SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 33, No. 7, pp. 1552–1562, 2014

Printed in the USA

Environmental Toxicology

EFFECTS OF ANIONIC POLYACRYLAMIDE PRODUCTS ON GILL HISTOPATHOLOGY IN JUVENILE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

Jennifer L. Kerr,† John S. Lumsden,‡ Spencer K. Russell,‡ Edyta J. Jasinska,§ and Greg G. Goss*§
†Clearflow Enviro Systems Group, Sherwood Park, Alberta, Canada
‡Fish Pathology Laboratory, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada
§Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada

(Submitted 23 February 2012; Returned for Revision 19 March 2012; Accepted 13 March 2014)

Abstract: Anionic polyacrylamide (PAM) products are commonly used to remove suspended materials from turbid waters and to help mitigate soil erosion. In the present study, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to 3 mg/L to 300 mg/L of 10 commercially available PAM products (Clearflow Water Lynx Polymer Log and Clearflow Soil Lynx Granular Polymer; Clearflow Enviro Systems Group), and gill histological parameters were measured following either 7 d or 30 d of polymer exposure. A cationic polymer product (≤0.38 mg/L MagnaFloc 368; Ciba Specialty Chemical) was also tested for comparison. Mild gill lesions were observed in fish exposed to polymer products. Lamellar fusion, interlamellar hyperplasia, epithelial lifting, mucous cell metaplasia, and cell counts of epithelial swelling and necrosis/apoptosis were minimal in fish exposed to environmentally relevant concentrations of anionic polymer (≤30 mg/L). Gill morphology was largely unaffected by exposure to concentrations up to 300 mg/L of many PAM products. Several anionic polymer products noticeably affected gill tissue by increasing epithelial hypertrophy, interlamellar hyperplasia, mucous cell metaplasia, and the frequency of necrotic cells. The severity of the lesions lessened with time, suggesting that fish may have experienced a short-term irritant effect. Similar levels of gill pathology were frequently observed in fish exposed to cationic polymer MagnaFloc 368 despite the concentration being 1000-fold lower than that of the PAM products. These observations highlight the increased toxicity of cationic polymers to aquatic life compared with anionic PAMs. *Environ Toxicol Chem* 2014;33:1552–1562. © 2014 SETAC

Keywords: Polymer Polyacrylamide Aquatic toxicology Fish Flocculant

INTRODUCTION

High sediment loads within aquatic environments have been shown in some studies to result in severe and negative effects on aquatic organisms [1]. These effects include clogging of gills and preventing eggs from hatching in certain invertebrate and fish species. Different types of flocculants, especially polymers, are currently in use to help remove suspended sediments from turbid waterways or discharge waters. These same products are often used to mitigate soil erosion, thereby reducing the amount of sediment deposited into aquatic environments. The polymer flocculants can be categorized into 3 groups based on their charge alone: anionic, cationic, and neutral [2]. One of the most commonly used types of flocculant for soil stabilization/erosion control in agriculture is anionic polyacrylamide (PAM). Polyacrylamides have been shown to be highly effective in reducing soil erosion in exposed environments and as flocculants of suspended sediments in runoff water or other turbid wastewaters [3–7]. Anionic PAMs used in erosion control or water clarification are typically water-soluble and linear copolymers of acrylamide and acrylic acid with very high molecular weights (typically 12–15 mg/mol with more than 150 000 monomer units per molecule). With the acrylamide monomer known to be a potent carcinogen [8], manufacturing processes restrict residual acrylamide monomers within all PAMs to be used for drinking water clarification to no more than 0.05% wt/wt [3]. These products can be produced in a number of forms such as granular, emulsion, or gel block. In general, gel block PAMs have much lower solubility than other forms and are only used for in-flow treatment of turbid water. Polyacrylamides increase settling and removal of suspended sediments from turbid waters by flocculation, as opposed to cationic polymers, which primarily function as coagulants [3].

Anionic PAMs have very low toxicity in mammals [9], freshwater teleosts [3,10–12], and aquatic invertebrates [11,12]. Polyacrylamides are considered low risk for bioaccumulation because of the large size of the molecules [13,14]. Furthermore, PAMs do not degrade into acrylamide monomers or other known toxic compounds under environmental conditions [15]. When used at prescribed rates, PAMs are considered to be environmentally safe [3,6]. Cationic polymers, in contrast, are recognized as having observable toxic effects on aquatic life [10,16,17] and consequently are generally recommended only for closed-loop systems or when there is no risk of exposure to aquatic life.

While a number of toxicity tests have been conducted on anionic PAM products, these studies have focused primarily on either the acute toxicity (<96 h) of the products or the effects of polymer exposure on growth rate and reproductive success. Very few studies have investigated the effects of chronic exposure on parameters such as gill morphology and/or lesions. In the present study, we describe the effects of sublethal concentrations of a variety of anionic polymer products or a reference cationic polymer previously shown to induce gill pathology [10] on juvenile rainbow trout (*Oncorhynchus mykiss*). Fish were exposed for either 7 d or 30 d (static renewal), and their gill tissue was examined for light microscopic lesions commonly associated with irritant exposure and chemical pollutants. Histopathological parameters were measured qualitatively based on damage rankings (lamellar fusion, interlamellar hyperplasia, epithelial lifting, and epithelial hypertrophy), while others were

All Supplemental Data may be found in the online version of this article.
* Addressed correspondence to greg.goss@ualberta.ca.
Published online 19 March 2014 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.2582

measured quantitatively (mucous metaplasia, cells exhibiting epithelial swelling, necrosis, and apoptosis). Gill tissue was chosen because it comes in direct contact with chemicals in the water and is a critical organ for teleost homeostasis. No other organs of the fish were examined for histopathology because previous research [10] has indicated that neither large–molecular weight cationic nor anionic PAMs cause histopathological changes in the internal organs of exposed fish. Presumably, the large size of PAMs prevents them from passing through the external epithelium. Supporting this, no evidence of bioaccumulation of anionic PAMs has been reported in the literature [13,17].

MATERIALS AND METHODS

Experimental animals

Rainbow trout eggs (*O. mykiss*) were obtained from Raven Brood Trout Station. The eggs were hatched in the Aquatics Facility of the Department of Biological Sciences at the University of Alberta (Canada), grown to parr stage, and maintained in flow-through 450-L fiberglass tanks filled with aerated and dechlorinated City of Edmonton tap water. Water temperature in the large tanks was maintained at 10 °C to slow growth until approximately 2 wk prior to exposure experiments, at which point the fish were slowly acclimated to 15 °C. The photoperiod mimicked that of Edmonton, Alberta, Canada. Prior to the start of the exposure experiments, fish were fed once per day with partially ground, dry, commercial trout pellets. Following the start of the exposure study, fish were fed ad libitum every Monday, Wednesday, and Friday. Uneaten food was removed 10 min after addition to tanks, in accordance with Biological Sciences Animal Services (BSAS) animal care protocol #571802.

Test compounds and materials

Unless otherwise noted, all general laboratory chemicals were supplied by Sigma. The toxicity of 11 different polymer products was tested in the present experiment, of which 10 were anionic PAMs. Soil Lynx products (CFGP 298, CFGP 295, CFGP 288, CFGP 270, CFGP 260, and CFGP 255) and Water Lynx products (CFPL 297, CFPL 297.3, CFPL 294, and CFPL 293) are anionic, water-soluble, PAM copolymer products supplied by Clearflow Enviro Systems Group. They are granular and gel-like in nature, respectively. The cationic polymer MagnaFloc 368 is a coagulating polydimethyldiallylammonium chloride polymer manufactured by Ciba Specialty Chemical.

Experimental design

For each polymer tested, 25 juvenile rainbow trout (size range, 45–65 mm, 4.2–6.4 g) were exposed in a single tank to different concentrations of polymer solutions for up to 30 d in glass aquaria (9.0 L; Hagen). All experiments were run in a 4-mo window, and fish were selected from a group brood stock aged 7 mo to 11 mo, with similar sizes matched between and within experiments. Six liters of polymer solution or dechlorinated tap water (control) were maintained at approximately 15 °C under constant aeration. Water was replaced every 2 d to prevent buildup of fish nitrogenous waste and other pollutants. Basic water chemistry (pH, temperature, dissolved oxygen, and conductivity) was measured every second day using a hand-held electronic probe (model 85-10FT; YSI) or pH meter (Accumet, model AB15; Fisher Scientific).

Fish were exposed in 3 separate experiments, with at least 1 control tank (dechlorinated City of Edmonton tap water) and 1 tank of fish exposed to the cationic polymer MagnaFloc 368 in each experiment serving as a paired control. Different formulations of either granular Soil Lynx CFGP or gel block Water Lynx CFPL blend were tested within each experiment. Cationic polymer exposure consisted of trout exposed to either 0.38 mg/L or 0.30 mg/L of MagnaFloc 368. Our first experiment indicated that 0.38 mg/L of MagnaFloc resulted in higher than expected mortality; therefore, the MagnaFloc concentration was lowered slightly in experiments 2 and 3 to 0.30 mg/L to ensure that we had enough fish to sample after 30 d.

Anionic polymer exposures consisted of fish immersed in 3 mg/L, 30 mg/L, or 300 mg/L of granular Soil Lynx CFGP 298, CFGP 295, and CFGP 288. Stock solutions (3000 mg/L) were allowed to mix for 24 h to 48 h prior to dilution. Because of the hydroscopic nature of the granular CFGP 270, CFGP 260, and CFGP 255 products and the resulting high viscosity of the exposure solutions, trout were only exposed to 3 mg/L, 30 mg/L, or 100 mg/L. For polymers less commonly used in aquatic environments (CFGP 288, CFGP 270, CFGP 260, and CFGP 255), only fish from the treatments subjected to the highest concentrations of these polymers had their gills examined using light microscopy. For polymers commonly used directly in aquatic environments (Water Lynx gel blocks CFPL 297, CFPL 297.3, and CFPL 294), fish from a range of concentrations of these polymers had their gills examined histologically.

The low solubility of the Water Lynx anionic copolymer gel products prevented us from creating stock solutions. Instead, for gel block products CFPL 297, CFPL 297.3, and CFPL 294, an appropriate weight was added to dechlorinated tap water such that if the CFPL product were to fully dissolve, this stock solution would have a concentration of 300 mg/L. The solution was gently mixed by aeration for approximately 1 wk at 15 °C. Fish were exposed to either a 1%, a 10%, or a 100% solution of this mixture corresponding to 3 mg/L, 30 mg/L, or 300 mg/L. The CFPL 293 fish were exposed to solutions that would correspond to 3 mg/L, 30 mg/L, and 200 mg/L because of the higher reported toxicity of the CFPL 293 product (*Pimephales promelas* 48-h 50% lethal concentration = 294 ppm; data supplied by manufacturer). However, gelatinous masses of residual CFPL product often remained within the individual 300 mg/L stock solutions for the entire 30 d of fish exposure despite constant mixing by aeration.

Light microscopy of gill tissue

At 7 d or 30 d of exposure, 5 fish from each exposure group were euthanized by immersion in a solution of tricaine methane sulfonate (1 g/L MS-222; Syndel Labs). Gill baskets were removed, rinsed in cold phosphate-buffered saline (pH 7.0), and placed overnight in Bouin's fixative (75% saturated picric acid, 20% formalin, 5% glacial acetic acid; Fisher). The tissues were then transferred to 70% ethanol, rehydrated, and processed using a Histomatic Tissue Processor (Leica TP 1020). The tissues were embedded in paraffin blocks, sectioned in 4 μM using a microtome, and placed on microscope slides using routine methods. The slides were stained using Harris' hematoxylin and acidified eosin and coded. The gill lesions were evaluated as described below without knowledge of groups and/or treatments. A second pathologist interpreted a portion of the slides (also without knowledge of treatments) to ensure consistency.

Histopathology of 3 full-length gill filaments at 3 separate and nonoverlapping locations was evaluated at either 10× magnification (lamellar fusion, interlamellar hyperplasia, epithelial lifting) or 40× magnification (epithelial hypertrophy, mucous cell metaplasia, epithelial swelling, and pyknosis/karyorrhexis/apoptosis). Ranked lesions were ascribed as described in the following paragraphs.

For lamellar fusion (synechia), the following criteria were used: none (0), no fusion; mild (1), 1% to 25% of lamellae were fused (included directly apposed lamellae—synechia), and if only 1 or 2 were fused, this category was still used; moderate (2), assigned when gills had 25% to 50% fusion; severe (3), 50% to 75% fusion; and global (4), ascribed when >75% fusion occurred. The ranking of 4 also applied when an arch and its filaments/lamellae had undergone global hyperplasia as described below. Interlamellar hyperplasia was evaluated in a similar fashion: none (0), interlamellar epithelium was 1 to 2 cell layers thick, expected in controls; mild (1), 4 to 5 cell layers thick, diffusely or multifocal hyperplasia to moderate severity; moderate (2), diffuse hyperplasia filling up to one-third to one-half of the lamellar space or the multifocal pattern that affects a greater number of lamellar spaces or multiple foci; severe (3), diffuse hyperplasia filling one-half or more of the interlamellar space; and global (4), total filling of all lamellar spaces or complete arch fusion.

Epithelial lifting was given rankings of 0 to 4: none (0) indicated no lifting; mild (1) was assigned when the epithelium of 1% to 25% of lamellae were lifted along 1 or both sides of the pillar cell channel (typically at least several epithelial cells affected along the length); moderate (2) indicated 25% to 50% of lamellae had epithelial lifting; severe (3) indicated 50% to 75% of lamellae had epithelial lifting; and global (4) was used when >75% of lamellae had epithelial lifting.

Epithelial hypertrophy was given a reduced ranking of 0 to 2 to better achieve operational consistency. A ranking of none (0) indicated that the thickness of the epithelium (on 1 side of the pillar cell channel) was up to approximately one-half the thickness of the pillar cell channel on that lamella and was the situation expected in most controls. A ranking of mild–moderate (1) was given when the lamellar epithelial thickness was greater than one-half and up to 1 full pillar cell width in thickness, and a ranking of severe (2) was assigned when the lamellar epithelial thickness was greater than 1 full pillar cell width in thickness.

Mucous cell metaplasia was also evaluated by counting the number of goblet cells along 3 lamellar arrays (1 complete filament and one-half filament) on a subsection of the lamellae examined above. Goblet cells in the arch epithelium at the base of filaments or on the filament tips were specifically excluded from the count. The number of cells undergoing marked epithelial swelling (hydropic swelling or degeneration) and pyknosis/karyorrhexis/apoptosis (a measure of cell death) was counted along the same 3 lamellar arrays up to a maximum of 50 affected cells.

Statistics

Differences between histopathology in control and treated fish at 7 d and 30 d postexposure were compared using two-way analysis of variance (ANOVA) using Prism version 5.03 for Windows (GraphPad Software), in which data were continuous and passed tests for homogeneity of variance and distribution. If two-way ANOVA indicated statistical significance, sample data from each day were analyzed separately using one-way ANOVA followed by Tukey posteriori tests ($p \leq 0.05$). For data that were discontinuous (e.g., ranked data), we used the nonparametric Kruskal-Wallis test to look for statistically significant differences. All data are presented as mean ± standard error. Unless otherwise noted, statistically significant differences were considered when $p \leq 0.05$. Only general trends were noted for cell counts of epithelial swelling and necrosis/apoptosis because of the unquantified and nonranked nature of the data when >50 cells were noted.

RESULTS

Water chemistry and survival

Supplemental Data, Tables S1 and S2, outline water-quality parameters (Supplemental Data, Table S1) for all experiments or mortality (Supplemental Data, Table S2) in either days 0 to 7 or days 8 to 30. Water temperature and pH conditions were the same through all experiments (14 ± 1 °C). Dissolved oxygen was kept between 85% and 91% solubility in all treatments. Increased average levels of conductivity relative to time-matched clean-water controls were found in water containing 300 mg/L of CFPL 297, CFGP 288, CFPL 297.3, or CFPL 294. Mortality of fish exposed to both types of anionic polymer products (CFGP and CFPL) was low and not generally dose- or time-dependent (Supplemental Data, Table S2). Of note, 5 fish exposed to 300 mg/L of CFGP 298 died on day 1 postexposure (21% mortality); however, no further deaths were observed within this treatment group over the remaining 29 d of the experiment. The reasons for these fish deaths are unknown. Fish exposed to very low concentrations of the cationic polymer MagnaFloc 368 at 0.38 mg/L experienced substantially higher levels of mortality than any of the anionic polymer exposure groups or the clean-water controls (34%, experiment 1; data not shown). Because of the higher than anticipated levels of mortality at 0.38 mg/L, the concentration of the cationic MagnaFloc 368 exposure group was lowered to 0.30 mg/L for experiment 2 and experiment 3. Mortality levels within this 0.30 mg/L exposure group were <7% over 30 d (Supplemental Data, Table S2).

Lamellar fusion

In general, only very low levels of lamellar fusion were seen within the anionic polymer exposure groups at either the day 7 or the day 30 sampling time (≤0.76 mean ranked damage, whereby a value of 1 indicates mild damage, with 1%–25% lamellar fusion; Figure 1). Although a number of statistically significant differences exist between exposure groups and time-matched controls or between different concentrations of the same polymer product (Figure 1), we will focus only on exposure groups that showed the highest average levels of lamellar fusion, for brevity. In general, higher levels of lamellar fusion were observed in fish exposed to either the cationic MagnaFloc 368 at 0.3 mg/L or the highest concentrations of anionic CFPL 297 (300 mg/L), CFPL 297.3 (300 mg/L), and CFPL 293 (200 mg/L), although the overall level of gill lamellar fusion within these fish was very mild (generally ≤0.4 average ranked damage units out of a maximum ranking of 4; Figure 1 with the exception of CFPL 293, which reached ~0.8 at 300 mg/L at day 7). Whereas fish exposed to MagnaFloc 368 (experiments 2 and 3) and fish exposed to 300 mg/L of CFPL 297.3 had increases in mean ranked lamellar damage by day 30, lamellar damage in fish exposed to 200 mg/L CFPL 293 was significantly abated at 30 d postexposure (Figure 1C).

Interlamellar hyperplasia

The average ranked level of interlamellar hyperplasia in gill tissue of polymer-exposed fish was generally low. A ranked

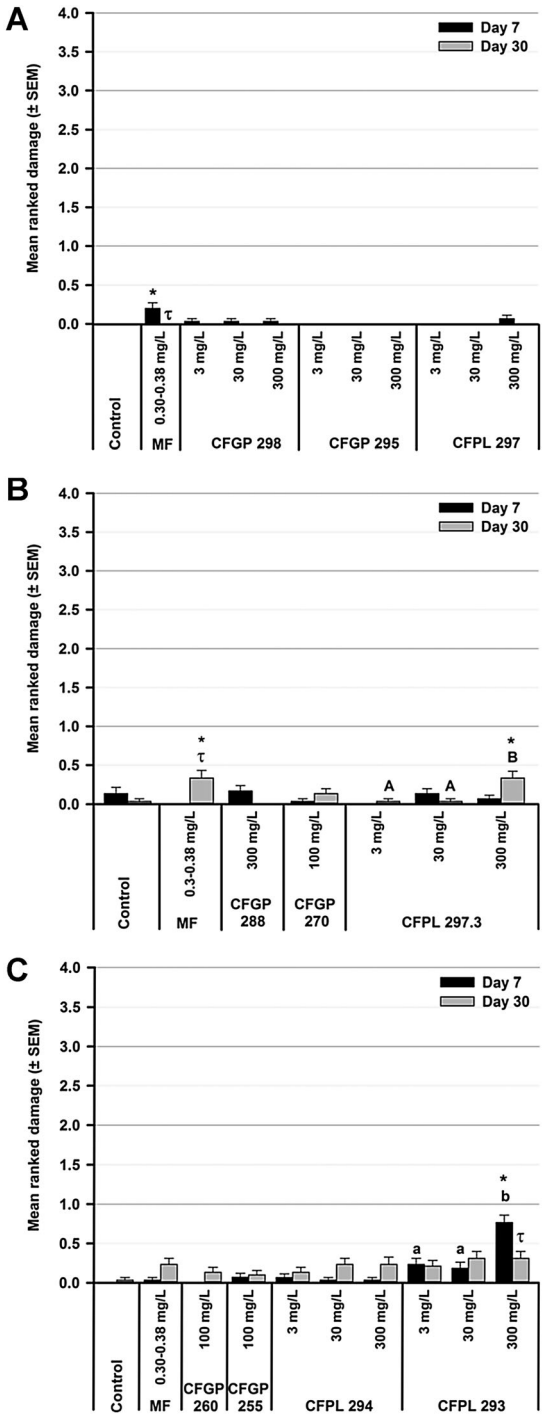

Figure 1. Lamellar fusion (synechia) in fish exposed to different polymer products for either 7 d or 30 d within experiment 1 (**A**), experiment 2 (**B**), or experiment 3 (**C**). Approximately 30 separate areas representing 5 different fish gill tissues were examined under 10× magnification, and damage was ranked as described in *Materials and Methods*. Data are represented by mean ± standard error of the mean (SEM). Asterisk (∗) indicates statistical significance ($p < 0.05$) between polymer groups and time-matched controls; τ indicates statistical significance between 30-d exposure groups and their 7-d counterparts; differing lowercase letters indicate significance between different concentration exposure groups of the same polymer product at 7 d postexposure, whereas differing uppercase letters indicate significance at 30 d postexposure.

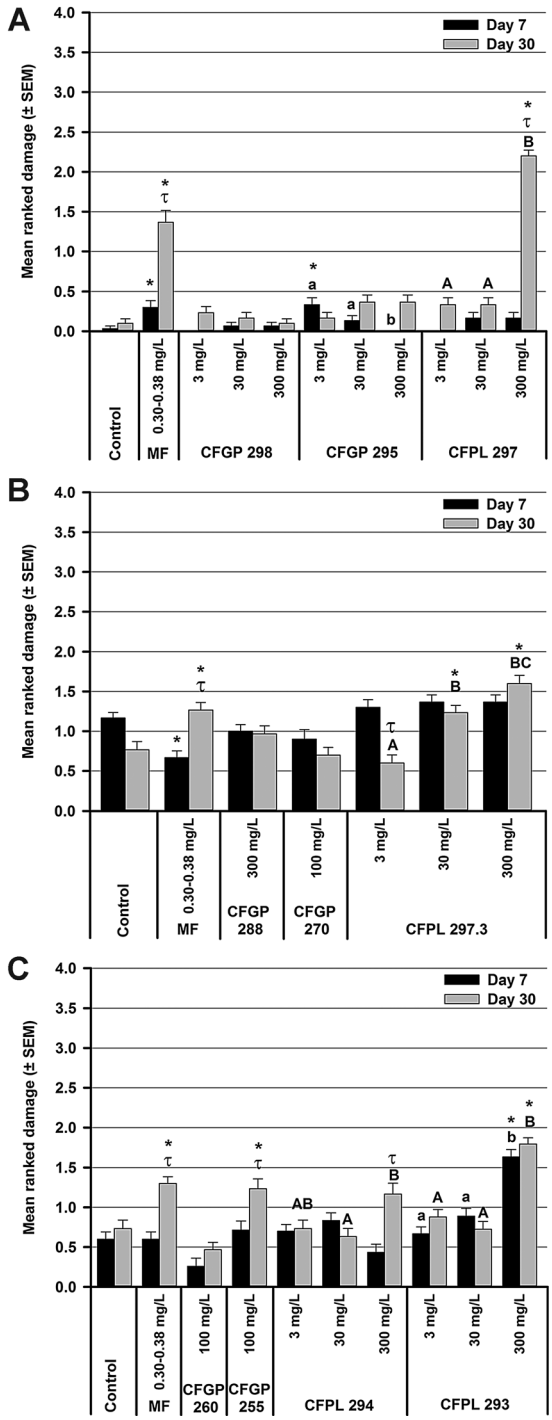

Figure 2. Interlamellar hyperplasia in fish exposed to different polymer products for either 7 d or 30 d within experiment 1 (**A**), experiment 2 (**B**), or experiment 3 (**C**). Thirty separate areas representing 5 different fish gill tissues were examined under 10× magnification, and damage was ranked as described in *Materials and Methods*. Data are represented by mean ± standard error of the mean (SEM). Asterisk (∗) indicates statistical significance ($p < 0.05$) between polymer groups and time-matched controls; τ indicates statistical significance between 30-d exposure groups and their 7-d counterparts; differing lowercase letters indicate significance between different concentration exposure groups of the same polymer product at 7 d postexposure, whereas differing uppercase letters indicate significance at 30 d postexposure.

damage level of 1 indicates only mild interlamellar hyperplasia where we would see a diffuse pattern 4 to 5 cell layers thick or multifocal hyperplasia of moderate severity. We have arbitrarily set a value of >1.0 as likely to be of biological significance, given that control fish often had some interlamellar hyperplasia as determined by our ranking criteria.

Overall, very low levels of interlamellar hyperplasia were observed in fish following either a 7-d or a 30-d exposure to polymer products (CFGP 298, CFGP 295, 3 mg/L or 30 mg/L CFPL 297, CFGP 288, CFGP 270, 3 mg/L CFPL 297.3, CFGP 260, 3 mg/L or 30 mg/L CFPL 294, or 3 mg/L or 30 mg/L CFPL 293; Figure 2). Fish exposed to very low concentrations of the cationic MagnaFloc 368 for 30 d consistently had significantly higher levels of interlamellar hyperplasia than both clean-water controls and fish exposed to MagnaFloc 368 for the shorter 7-d time period (Figure 2). Significantly higher levels of interlamellar hyperplasia were observed only in fish exposed to the higher concentrations of specific anionic polymer logs for 30 d (300 mg/L CFPL 297, Figure 2A; 30 and 300 mg/L CFPL 297.3, Figure 2B; and 200 mg/L CFPL 293, Figure 2C). Regardless, levels of hyperplasia within most treatments were generally mild or mild to moderate and not considered high (e.g., a ranking <2). The highest level of interlamellar hyperplasia was observed in fish exposed to 300 mg/L CFPL 297 for 30 d; these fish had moderate levels of interlamellar hyperplasia (mean ranked damage = 2.2 ± 0.07; Figure 2A).

Epithelial lifting

In experiment 1 and experiment 2, the interaction between the length of exposure and the treatment group was found to be significant; therefore, the data for day 7 and day 30 were analyzed separately. In experiment 3, fish from the 30-d treatment had significantly higher ($p < 0.05$) levels of epithelial lifting compared with those from the 7-d treatment. The mean ranked levels of epithelial lifting for individual exposure groups are displayed in Table 1.

Low levels of epithelial lifting were observed on the gills of fish exposed to anionic polymer products for either 7 d or 30 d (Table 1). Images of gill tissue from clean-water control fish as well as affected and unaffected polymer-exposed fish are included in Figure 3. Biological relevance was set at the mean ranked damage level of 0.5, whereby a value of 1 represents mild levels of lifting (1–25% lamellae having epithelium that is lifted along 1 or both sides of the pillar cell channel; typically, at least several epithelial cells affected along the length). Unlike the effects of high concentrations of anionic CFPL products on gill interlamellar hyperplasia, exposure of juvenile rainbow trout to high concentrations of CFPL products did not result in increased levels of epithelial lifting (Table 1). However, fish exposed to the cationic polymer MagnaFloc 368 (0.30 mg/L) in experiment 2 experienced a significant increase in epithelial lifting by 30 d compared with fish exposed for 7 d (mean ranked damage of 0.74 ± 0.08 vs 0.23 ± 0.09, respectively; Table 1 and Figure 3).

Table 1. Mean ranked level of epithelial lifting for individual exposure groups[a]

Experiment	Treatment	Concentration (mg/L)	Day 7 (Mean ± SEM)	Day 30 (Mean ± SEM)
1	Control		0.03 ± 0.03	0.03 ± 0.03
	MagnaFloc 368	0.38	0.43 ± 0.09	0.30 ± 0.09
	CFGP 298	3	0.47 ± 0.09 a	0.17 ± 0.07
		30	0.37 ± 0.09 ab	0.07 ± 0.05
		300	0.03 ± 0.03 b	0.07 ± 0.05
	CFGP 295	3	0.17 ± 0.07	0.33 ± 0.10 *A
		30	0.07 ± 0.05	0.07 ± 0.05 AB
		300	0.07 ± 0.05	0.03 ± 0.03 B
	CFPL 297	3	0.43 ± 0.09 a	0.20 ± 0.07
		30	0.33 ± 0.09 ab	0.13 ± 0.06
		300	0.00 ± 0.00 *b	0.00 ± 0.00
2	Control		0.30 ± 0.10	0.37 ± 0.10
	MagnaFloc 368	0.30	0.23 ± 0.09	0.73 ± 0.08 †
	CFGP 288	300	0.27 ± 0.08	0.17 ± 0.07
	CFGP 270	100	0.60 ± 0.12	0.27 ± 0.12
	CFPL 297.3	3	0.23 ± 0.08	0.30 ± 0.09
		30	0.13 ± 0.06	0.30 ± 0.09
		300	0.27 ± 0.08	0.17 ± 0.07
3	Control		0.00 ± 0.00	0.00 ± 0.00
	MagnaFloc 368	0.3	0.07 ± 0.05	0.13 ± 0.06
	CFGP 260	100	0.00 ± 0.00	0.23 ± 0.09
	CFGP 255	100	0.07 ± 0.05	0.17 ± 0.07
	CFPL 294	3	0.17 ± 0.07	0.13 ± 0.06
		30	0.10 ± 0.06	0.13 ± 0.06
		300	0.03 ± 0.03	0.10 ± 0.06
	CFPL 293	3	0.07 ± 0.05	0.45 ± 0.28
		30	0.04 ± 0.04	0.24 ± 0.08
		200	0.20 ± 0.07	0.00 ± 0.00

[a]Approximately 30 separate areas representing 5 different fish gill tissues were examined under 10× magnification and damage ranked upon the following system: none (0), no lifting; mild (1), 1% to 25% lamellae have epithelium which is lifted along 1 or both sides of the pillar cell channel; moderate (2), 25% to 50% of lamellae have epithelial lifting; severe (3), 50% to 75% of lamellae have epithelial lifting; global (4), >75% of lamellae have epithelial lifting.

*Indicates significance between polymer-exposed groups and time-matched clean water controls ($p \leq 0.05$ using a 1-way analysis of variance and Tukey's posteriori test).

†Indicates significance between day 30 and the corresponding day 7 value for a treatment group ($p < 0.05$); differing lowercase letters indicate significance between different concentration exposure groups of the same polymer product at day 7 postexposure, while differing uppercase letters indicate significance at day 30 postexposure.

SEM = standard error of the mean.

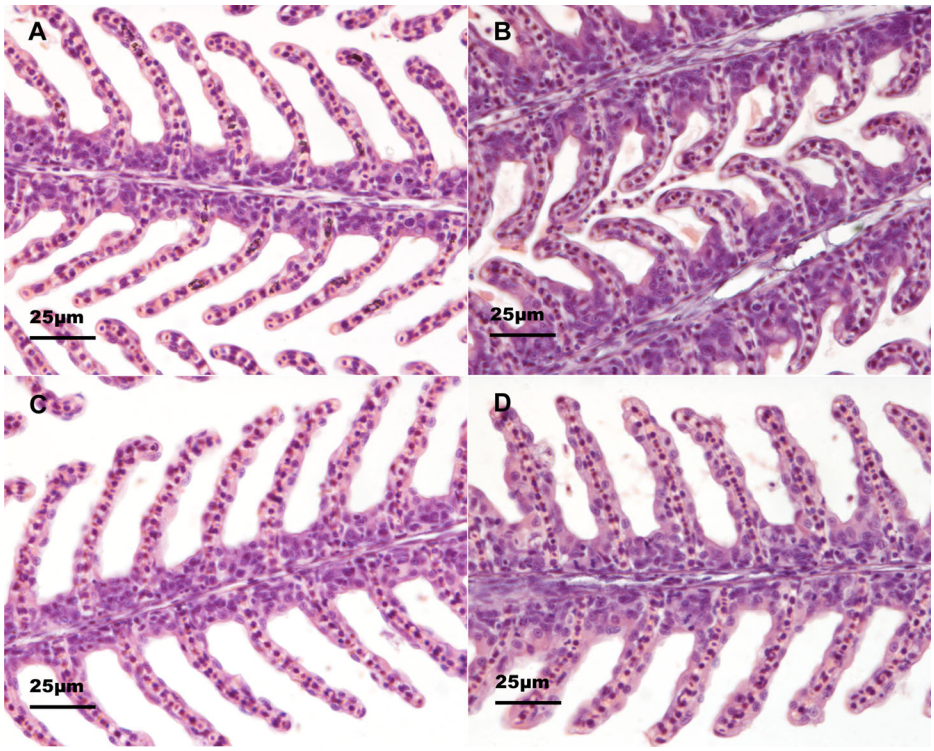

Figure 3. Hematoxylin and eosin–stained gill filaments (40× magnification) from fish exposed to clean-water control (**A**), the cationic polymer MagnaFloc 368 (0.30 mg/L) (**B**), or 2 high concentrations of anionic polymer log products: 300 mg/L of gel block CFPL 297.3 (**C**) and 300 mg/L of granular CFGP 288 (**D**). Examples of control fish and fish exposed to anionic polymer products have epithelial lifting damage ranks of 0/4, while fish exposed to MagnaFloc 368 show mild epithelial lifting (1/4). All images were taken from fish exposed for 30 d during experiment 2. Scale bar = 25 µm. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

This epithelial lifting in fish exposed to MagnaFloc 368 was variable as fish exposed to the cationic polymer in experiment 1 and experiment 3 did not demonstrate statistically significant increases compared with time-matched controls or 7-d cohorts (Table 1).

Epithelial hypertrophy

An increased level of epithelial hypertrophy compared with control fish was observed in fish exposed to CFGP 298 (3 mg/L, 30 mg/L), CFGP 295 (3 mg/L, 30 mg/L), CFPL 297 (3 mg/L, 300 mg/L), CFPL 297.3 (3 mg/L), and CFPL 293 (200 mg/L) at the 7-d sampling time point (Table 2). All other polymer exposure groups and concentrations had epithelial hypertrophy levels less than or similar to the appropriate 7-d clean-water control (Table 2). Examples of unaffected and affected gill tissues are shown in Figure 4.

Exposure to the cationic polymer MagnaFloc 368 for 30 d consistently resulted in a significant increase in the level of gill epithelial hypertrophy compared with both time-matched controls and 7-d cationic polymer–exposed fish (Table 2). Fish exposed to 3 mg/L or 30 mg/L of CFGP 298 and CFGP 295 had significant reductions in the averaged mean ranked level of epithelial hypertrophy at 30 d compared with results at 7 d. Conversely, many high concentration–exposure groups had significant increases in the level of epithelial hypertrophy by 30 d compared with 7 d (300 mg/L CFGP 298, 300 mg/L CFGP 295, 300 mg/L CFPL 297, 300 mg/L 297.3, and 100 mg/L CFGP 255). Fish exposed to these high concentrations of polymer products frequently had levels of epithelial hypertrophy that were significantly greater than those of the time-matched 30-d clean-water control (Table 2).

Mucous cell metaplasia

In addition to ranked histopathology data, direct counts of metaplastic goblet cells were enumerated. Clean-water controls had variable numbers of cells exhibiting mucous cell metaplasia depending on the exposure experiment. In general, a short-term increase in the number of affected cells was observed in fish exposed to lower concentrations of CFGP 298 (3 mg/L, 30 mg/L) and CFGP 295 (3 mg/L; Figure 5A) for 7 d. Exposure to the highest concentration of CFPL 297 (300 mg/L) and CFPL 293 (200 mg/L) produced significantly increased numbers of affected mucous cells relative to time-matched controls at 7 d (Figure 5).

Following 30 d of polymer exposure, mucous cell metaplasia significantly abated in many of the previously affected exposure groups, with the exception of fish exposed to 300 mg/L cell CFPL 297.3, which had similar numbers of metaplastic mucous cells at both sampling time points (Figure 5). Interestingly, exposure to low concentrations of cationic polymer (0.30 mg/L, up to 1000-fold lower concentrations than those of anionic polymers) consistently resulted in significantly higher mucous cell metaplasia at 30 d than at 7 d, regardless of exposure experiment.

Epithelial swelling, necrosis/apoptosis

The number of cells exhibiting epithelial swelling or necrosis/apoptosis was determined within each field of view, up to a maximum of 50 affected cells (Figure 6). Initially, a higher incidence of affected gill cells was observed in fish exposed to CFGP 298 (3 mg/L, 30 mg/L, 300 mg/L), CFGP 295 (3 mg/L, 30 mg/L, 300 mg/L), and 300 mg/L of the polymer logs

Table 2. Mean ranked level of epithelial hypertrophy for individual exposure groups[a]

Experiment	Treatment	Concentration (mg/L)	Day 7 (Mean ± SEM)	Day 30 (Mean ± SEM)
1	Control		0.00 ± 0.00	0.30 ± 0.09
	MagnaFloc 368	0.38	0.00 ± 0.00	1.33 ± 0.13 *†
	CFGP 298	3	0.73 ± 0.08 *a	0.20 ± 0.07 A†
		30	0.97 ± 0.03 *a	0.00 ± 0.00 A†
		300	0.20 ± 0.07 b	0.67 ± 0.09 B†
	CFGP 295	3	1.57 ± 0.09 *a	0.13 ± 0.06 A†
		30	1.03 ± 0.06 *b	0.00 ± 0.00 A†
		300	0.17 ± 0.07 c	0.80 ± 0.09 *B†
	CFPL 297	3	0.67 ± 0.11 *a	0.90 ± 0.10 *A
		30	0.00 ± 0.00 b	0.70 ± 0.13 *A†
		300	1.00 ± 0.00 *c	2.00 ± 0.00 *B†
2	Control		1.27 ± 0.08	1.00 ± 0.07
	MagnaFloc 368	0.30	0.13 ± 0.06 *	1.47 ± 0.09 *†
	CFGP 288	300	1.13 ± 0.08	0.97 ± 0.03
	CFGP 270	100	0.73 ± 0.08 *	0.73 ± 0.08
	CFPL 297.3	3	0.90 ± 0.07 *	0.60 ± 0.09 *A
		30	1.13 ± 0.08	0.83 ± 0.12 A
		300	1.00 ± 0.00	1.80 ± 0.07 *B†
3	Control		0.53 ± 0.09	0.83 ± 0.12
	MagnaFloc 368	0.3	0.33 ± 0.09	1.20 ± 0.07†
	CFGP 260	100	0.15 ± 0.07	0.63 ± 0.10
	CFGP 255	100	0.46 ± 0.10	0.97 ± 0.09†
	CFPL 294	3	0.37 ± 0.09	0.57 ± 0.09 A
		30	0.60 ± 0.09	0.57 ± 0.09 A
		300	0.73 ± 0.10	1.10 ± 0.11 B
	CFPL 293	3	0.83 ± 0.07 a	0.30 ± 0.08 A†
		30	0.74 ± 0.16 a	0.83 ± 0.09 B
		200	1.83 ± 0.07 *b	1.52 ± 0.09 *C

[a]Approximately 30 separate areas representing 5 different fish gill tissues were examined under 40× magnification and damage ranked upon the following system: none (0), thickness of the epithelium (on 1 side of the pillar cell channel) is up to approximately one-half the thickness of the pillar cell channel on that lamella; mild to moderate (1), epithelial thickness was greater than one-half and up to 1 full pillar cell width in thickness; severe (2), thickness was greater than 1 full pillar cell width in thickness.
*Indicates significance between polymer-exposed groups and time-matched clean water controls ($p \leq 0.05$ using a 1-way analysis of variance and Tukey's posteriori test).
†Indicates significance between day 30 and the corresponding day 7 value for a treatment group ($p < 0.05$); differing lowercase letters indicate significance between different concentration exposure groups of the same polymer product at day 7 postexposure ($p < 0.05$), while differing uppercase letters indicate significance at day 30 postexposure ($p < 0.05$).
SEM = standard error of the mean.

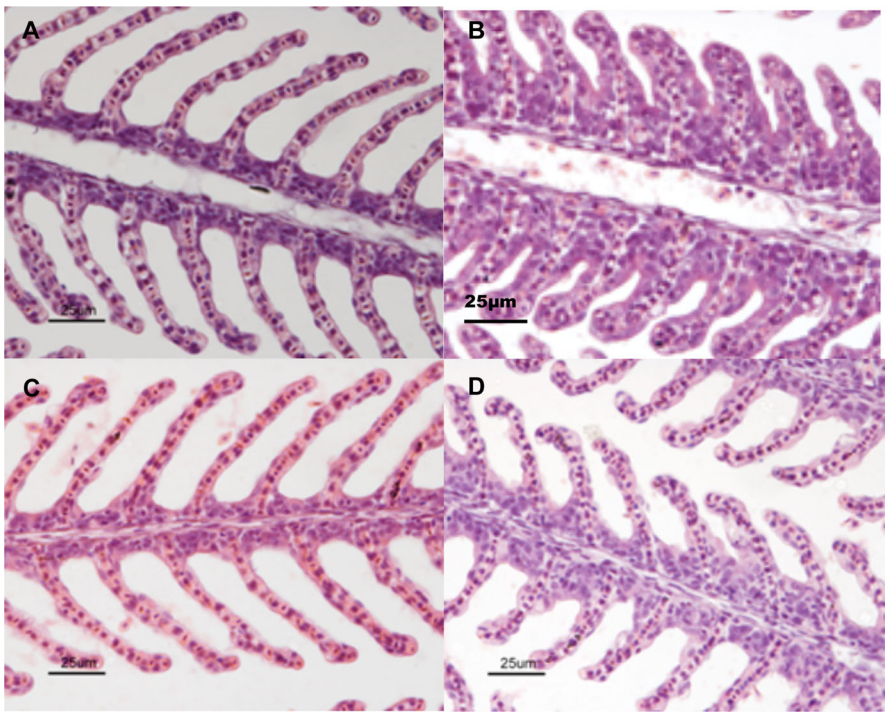

Figure 4. Hematoxylin and eosin–stained gill filaments (40× magnification) from fish exposed to clean-water control (**A**), the cationic polymer MagnaFloc 368 (0.30 mg/L) (**B**), an anionic polymer exposure group that did not affect epithelial hypertrophy (30 mg/L CFPL 294) (**C**), and an anionic polymer exposure group that did induce epithelial hypertrophy (300 mg/L CFPL 294) (**D**). Control fish and fish exposed to 30 mg/L of CFPL 294 (**C**) showed epithelial hypertrophy damage ranks of 0/2, whereas fish exposed to MagnaFloc 368 (**B**) or 300 mg/L of CFPL 294 (**D**) had the greatest degree of epithelial hypertrophy (moderate, 2/2). All images were taken from fish exposed for 30 d during experiment 3. Scale bar = 25 µm. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

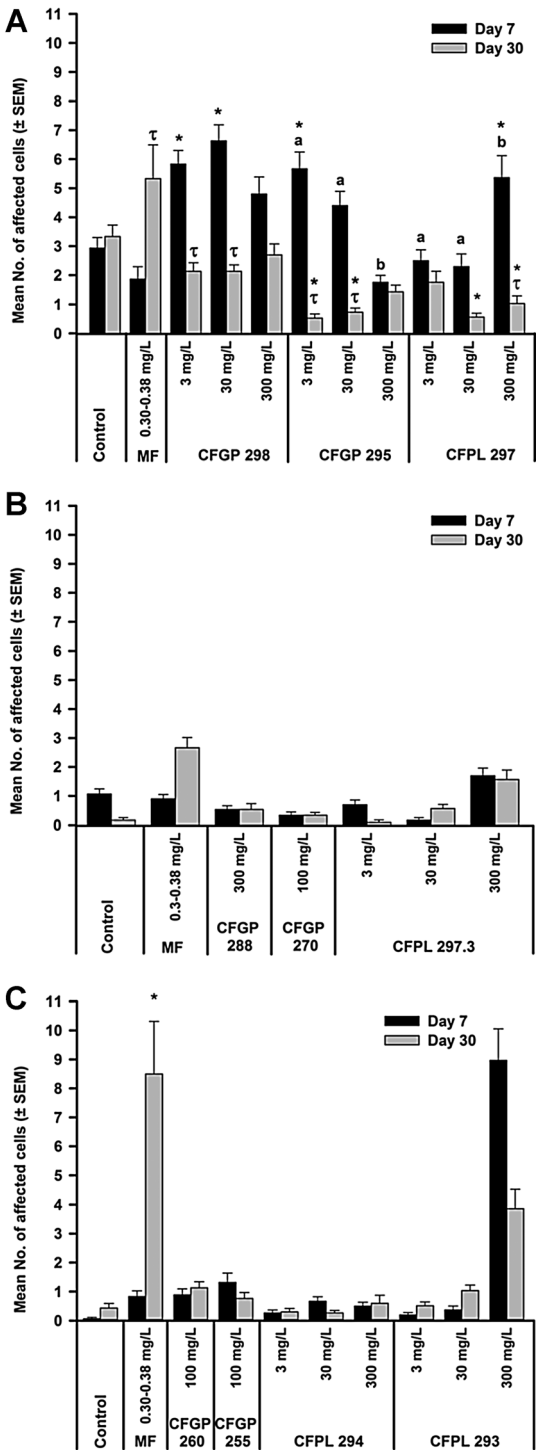

Figure 5. Mean number of cells with mucous cell metaplasia (± standard error of the mean [SEM]) in fish exposed to different polymer products for either 7 d or 30 d within experiment 1 (**A**), experiment 2 (**B**), or experiment 3 (**C**). Approximately 30 separate areas representing 5 different fish gill tissues were examined under 40× magnification. Asterisk (*) indicates tatistical significance ($p < 0.05$) between polymer groups and time-matched controls; τ indicates statistical significance between 30-d exposure groups and their 7-d counterparts; differing lowercase letters indicate significance between different concentration exposure groups of the same polymer product at 7 d postexposure, whereas differing uppercase letters indicate significance at 30 d postexposure.

CFPL 297 and CFPL 293 at 7 d postexposure (Supplemental Data, Figure S1). By 30 d, abnormal cells were less frequently observed within the CFGP 298 (3 mg/L, 30 mg/L, 300 mg/L) and CFGP 295 (3 mg/L, 30 mg/L) exposure groups. However, increases in abnormal cell counts were noted in fish exposed to the highest concentrations of CFGP 295 (300 mg/L), CFPL 297 (300 mg/L), CFPL 297.3 (300 mg/L), and CFPL 293 (200 mg/L), relative to the time-matched clean-water control and 7-d cohorts (Figure 6). The remaining exposure groups were unaffected by polymer exposure at either sampling time point (MagnaFloc 368, CFGP 288, CFGP 270, CFGP 240, CFGP 255, CFPL 294; Figure 6). Images of gill tissues from clean-water controls as well as from polymer treatment groups can be seen in Figure 7.

DISCUSSION

Gill tissue is commonly examined in toxicological studies because of its large surface area that is intimately exposed to the environment. Short-term exposure to waterborne irritants or pollutants may cause physical changes such as cell swelling (epithelial hypertrophy), potentially increasing the thickness of the lamellae within the gill and reducing gas exchange efficiency [18,19]. Epithelial hypertrophy is also often associated with epithelial lifting because of interstitial edema [20]. Severe or chronic exposure to irritants and pollutants can cause profound changes in gill structure such as interlamellar hyperplasia. Severe interlamellar hyperplasia can eventually result in lamellar fusion with loss of the interlamellar space, greatly reducing the surface area of the gill [18,20]. Common gill responses to irritant and pollutant exposure are mucous cell metaplasia, increased mucous production, and necrosis or apoptosis [18,19].

Epithelial hypertrophy was found to be one of the most affected histopathological parameters measured. Epithelial hypertrophy is considered one of the earliest features of external irritant exposure [19]. This pathology has been previously observed in fish gill tissue following exposure to the flocculent polymer chitosan. Chitosan is generally considered to be nontoxic in most situations but is acutely toxic to rainbow trout when dissolved in acetic acid, a common method of liquid dispersal [21]. Exposure to concentrations as low as 0.019 mg/L chitosan acetate resulted in epithelial lifting, epithelial hypertrophy, and epithelial hyperplasia, while higher concentrations caused lamellar fusion as well as the proliferation and hypertrophy of mucous cells [21].

Cationic polymers have also been shown to induce physiological changes in gill tissue at very low concentrations and short exposure times (0.307–7.2 mg/L and 20–40 min, respectively [17]). Cationic polymer–induced changes in gill integrity resulted in a significant decrease in blood pH, an increase in blood ammonia, and fluctuations in blood sodium, potassium, and chloride ion concentrations [17]. Muir et al. [17] also observed mucous secretions streaming from the gills of trout exposed to cationic polymers; increased production of mucous by goblet cells is a well-known irritant effect of pollutants in fish [18]. No excessive production of mucous was observed following the exposure of juvenile rainbow trout to polymer products in the present study. However, increased numbers of lamellar goblet cells were consistently noted in fish chronically (30 d) exposed to very low concentrations of the cationic polymer MagnaFloc 368. Mucous cell metaplasia was also noted in fish exposed to specific product blends of Soil Lynx CFGP and Water Lynx CFPL products, although in general the degree

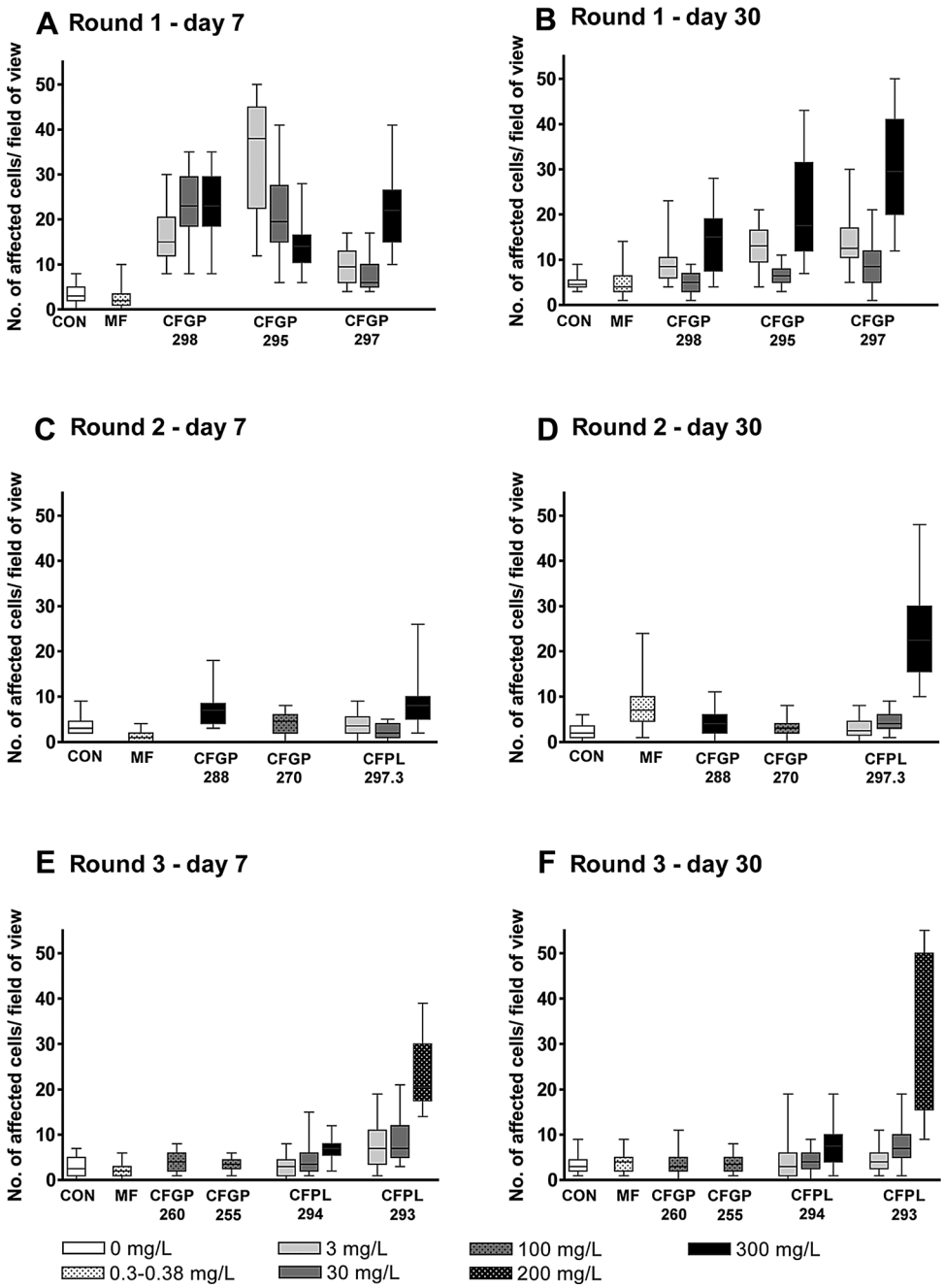

Figure 6. Mean number of cells undergoing epithelial swelling and necrosis/apoptosis (±standard error of the mean [SEM]) in fish exposed to different polymer products for 30 d within experiment 1 (**A**), experiment 2 (**B**), or experiment 3 (**C**). Approximately 30 separate areas representing 5 different fish gill tissues were examined under 40× magnification. The number of cells exhibiting both characteristics was counted up to a maximum of 50.

of metaplasia was reduced at 30 d relative to 7 d. This suggests that trout exposed to anionic polymer products may have experienced a short-term irritant effect at 7 d (manifested as mild mucous cell metaplasia) but had adapted by 30 d. The consistent and more profound increase in mucous cell metaplasia at 30 d versus 7 d in fish exposed to MagnaFloc 368 suggests that adaptation does not occur and that the cationic polymer may have induced more long-term changes in the gill structure despite its far lower concentration.

While the toxicity of cationic polymers to aquatic organisms is well known, gill histopathology studies involving the exposure of fish to anionic polymer products are comparatively rare. Liber et al. [10] exposed lake trout fry (*Salvelinus namaycush*) to different concentrations of an anionic PAM (MagnaFloc 156) or a cationic polymer (MagnaFloc 368) using either acute (96 h) or chronic (30 d) exposures. These authors reported both cationic and anionic polymer–induced effects on cloudy swelling of epithelial cells (epithelial hypertrophy) and a loss of filament structure as a result of the thickening and shortening of the gill filaments. Concentrations of anionic PAM required to induce these histopathological effects were high (≥150 mg/L). Following 30 d of exposure, few significant differences were observed between cationic or anionic polymer products and clean-water controls [10]. However, an increased lamellar height in fish exposed to high concentrations of anionic PAM (150 mg/L, 300 mg/L) was suggested by the authors to possibly represent a physiological adaptation to the polymers over time [10]. Although we did not measure lamellar height, the

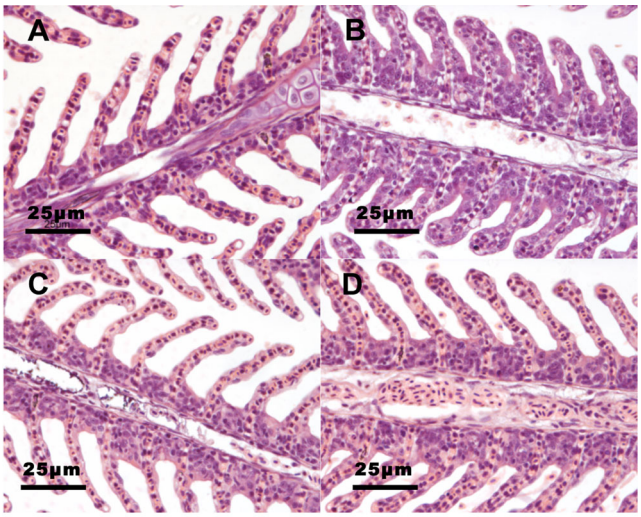

Figure 7. Hematoxylin and eosin–stained gill filaments (400× magnification) from fish exposed to clean-water control (**A**), the cationic polymer MagnaFloc 368 (0.30 mg/L) (**B**) showing damage (arrows), an anionic polymer exposure group that did not affect epithelial swelling and necrosis/apoptosis (30 mg/L CFGP 295) (**C**), and an anionic polymer exposure group that did induce epithelial swelling and necrosis/apoptosis (arrows) (300 mg/L CFGP 295) (**D**). All images were taken from fish exposed for 30 d during experiment 1. Scale bar = 25 μm. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

present study was also able to show a reduced level of pathology between fish sampled on day 7 and those sampled on day 30, though this varied with the polymer product blend, exposure concentration, and the histopathological parameter examined.

Exposure to some anionic polymer blends (but generally only CFPL products) at very high concentrations resulted in significant increases in the number of cells exhibiting marked epithelial swelling and necrosis/apoptosis. Unlike mucous cell metaplasia, which was reduced from 7 d to 30 d, the number of dead cells within the gills of these groups of fish tended to increase with time. Despite this observation, there was no significant mortality noted in the fish exposed to the highest concentration of CFPL products. The increased epithelial swelling and necrosis/apoptosis may have potential negative consequences, such as increased morbidity or mortality, if combined with secondary environmental stressors, such as low environmental oxygen, disease, or parasitemia. To determine if these noted changes in necrosis/apoptosis following exposure to these very high concentrations are of concern, future studies should examine the physiological status of the fish (e.g., changes in osmolarity, ion balance, pH regulation) in combination with normal environmental mild stressors.

Individual polymer products were variable in terms of their effects on gill histopathology—high concentrations of some polymer products, such as CFPL 293, were more likely to elicit significant pathological lesions in gill tissue, whereas others (CFGP 288, CFPL 294) rarely demonstrated effects. The reason for such variability within individual anionic polymer product blends on gill histopathology is unknown because the specific chemical makeup of each anionic polymer blend is proprietary. However, studies by Hall and Mirenda [22] suggest that the charge density of polymer may be the most important factor in controlling polymer toxicity to fathead minnow (*Pimephales promelas*), with increasing charge density resulting in higher toxicity. Smaller–molecular weight polymers may also have higher toxicity to aquatic organisms [23], although other studies [22] have not seen such a correlation. It is important to note that many of these studies focused on cationic polymers rather than anionic polymers because of their increased toxicity to aquatic life. The high toxicity of cationic polymers to fish is hypothesized to be the result of electrostatic attraction between the polymers and negatively charged gill tissue (Yamamoto [24], as cited in Beisinger and Stokes [25]). Research by Muir et al. [17] found that cationic polymers accumulated on gill tissue, lending support to this hypothesis. Accordingly, anionic polymer products would experience charge repulsion with the negatively charged gill tissue, thus potentially resulting in less polymer-induced irritation.

While anionic PAMs appear to have very little effect on fish species at concentrations used in most environmental applications, high concentrations of PAM products following a spill event could potentially pose a risk to fish because of the extreme viscosity of these solutions. Buchholz [26] found that 2500 mg/L of anionic PAM caused deaths in exposed minnows and hypothesized that the deaths were likely caused by an increased energy requirement for movement and insufficient gas exchange in the viscous solution. Smaller aquatic organisms such as invertebrates are likely more acutely affected by viscosity than larger organisms [27]. Field studies mimicking real-world conditions (recommended application rates as well as worst-case scenarios) and toxicity tests on a variety of organisms from different trophic levels may provide the best methods to assess long-term impacts of anionic polymer product use on the receiving environment.

CONCLUSIONS

In general, chronic, sublethal exposure of juvenile rainbow trout to environmentally relevant doses of anionic polymer products resulted in either no damage or very low levels of damage to gill tissue. In instances where mild to moderate levels of gill pathology were observed, the effects were found only in fish exposed to the higher concentrations of anionic polymer products ($\geq$100 mg/L). To put this concentration in perspective, the recommended application rate of anionic PAM for erosion control in agricultural irrigation furrows is between 1 mg/L and 10 mg/L [7,28–30]. Other studies have shown minimal toxicity of anionic PAMs to aquatic life despite concentrations far higher than that used in irrigation water [12]. Fish exposed to $\leq$0.38 mg/L of the cationic polymer product MagnaFloc 368 also generally experienced low levels of pathology; in our preliminary experiments, concentrations greater than 0.38 mg/L caused high levels of mortality. We believe that the toxicity of MagnaFloc 368 may be a result of buildup of cationic polymer on the negatively charged gill surface, leading to impairment of O_2 uptake and subsequent death (as suggested by Beisinger and Stokes [25]). Disruptions in ion regulation at the gill surface by cationic polymer exposure [17] also cannot be ruled out. Importantly, however, in instances when exposure to the cationic polymer did result in significant increases in pathology, these effects were observed at far lower concentrations than that of anionic polymer products—up to 1000-fold lower concentrations than anionic products ($\leq$0.38 mg/L vs 300 mg/L, respectively). This concentration discrepancy between cationic

polymer and anionic polymer products highlights the increased toxicity and potential risk to aquatic organisms posed by cationic polymer products.

SUPPLEMENTAL DATA

Tables S1–S2.
Figure S1. (193 KB PDF).

Acknowledgment—The authors gratefully acknowledge funding from the National Research Council of Canada's Industrial Research Assistance Program to Clearflow Enviro Systems Group. During the time the present study was conducted, J.L. Kerr was an employee of Clearflow Enviro Systems Group.

REFERENCES

1. Newcombe CP, MacDonald DD. 1991. Effects of suspended sediments on aquatic ecosystems. *N Am J Fish Manag* 11:72–82.
2. Bolto B, Gregory J. 2007. Organic polyelectrolytes in water treatment. *Water Res* 41:2301–2324.
3. Barvenik FW. 1994. Polyacrylamide characteristics related to soil applications. *Soil Sci* 158:235–243.
4. Lentz RD, Sojka RE. 1994. Field results using polyacrylamide to manage furrow erosion and infiltration. *Soil Sci* 158:274–282.
5. Green VS, Stott DE. 1999. Polyacrylamide: A review of the use, effectiveness and cost of a soil erosion control amendment. In Stott DE, Mohtar RH, Steinhardt GC, eds, *Sustaining the Global Farm: Selected papers from the 10th International Soil Conservation Organization Meeting*, Perdue University and the USDA-ARS National Soil Erosion Research Laboratory, West Lafayette, IN, USA, May 24–29, 1999, pp 384–389.
6. Entry JA, Sojka RE, Watwood M, Ross C. 2002. Polyacrylamide preparations for protection of water quality threatened by agricultural runoff contaminants. *Environ Pollut* 120:191–200.
7. Sojka RE, Entry JA, Orts WJ, Morishita DW, Ross CW, Horne DJ. 2005. Synthetic- and bio-polymer use for runoff water quality management in irrigated agriculture. *Water Sci Technol* 51:107–115.
8. Exon JH. 2006. A review of the toxicology of acrylamide. *J Toxicol Environ Health B Crit Rev* 9:397–412.
9. McCollister DE, Hake CL, Sadek SE, Rowe VK. 1965. Toxicological investigations of polyacrylamides. *Toxicol Appl Pharmacol* 7:639–651.
10. Liber K, Weber L, Levesque C. 2005. Sublethal toxicity of two wastewater treatment polymers to lake trout fry (Salvelinus namaycush). *Chemosphere* 61:1123–1133.
11. Krauth DM, Bouldin JL, Green VS, Wren PS, Baker WH. 2008. Evaluation of a polyacrylamide soil additive to reduce agricultural-associated contamination. *Bull Environ Contam Toxicol* 81:116–123.
12. Weston DP, Lentz RD, Cahn MD, Ogle RS, Rothert AK, Lyde MJ. 2009. Toxicity of anionic polyacrylamide formulations when used for erosion control in agriculture. *J Environ Qual* 38:238–247.
13. Stephens SH. 1991. Final report on the safety assessment of polyacrylamide. *J Am Coll Toxicol* 10:193–202.
14. National Industrial Chemicals Notification and Assessment Scheme. 2002. Acrylamide. Priority Existing Chemical Assessment Report No. 23. Commonwealth of Australia, Canberra.
15. Caulfield MJ, Qiao GG, Soloman DH. 2002. Some aspects of the properties and degradation of polyacrylamides. *Chem Rev* 102:3067–3083.
16. Beim AA, Beim AM. 1994. Comparative ecological–toxicological data on determination of maximum permissible concentrations (MPC) for several flocculants. *Environ Technol* 15:195–198.
17. Muir MM, Kosteretz KG, Lech JJ. 1997. Localization, depuration, bioaccumulation and impairment of ion regulation associated with cationic polymer exposure in rainbow trout (Oncorhynchus mykiss). *Xenobiotica* 27:1005–1014.
18. Ferguson HW. 2006. *Systemic Pathology of Fish: A Text and Atlas of Normal Tissues in Teleosts and Their Responses in Disease*, 2nd ed. Scotian Press, London, UK, pp 24–62.
19. Roberts RJ. 2004. *Fish Pathology*, 3rd ed. WB Saunders, Philadelphia, PA, USA.
20. Mallat J. 1985. Fish gill structural changes induced by toxicants and other irritants: A statistical review. *Can J Fish Aquat Sci* 42:630–648.
21. Bullock G, Blazer V, Tsukuda S, Summerfelt S. 2000. Toxicity of acidified chitosan for cultured rainbow trout (Oncorhynchus mykiss). *Aquaculture* 185:273–280.
22. Hall WS, Mirenda RJ. 1991. Acute toxicity of wastewater treatment polymers to Daphnia pulex and the fathead minnow (Pimephales promelas) and the effects of humic acid on polymer toxicity. *Research Journal of the Water Pollution Control Federation* 63:895–899.
23. Goodrich MS, Dulak LH, Friedman MA, Lech JJ. 1991. Acute and long-term toxicity of water soluble cationic polymers to rainbow trout (Oncorhynchus mykiss) and the modification of toxicity by humic acid. *Environ Toxicol Chem* 10:509–515.
24. Yamamoto S. 1979. Safety of cationic polymeric coagulants. *Seikatsu to Kankyo* 24:32–38.
25. Beisinger KE, Stokes GN. 1986. Effects of synthetic polyelectrolytes on selected aquatic organisms. *Journal of the Water Pollution Control Federation* 58:207–213.
26. Buchholz FL. 1992. Polyacrylamides and polyacrylic acids. In Elvers B, Hawkins S, Schulz G, eds, *Ullmann's Encyclopaedia of Industrial Chemistry*, Vol A21. VCH, Weinheim, Germany, pp 143–146.
27. Acharya K, Schulman C, Young MH. 2010. Physiological response of Daphnia magna to linear anionic polyacrylamide: Ecological implications for receiving waters. *Water Air Soil Pollut* 212:309–317.
28. Barvenik FW, Sojka RE, Lentz RD, Andrawes FF, Messner LS. 1996. Fate of acrylamide monomer following application of polyacrylamide to cropland. *Proceedings*, Managing Irrigation-Induced Erosion and Infiltration with Polyacrylamide, College of Southern Idaho, Twin Falls, ID, USA, May 6–8, 1996, pp 103–110.
29. Orts WJ, Sojka RE, Glenn GM. 2000. Biopolymer additives to reduce erosion-induced soil losses during irrigation. *Ind Crop Prod* 11:19–29.
30. Entry JA, Sojka RE. 2000. The efficacy of polyacrylamide related compounds to remove microorganisms and nutrients from animal wastewater. *J Environ Qual* 29:1905–1914.