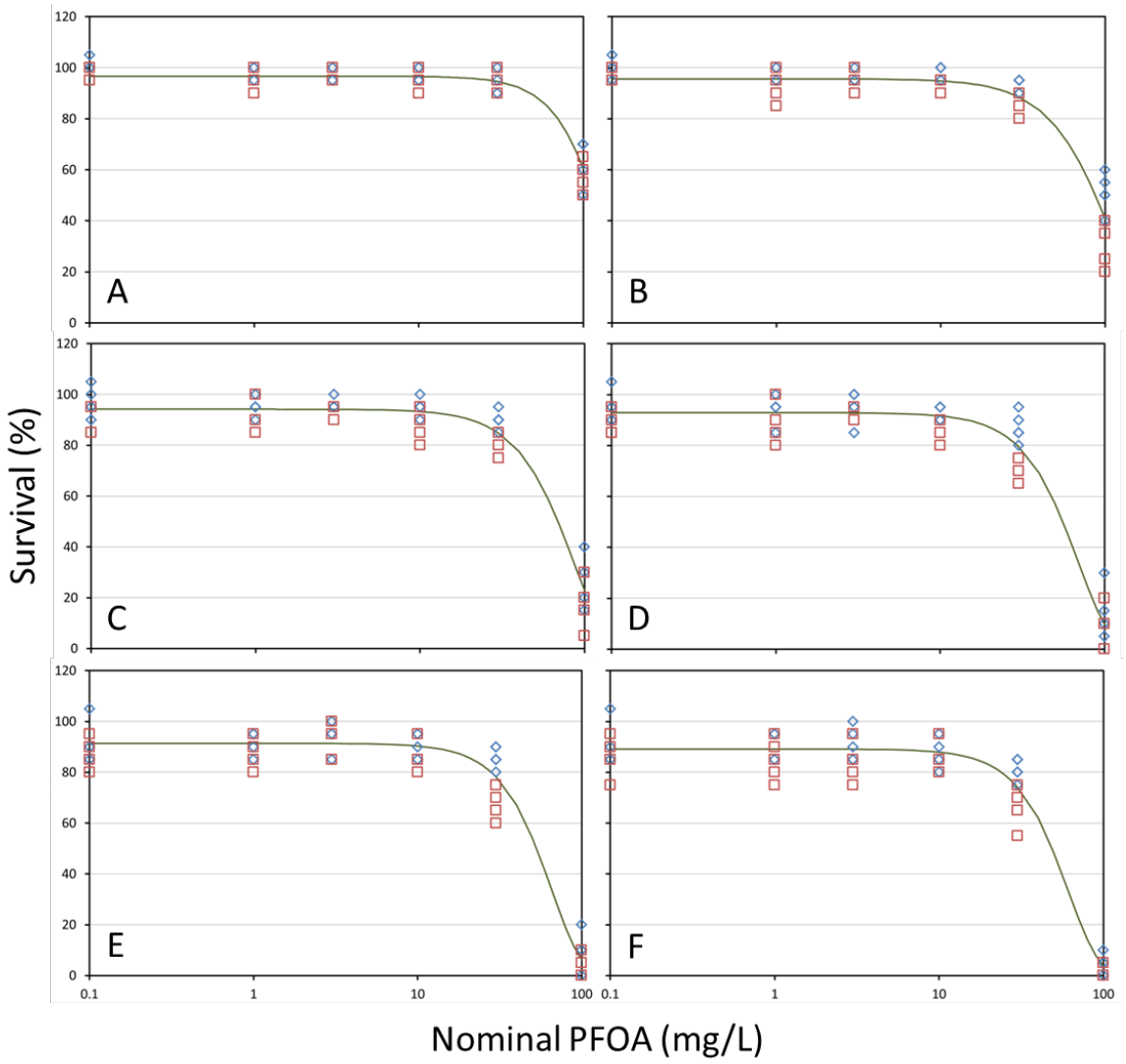

Figure 3. Survival of *Hyalella azteca* following 1-week (A), 2-week (B), 3-week (C), 4-week (D), 5-week (E), and 6-week (F) aqueous static-renewal exposures to PFOA. Different symbols represent different experiments. Controls are data points on the y-axis. Lines are the nonlinear regression models used to calculate toxicity endpoints.

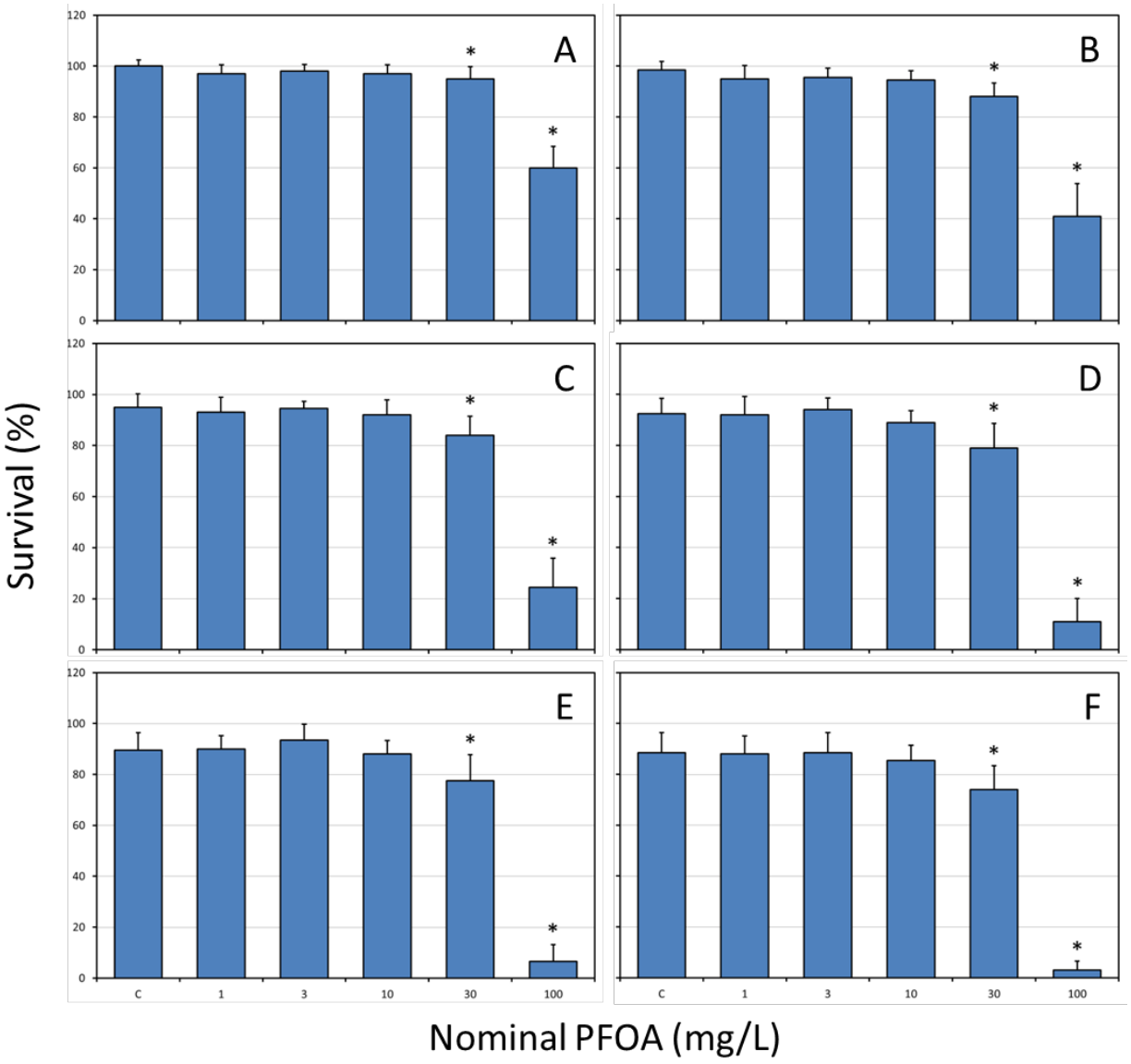

Figure 4. Survival of *Hyalella azteca* following 1-week (A), 2-week (B), 3-week (C), 4-week (D), 5-week (E), and 6-week (F) aqueous static-renewal exposures to PFOA. C = Control. Error bars are standard deviations. Asterisks are significantly different from controls (ANOVA, $p < 0.05$).

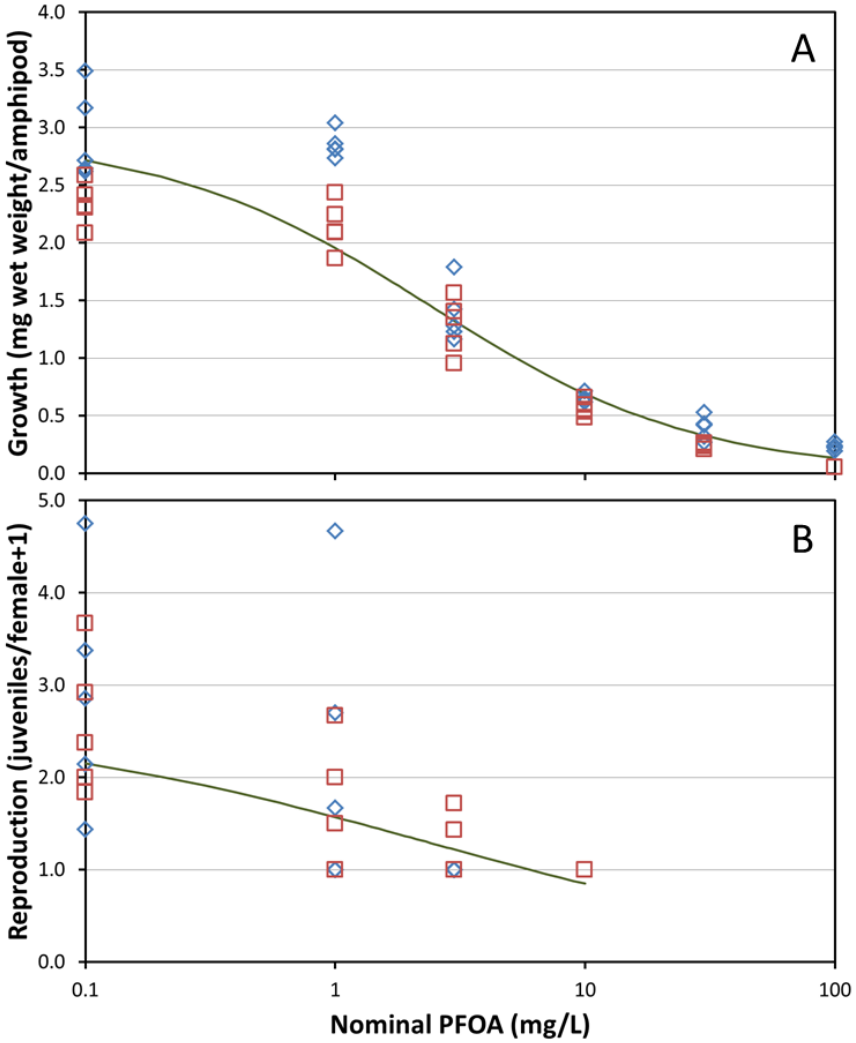

Figure 5. Growth (A) and reproduction (B) of *Hyalella azteca* following 6-week aqueous static-renewal exposures to PFOA. Different symbols represent different experiments. Controls are data points on the y-axis. Lines are the nonlinear regression models used to calculate toxicity endpoints.

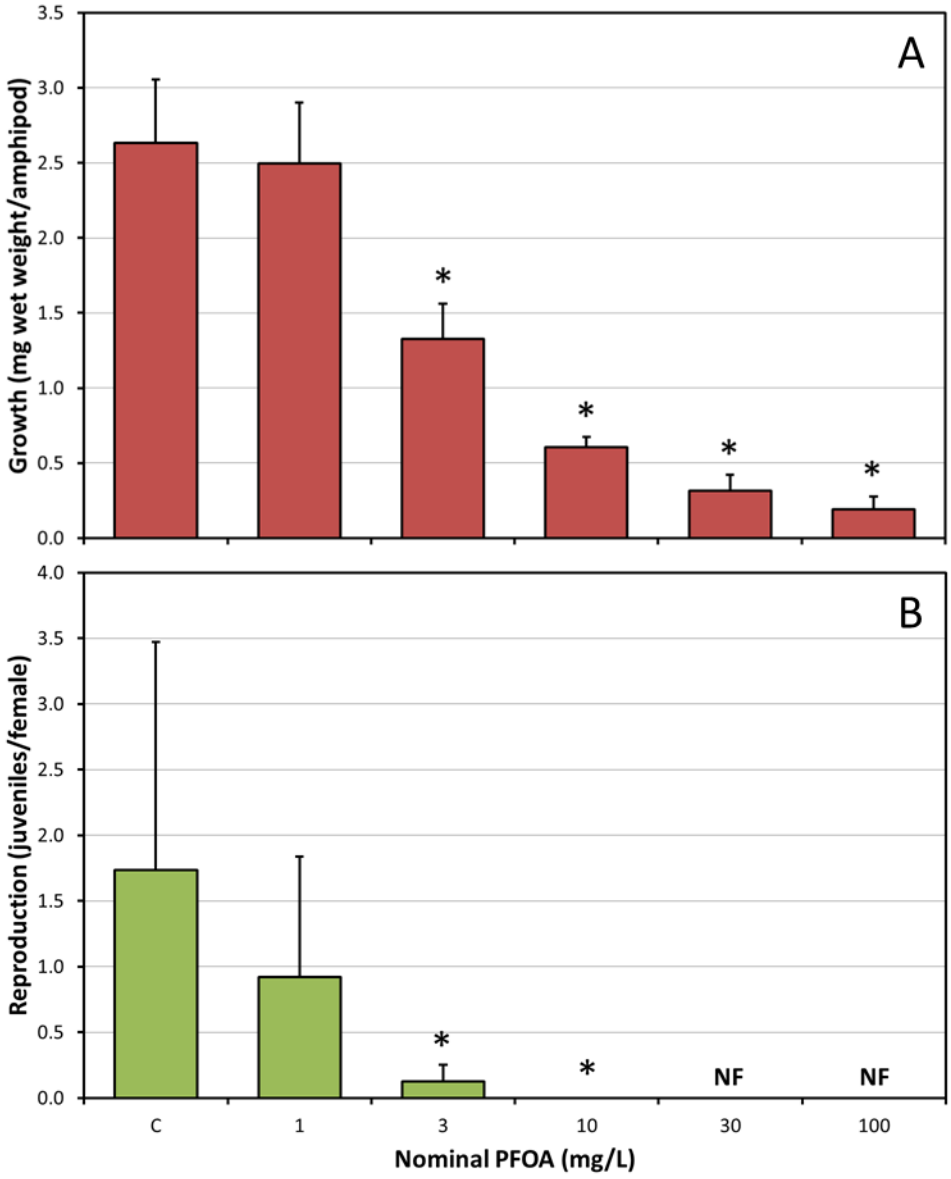

Figure 6. Growth (A) and reproduction (B) of *Hyalella azteca* following 6-week aqueous static-renewal exposures to PFOA. C = Control, NF = no females could be identified. Error bars are standard deviations. Asterisks are significantly different from controls (ANOVA, $p < 0.05$).

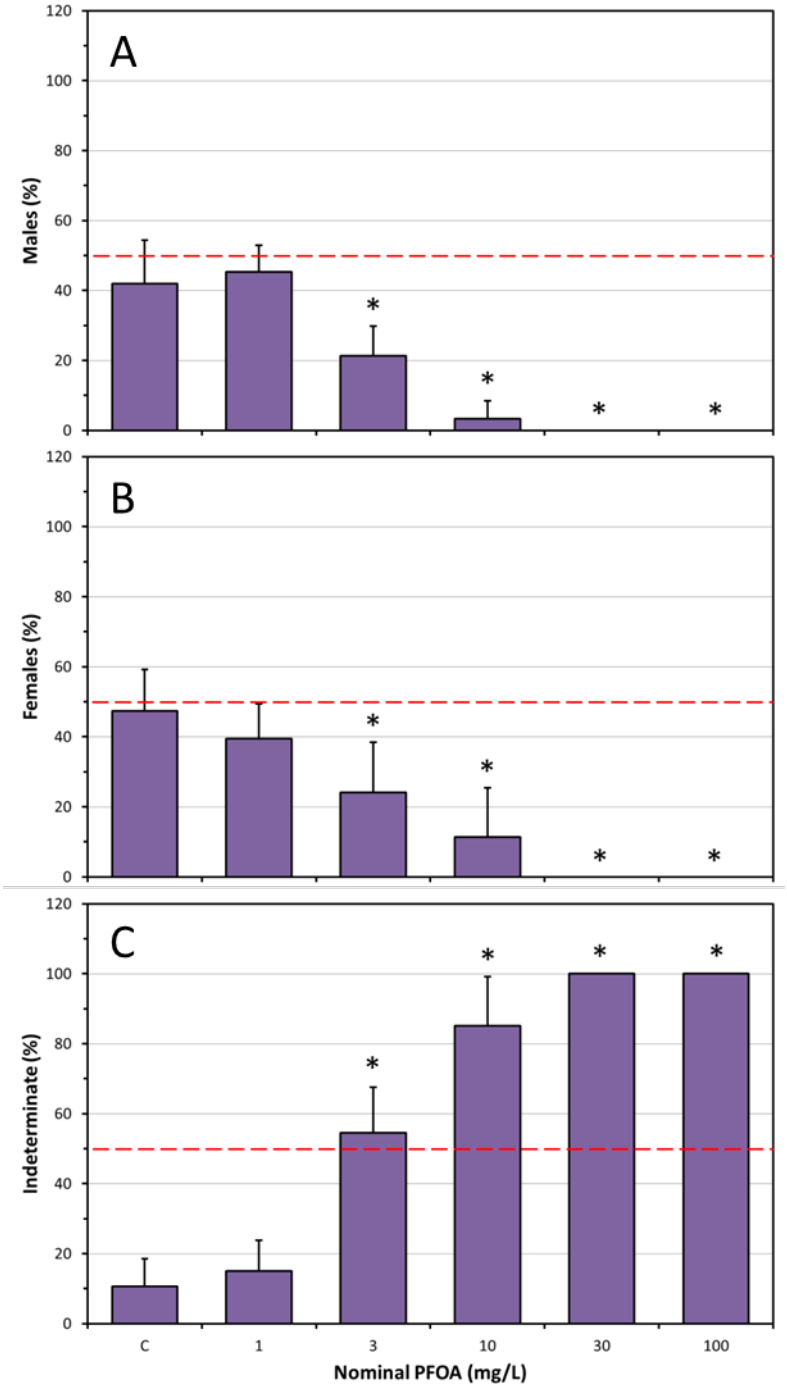

Figure 7. Proportion of *Hyalella azteca* that were identified as male (A), female (B), and indeterminate (C) following 6-week aqueous static-renewal exposures to PFOA. C = Control. Error bars are standard deviations. Asterisks are significantly different from controls (ANOVA, $p < 0.05$). The red dashed line = 50%.

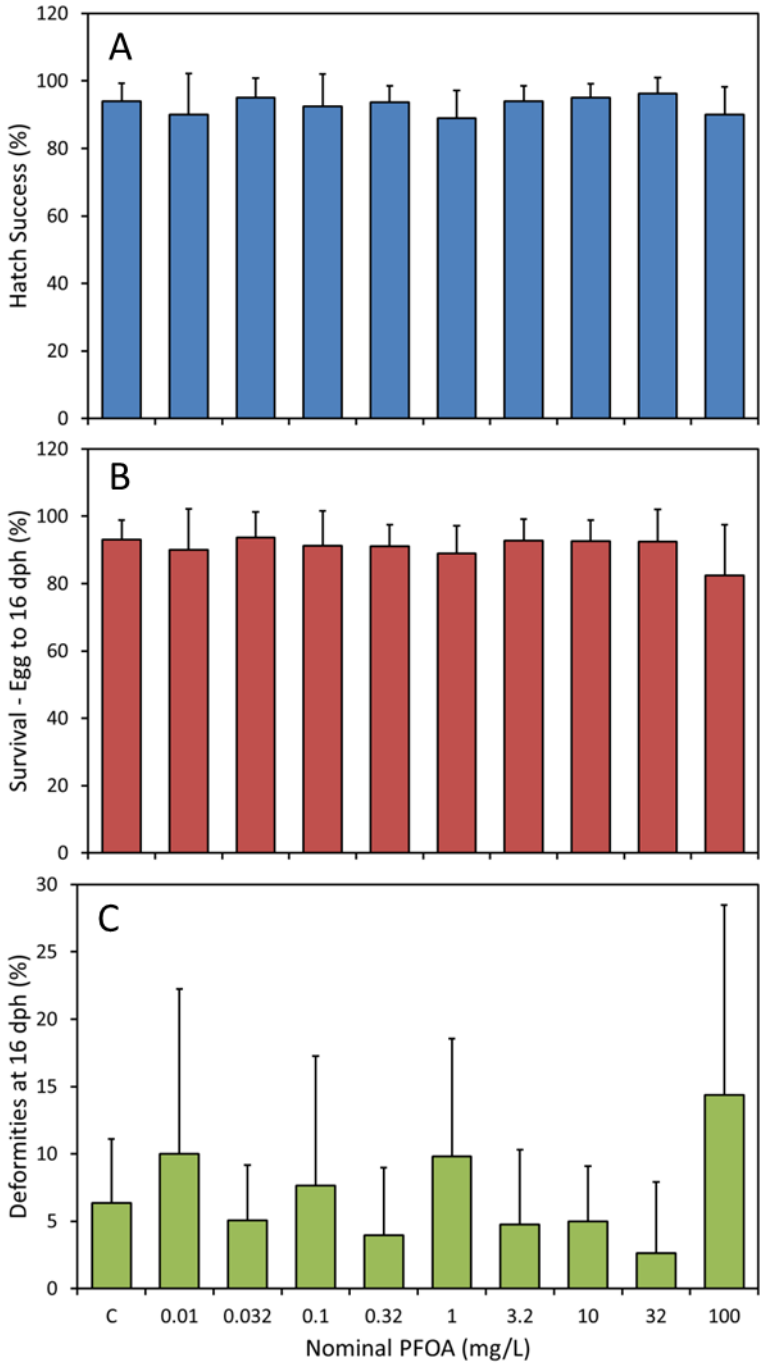

Figure 8. Hatch success (A), survival (egg to 16 days post-hatch [dph], B), and deformities at 16 dph (C) of *Pimephales promelas* following a 21-d aqueous static-renewal exposure to PFOA. C = Control. Error bars are standard deviations.

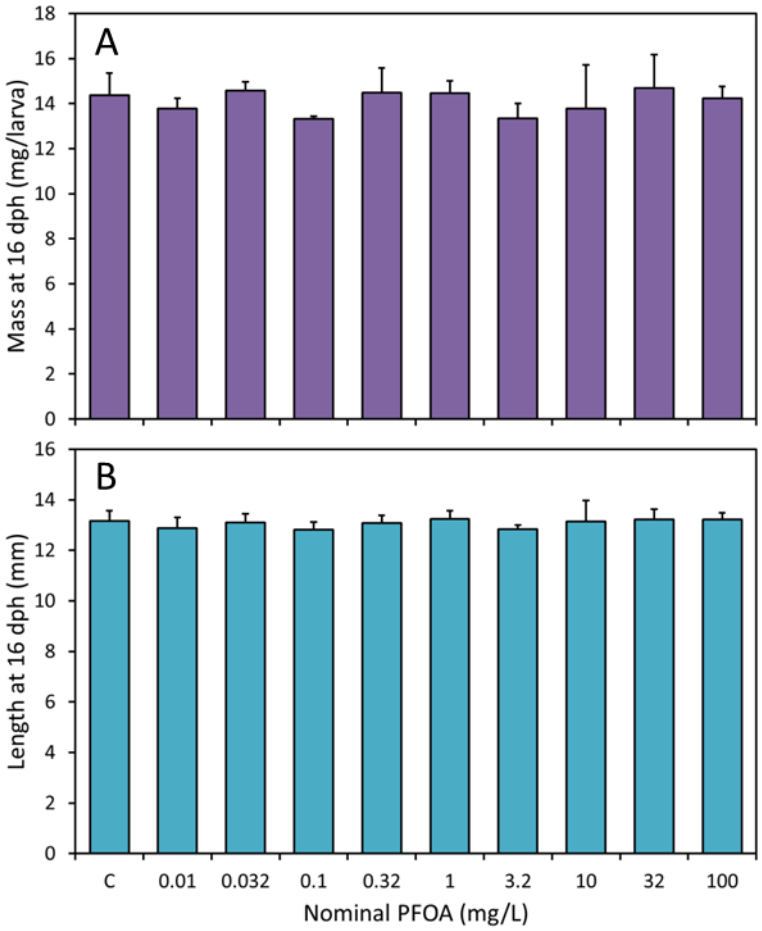

Figure 9. Growth of *Pimephales promelas*, expressed as mass (A) and length (B) at 16 days post-hatch (dph) following a 21-d aqueous static-renewal exposure to PFOA. C = Control. Error bars are standard deviations.