Effects of Cationic Polyacrylamide and Cationic Starch on Aquatic Life

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ABSTRACT: Geotextile tubes with polyacrylamide flocculants are widely used in dewatering applications. Due to variations in solid concentrations during dredging, excess flocculant is sometimes released into the environment, where it might have toxic effects. This study determined optimum doses for a cationic polyacrylamide (CPAM) and a natural-based polymer alternative, cationic starch (C. Starch). Slurry samples were treated with optimum and 50% overdoses of each compound, and residual polymer concentrations were measured. Overdosed C. Starch resulted in low residuals (<2 ppm), but overdosed CPAM resulted in 17.4 ppm residual polymer. The relative toxicity of CPAM and C. Starch was also tested using zebrafish embryos. 100% of embryos that had their chorion removed and 71.8% of embryos that retained their chorions, were dead or dying after 7 days of exposure to CPAM. In contrast, there was no statistically significant difference in the numbers of embryos that were dead or dying, when exposed to C. Starch, compared to controls.

These data strongly suggest that C. Starch should be considered as a replacement to CPAM in dewatering applications.

Keywords: Geosynthetics, geotextile tube, dewatering, cationic starch, cationic polyacrylamide, toxicity, zebrafish, lethality

1. INTRODUCTION

Geotextile tubes are widely used in dewatering applications to decontaminate or "clean up" lakes, rivers, and ponds. High water content dredged slurries are pumped into geotextile tubes, usually in multiple intervals, and the water filters through the pores of the permeable geotextile while the sediments are retained. For geotextile tubes to dewater fine sediments, often flocculants are used to improve sediment retention and dewatering performance (Kang & McLaughlin, 2016). Flocculants help bridge small sediments (i.e. silt and clay) together to form larger flocs. Synthetic polymers, especially acrylamide-based, are used extensively in geotextile tube dewatering. Several studies (Kang & McLaughlin, 2016; Khachan et al., 2011; Koerner & Koerner, 2006; Lee et al., 2011) have shown that polyacrylamide is highly effective in reducing dewatering time and increasing sediment retention in geotextile tubes.

Kang & McLaughlin (2016) studied the effects of passive dosage of a commercial biopolymer (chitosan) using porous socks and a charging agent in comparison to the active dosage of dissolved polyacrylamide (PAM) through injection. Turbid water (cloudy water caused by small suspended sediments) was pumped through a corrugated pipe into a geotextile bag (61cm x 61cm) at 57 L/min, and water samples were collected at the pipe entrance, pipe exit (where it entered the bag), and after it drained from the bag. Each sample was shaken for 10 seconds then allowed to settle for 30 seconds before turbidity was measured using a turbidity probe. These authors found that the control system, which used only the geotextile bag (no flocculant), showed a 70% reduction in turbidity. However, the addition of PAM or chitosan resulted in a 97% reduction in the turbidity of the effluent by the time it exited the geotextile bag. The authors concluded that both passive and active dosing were effective methods of adding a flocculant, and that both the

PAM and chitosan provided sufficient flocculation in construction site water (i.e. turbidity > 2000 NTU) to improve dewatering performance.

Khachan et al. (2011) studied dewatering performance of cationic polyacrylamide versus a starch-based polymer (starch phosphate). Varying doses of each flocculant were mixed into silt slurries of 33% solid concentration for 180 seconds. The slurry was then poured into the testing apparatus which consisted of a 7.2 cm diameter geotextile specimen at the bottom of a pressure chamber. An applied pressure of 34.5 kPa was used to imitate internal pressures during geotextile tube dewatering. The filter cake height, filter cake moisture content, percent solids retained, flow rate, and percent solids passing were measured and evaluated. These authors found a linear relationship between the amount of flocculant added and the water content of retained soil and the height of the filter cake. It was also observed that an increase in polymer flocculant results in a decreased dewatering time; dewatering time was reduced from 150 minutes to 50 and 30 minutes for the starch-based polymer and synthetic polymer, respectively.

These studies, along with others, have shown the effectiveness of acrylamide-based flocculants for increasing dewatering rates and soil retention in geotextile tube dewatering applications. However, there is a growing concern about the safety and environmental impact of synthetic polymers (Albassam et al. 1987; Bhatia et al. 2014). In geotextile tube dewatering applications, laboratory tests are conducted in advance to determine the optimum dose of the polymer needed to adequately flocculate the soil sediments. The optimum dose depends on the percentage of soil by mass in the soil and water slurry that would, for example, be dredged from the bottom of a lake. However, when the dredging takes place and the slurry is conveyed to the geotextile tubes, the soil percentage does not necessarily remain constant (i.e. there is no way to control the percent solids in the field). If there is more soil mass than expected, there will not be

enough polymer for effective flocculation, and this will allow for some small unflocculated soil sediments to escape the tube. On the other hand, if there is a lower concentration of slurry than expected, there will be excess polymer, and this will be released in the supernatant draining from the geotextile tube (Bhatia et al. 2014). Recently, there is increasing concern about the effect of cationic acrylamide-based flocculants on human and environmental health, and this has lead to an interest in studying these potential effects (Buczek et al. 2017; Harford et al. 2011).

Harford et al. (2011) studied the biological impacts of cationic and anionic polyelectrolyte polymers on aquatic life. These polymers have been used in water treatment processes through flocculant block formulation to flocculate/coagulate the particles to make removal easier, similar to geotextile tube dewatering. In this study, the two components of a commercial flocculant block formulation, anionic polyacrylamide (PAM) and polyethylene glycol (PEG), and the flocculant block itself were assessed to determine which ingredient was most toxic, and to find safe concentration levels. Five Australian freshwater species (unicellular green alga, duckweed, green hydra, cladoceran, and Northern trout gudgeon) were exposed to varying concentrations of the flocculant block and its components for varying durations depending on each species. The highest exposures for the flocculant block, PAM, and PEG were 2000, 5000, and 12000 mg/L (ppm), respectively. The researchers found that the flocculant block affected these species in different ways. For example, cladoceran was the most sensitive, showing reproductive impairment and a reduced growth rate, while the duckweed showed a 27% increase in growth rate (Harford et al. 2011). The analyses of the two individual components also showed a wide range of toxic responses, suggesting that there are negative effects on at least some species from exposure to these chemicals, although this needs to be investigated in more detail.

This study aimed to evaluate and compare a commercially available cationic polyacrylamide (CPAM) and a natural-based alternative, cationic wheat starch (C. Starch). For this study, nine starch-based flocculants (wheat starch, corn starch, and potato starch) with different charge densities (CD) were prepared. The effectiveness of each starch-based flocculant was evaluated through performing jar tests and pressure filtration tests. Based on the measured turbidity of the supernatant in jar tests and the dewatering time obtained from pressure filtration tests, it was determined that the wheat starch-based flocculant is the most effective natural flocculant among the nine prepared flocculants. Khachan et al (2014, 2011) have shown that both flocculants are effective in geotextile tube dewatering. Here, the optimum dose necessary for effective flocculation, and thus dewatering rates and capabilities, were measured for each polymer. Residual polymer concentrations in the supernatant of settled slurries were measured at an optimum dose and a 50% overdose of each polymer flocculant. Finally, this study analyzed the toxicity of each polymer using zebrafish embryos as a model system, to determine if the alternative natural-based polymer (C. Starch) is less toxic to aquatic vertebrates than CPAM.

2. MATERIALS

2.1 Sediments

The sediments used for this study were obtained from Clarks Gravel Pit in Tully, New York. The sample was dry sieved through a No 200 standard sieve; anything passing was considered "Tully Fine" while anything retained on Sieve No. 200 was considered "Tully Coarse." This resulted in approximately 26% fines. A sieve analysis was performed on the Tully Coarse soil; this showed that the soil was a well graded silty sand (ASTM D2487, 2008). A hydrometer analysis of the Tully Fine soil was completed, showing that it consisted of approximately 23% clay-sized particles (<0.002 mm) and 77% silt-sized particles (0.002 - 0.06 mm). The particle size distribution for the two soils is shown in Figure 1. The specific gravity of the Tully soil was measured as 2.65.

2.2 Polymer Preparation

In a glass jar, 100 grams of deionized (DI) water and 0.25 grams of a commercially available cationic polyacrylamide (Zetag® 8185) were combined to produce a 2500 ppm solution. The ingredients were manually shaken for approximately 30 seconds and left overnight at room temperature to allow for complete mixing before use.

The cationic starch was prepared in the Syracuse University (SU) Department of Civil & Environmental Engineering. The cationizing agent used in this preparation was (3-chloro-2-hydroxypropyl) trimethylammonium chloride (CHPTAC), and a 1:3 ratio of glucose to CHPTAC was obtained. First, 10.08 grams of powdered wheat starch was added to 40 mL of ethanol in a 500-mL flask. In a separate 200 mL flask, 8.5 grams of sodium hydroxide (NaOH) was added to 20 mL of DI water and gently swirled to dissolve. This was then added to the 500-mL flask containing the ethanol and wheat starch solution and gently swirled to mix. An additional 40 mL

of ethanol was added, followed by 54 mL of the cationizing agent, CHPTAC. This was added dropwise using a syringe while the 500-mL flask sat on a stirring/hot plate with a magnetic stirrer.

The solution was then heated and stirred constantly using the hot plate and magnetic stirrer for six hours. A thermometer was placed through the cork stopper to monitor temperature; the solution was maintained at 60℃. After six hours, the hot plate was turned off and the solution was stirred slowly and left to cool to room temperature. Litmus paper was used to check the pH of the solution, and 2 mL increments of 1 M hydrochloric acid (HCl) were added until the solution was neutralized (pH ~ 7). Another 30-40 mL of ethanol was added to facilitate precipitation of the starch. The product was then filtered out using a sintered glass Buchner funnel (4-5.5 micron) and a 1 L flask under vacuum (~69 kN/m^2). The resulting C. Starch was collected and stored in a freezer overnight (Heinze et al. 2004; Khachan et al. 2014).

The following day, 12 grams of C. Starch were mixed in a glass jar with 12 grams of DI water. The resulting gel-like substance was placed into a 40-mm wide dialysis membrane (Spectra/Por® Biotech Cellulose Ester Dialysis Membranes MWCO: 6,000-8,000D). A stirring magnet was put inside, and the two ends were clamped shut. This was placed in a 1 L beaker of DI water and put on a slow stir. The DI water was changed every 2-4 hours. After this 24-hour dialysis process, the starch was freeze dried for approximately 72 hours and then stored in a regular freezer until further use.

Prior to beginning a toxicity test, a 2500 ppm stock of C. Starch was prepared by dissolving 0.25 grams of the freeze-dried starch in 100 grams of DI water. The starch and DI water were put into a glass jar. This was placed into a boiling pot of water for 15 minutes to completely dissolve the solids and obtain a homogeneous solution. The 2500 ppm solutions of CPAM and C. Starch were stored at room temperature in the lab and in a refrigerator in the lab, respectively.

2.3 Zebrafish Embryos

Danio Rerio (zebrafish) embryos were used in the toxicity experiments of this study. Zebrafish embryos are a commonly used vertebrate model system for toxicology studies (e.g., Bambino, Chu, & States, 2018; Hill, Teraoka, Heideman, & Peterson, 2005; Tiedeken & Ramsdell, 2007, 2009, 2010). They are particularly appropriate for this study as they are aquatic vertebrates and can be used to test the potential toxic effects of chemicals on aquatic species that have a permeable egg shell. In addition, they can be used to observe the potential toxic effects on aquatic species that develop without an egg shell because this outer covering can be removed without adversely affecting their development. Most aspects of zebrafish development and genetics are highly conserved with other vertebrates including mammals and toxic responses have also been shown to be conserved between zebrafish and other animals (Bambino et al., 2018; Howe et al., 2013; Meyers, 2018; Noyes, Garcia, & Tanguay, 2017; Tiedeken & Ramsdell, 2009, 2010). Finally, zebrafish embryos are an ethical choice for these studies as the National Institute of Health (NIH) has established that they are unlikely to be capable of experiencing suffering or distress before they are 8 days old (NIH ARAC, 2016).

Zebrafish embryos were obtained from natural paired spawnings of AB wild-type adults (Figure 2). Adult fish were maintained on a 14-hour light/10-hour dark cycle in an Aquaneering recirculating water system at 28.5°C in the Lewis Lab Aquarium in the SU Department of Biology. All zebrafish experiments in this research were approved by the Syracuse University Institutional Animal Care and Use Committee (IACUC). This freshwater species is a common model system used in biomedical research including toxicity testing (Hill et al., 2005). Adult fish are fed three times a day. Food includes brine shrimp and a mix of Aquaneering, Spirulina, AP, and Golden Pearl dry fish food. For this study, a male and female adult fish were placed in a breeding container,

separated by a plastic divider the evening before embryos were required. Each pair of fish was swapped to a clean breeding container with clean fish water the following morning and the dividers were removed to allow for natural spawning. After approximately 40-60 minutes, the adult fish were returned to their normal tanks and the embryos were collected using a tea net. Embryos were incubated in petri dishes in embryo medium (EM: 5mM NaCl, 0.17mM KCl, 0.33mM $CaCl_2 \cdot 2H_2O$, 0.33mM $MgSO_4 \cdot 7H_2O$ and 0.00004% methylene blue in autoclaved RO water) at 28.5℃. Infertile embryos were removed and embryos were staged in hours post fertilization (hpf) at 28.5°C or days post fertilization (dpf) according to Kimmel et al, 1995.

3. TEST SETUP & METHODS

3.1 Jar Test

A series of jar tests were performed to determine the optimum dose of both CPAM and C. Starch (ASTM D2035, 2003). The jar test apparatus (Phipps & Bird PB-700 Jartester) consisted of four stirring rods, on which only one test was performed at a time. A 15% by mass soil and water mixture was created by adding 83 grams of soil (i.e. Tully Fines and Tully Sand) to 468 grams of DI water in a 1000 mL beaker. The beaker of sample was put on the jar tester and allowed to mix for two minutes at approximately 200 rpm to ensure adequate mixing before beginning a test. Small increments of CPAM (0.2 mL for sand and 1 mL for fines) were added to the mixture, followed by one minute of mixing and a minute and a half of settling. A sample of the supernatant was obtained using a 10-mL pipette, and used to check the turbidity (HACH 2100N Turbidimeter). CPAM was added in increments until a turbidity of < 20 NTU was reached, and this was considered optimum dose. This process was repeated for three trials using CPAM. The same procedure was also conducted using C. Starch.

3.2 Residual Polymer

Residual polymer concentrations were determined using the streaming current detection method as presented by Bhatia et al. (2014). Prior to beginning the residual polymer tests, calibration curves were produced for the CPAM and C. Starch. A streaming current detector (Mutek PCD 02) was used to measure the particle charge and a potassium poly (vinyl alcohol) sulfate (KPVS) titrant was used for neutralization. The streaming current detector consists of two electrodes that measure the current when charged particles stick to the wall of a cylindrical measuring cell. A vertical piston moving in and out at a speed of 4 cycles per second creates an alternating current, and the detector presents the charge for the given solution (Bhatia et al. 2014). A known concentration of the polymer (10 mL) was put into the measuring cell and the piston was run for 15 minutes to allow the charge reading to stabilize. After that time, small increments (5-20 μL) of the KPVS titrant were added until the charge passed zero. Interpolation was used to determine which volume most closely neutralized the solution, and this was plotted with the known concentration. Calibration curves for CPAM and C. Starch are given in Figure 3.

Once calibration curves were complete, new 15% by mass soil and water mixture samples were treated with optimum dose and a 50% overdose of each polymer, and the concentration of residual polymer was measured. This test was only performed on Tully Fines as sand does not require flocculation since the particles are already large enough for dewatering (i.e. the sediments are larger than the geotextile tube's pore size). A 15% solid concentration slurry (i.e., Tully Fines + water) was placed on the jar test machine and mixed for two minutes. The optimum dose obtained from the jar test was then added to the sample. This was mixed for another two minutes, then the jar tester machine was turned off and the sample was given two minutes to settle. A 10-mL sample of the supernatant was collected and immediately transferred to the streaming current detector. The piston was run for 15 minutes to allow time for the reading to stabilize (Figure 4). If the charge

was positive, then there was residual polymer present and the KPVS titrant was added in increments to neutralize the sample (i.e., bring the charge to 0). The concentration of residual polymer in the supernatant sample was obtained from the calibration curve as the concentration associated with the volume of titrant needed to neutralize the sample. This process was repeated for three trials at optimum dose and another three trials at a 50% overdose, and with both CPAM and C. Starch.

3.3 Chemical Exposure of Zebrafish Embryos

The 2500 ppm solutions of CPAM and C. Starch were kept in glass jars at room temperature and +4^{o}C, respectively, for 24 hours. Each solution was diluted to 30 ppm using EM on the morning of application. Groups of 30 fertile embryos were aliquoted into 40 mm x 12 mm glass petri dishes. The EM was removed and 3 mL of EM (control), 30 ppm CPAM in EM, 15 ppm CPAM in EM, or 30 ppm C. Starch in EM was added to each dish using an electronic pipette (Eppendorf Repeater® M4). Each experiment contained both control and experimental dishes of embryos obtained from the same parent pair (Figure 5). Figure 6 shows examples of healthy zebrafish embryos at 1, 3, 5, and 7 dpf. Healthy embryos develop normal swim bladders by 5 dpf (e.g., Figure 6). In past experience, embryos with normal morphology and swim bladders usually survive to adulthood.

Previous preliminary experiments had suggested that the polymers might stick to the chorion (clear egg shell) that surrounds early stage zebrafish embryos (Figure 6E). Therefore, in some dishes the chorions were manually removed at 24 hpf using two pairs of fine forceps (embryos in Figure 6A-D all have their chorions removed). The chorion is pinned to the bottom of the dish with one pair of forceps and the second pair is used to make a tear in the chorion. If necessary, additional tears are made to make a hole in the chorion. The embryo is then gently

pushed out through the hole. The chorions were left in the dish so as not to remove any polymer that might be stuck to the chorions (e.g., see Figure 11). Embryos were not dechorionated until 24 hpf because it is possible to damage embryos when dechorionating before this time point. However, dechorionating zebrafish embryos after 24 hours does not damage them and has no effect on their development. As only some aquatic species have an egg shell/chorion, dechorionating some embryos is also a more effective method to test whether these chemicals might be toxic to aquatic animals that lack this protective outer covering.

All dishes were incubated at 28.5℃ and embryos were examined daily using a Nikon SMZ645 stereoscope. Obvious morphological phenotypes were noted, and dead embryos were removed. Death was determined by the absence of a heartbeat (2 dpf and older) or extensive necrosis. At 7 dpf, embryos were treated with 0.004% Tricaine (also called MS-222; Sigma-Aldrich A5040), to anesthetize them and facilitate identification of subtle morphological phenotypes. After analysis, all embryos were euthanized by freezing the dishes in a freezer for 24 hours. The dead embryos and liquid were then disposed of as hazardous waste.

The numbers of embryos that had died were combined with the number of embryos with obvious lethal phenotypes at 7 dpf, to estimate the lethality of each chemical. The cut-off for the experiments was 7 dpf since 8 dpf is the first point at which the Animal Research Advisory Committee (ARAC) guidelines for the use of zebrafish in the NIH Intramural Research Programme suggest zebrafish larvae should be considered capable of experiencing suffering (NIH ARAC, 2016). Phenotypes that were classified as lethal were heart edema (Figures 7A-C), severe body edema (Figure 7C), lack of a swim bladder (Figure 7E), and extensive necrosis (Figures 7D and 8). The swim bladder is used to maintain buoyancy (lateral stability), and lack of a swim bladder is a lethal phenotype. If three or more embryos died or had a lethal phenotype in any individual

control dish, then all the data from that experiment was not considered further, in case that batch of embryos was sick or otherwise compromised.

3.4 Data Analysis

For all experimental conditions (i.e., control, 30ppm CPAM, 30ppm C. Starch, 15ppm CPAM), individual experiments were conducted on at least three different days, on both embryos in their chorions and embryos dechorionated at 24 hpf, and at least 7 dishes were treated for each condition (Table 1, Fig. 5). Each experimental condition was compared to its own control dishes, developed in EM alone, that contained embryos from the same parent fish as the respective experimental dishes. In some dishes a few embryos stuck to the lid and were damaged – these are not included in these numbers or analyses. The percentage of embryos that died or had lethal phenotypes was calculated for each dish and are provided in the text as percent lethality +/- standard error. As the data were bimodally distributed, a logistical regression in R was used to determine whether results were statistically significantly different from each other (i.e., $P < 0.05$) (R Core Team, 2018). However, as this method does not work if there is zero variance within one predictor, one of the results for the 30 ppm CPAM exposure on dechorionated embryos was changed to be only 99% dead or dying (rather than 100%) to calculate the P value for the comparison between this experiment and controls and this condition and 30 ppm C. Starch.

4. RESULTS & DISCUSSION

4.1 Optimum Dose

The optimum doses of CPAM and C. Starch for Tully Fines and Tully Sand were determined using jar tests. The changing turbidity was recorded and plotted versus the addition of polymer. The optimum dose was selected as the dose which resulted in a turbidity of 10-20 NTU, as this is a typical, acceptable range for deposit into nearby waterways. Table 2 presents the ranges of optimum dose values obtained on 15% solids slurries of both Tully Fine and Tully Sand, using CPAM and C. Starch. As shown in Table 2, Tully Fine required higher doses of both polymers than the Tully Sand. Sand particle sizes are typically already large enough to be retained within the geotextile tube, and therefore do not typically require a large dose of flocculant. A small amount may be necessary to flocculate the low percentage of fine particles that remained in the sample, however, most of the fines were sieved out of the sand sample before testing.

The optimum doses of CPAM and C. Starch for Tully Sand were 1.1-2.0 ppm and 8.0-11.6 ppm, respectively. The optimum dose occurs where the plot begins to flatten, thus, optimum dose can often refer to a range of values which provide adequate flocculation for dewatering. C. Starch requires a larger dose in order to achieve similar flocculation and improvement in dewatering capabilities as CPAM (Khachan et al. 2014; Figure 9 and Figure 10). Further, for Tully Fine, the optimum dose of CPAM was 31-55 ppm, while it was 220-240 ppm for C. Starch. The fine particles require a larger dose to completely flocculate the sample for efficient dewatering, and more C. Starch is needed to achieve the same levels as CPAM. Since flocculation is not entirely necessary for Tully Sand, and only a small dose may be required if any, it likely will not result in any residual polymer. It was observed that flocs formed by using CPAM were larger than by using

C. Starch (Figure 9). Therefore, only Tully Fine was used for further testing of residual polymer concentration.

The relative efficiency of the cationic starch and cationic polyacrylamide (CPAM) can be attributed to the difference in their charge density (CD). The CD of the C. Starch used in this study is about 0.9 mEq/g and the CD of the CPAM is 2.85 mEq/g. In other words, the CD of the C. Starch is only 31% of CPAM's CD. Several studies (e.g., Khachan et al., 2014; Shirzad-Semsar, Scholz, & Kulicke, 2007) have shown that CD plays a significant role in the optimum dosage required for flocculation. In these studies, the optimum dosage of cationic starches was 3 to 6-fold more than CPAM. These findings agree with the findings in the current study (i.e., optimum dosage of cationic starch is about five times more than that of CPAM).

Although the optimum dosage of cationic starch is higher, the cost of the CPAM is approximately four times greater than that of the cationic starch. This indicates that the increase in optimum dosage does not lead to an increase in the overall cost. Additionally, based on findings in this study, cationic starch is more environmentally friendly and less toxic to aquatic organisms.

4.2 Residual Polymer

Residual polymer concentrations were determined using the Tully Fine soil and the streaming current detection method (Bhatia et al. 2014). Sediment samples were treated with either an optimum dose or a 50% overdose to represent potential field conditions. A 50% overdose is more extreme than is likely to occur in the field, so providing an upper limit of possible residual polymer. This was selected as a starting point in the chance that it resulted in no residual polymer, then no further testing would be needed. However, this was not the case, meaning this experiment should be repeated with lower percent overdoses that are more realistic in field conditions.

Table 3 shows the concentration of residual polymer in the samples when treated with optimum dose and 50% overdose of each polymer. The samples show substantially low residual polymer concentrations when treated with optimum dose of CPAM and C. Starch (i.e., 1.2 ppm and 1.1 ppm, respectively). Since optimum dose refers to the ideal amount of flocculant that achieves proper flocculation but does not result in excess polymer, these low values are expected. These results also verify that the optimum dose determined previously is substantially close to the actual optimum dose, but not perfectly accurate (exact optimum dose would theoretically result in ~0 ppm residual concentration).

The slurry samples that were treated with a 50% overdose of C. Starch showed similar results to the samples treated with the optimum dose (Table 3). The residual concentration of C. Starch ranged from 1.7-3.8 ppm, suggesting that residual polymer may not be a concern when using C. Starch in geotextile tube dewatering. However, the residual concentration of CPAM ranged from 1.5-17.4 ppm, where the upper end is more than a fifth of the original dose the sample received (i.e. 82.5 ppm). While a 50% overdose may be higher than what is likely to occur in the field, these results demonstrate that overdosing of CPAM is a potential concern.

For C. Starch and CPAM (30 ppm solution in deionized water), the Total Organic Carbon (TOC) was measured and the Chemical Oxygen Demand (COD) were calculated using the relationship given by Dubber & Gray, 2010. The COD value for the 30 ppm CPAM was 23 ppm and for C. Starch was 1 ppm. These values are much lower than the limit of 200 ppm in USA. Therefore, higher COD values are not a concern in this study.

4.3 Toxicity

To test the toxicity of C. Starch and CPAM, zebrafish embryos were exposed to 30 ppm of one or the other polymer from a few hours after fertilization until 7 dpf. Zebrafish embryos have

a clear, permeable egg shell called a chorion that may provide some protection from toxins and is not present in some other aquatic species (Figure 6E). Therefore, toxicity was tested in both the presence and absence of the chorion. In addition, in previous preliminary experiments it had appeared that CPAM might stick to the chorion (data not shown), suggesting that it might not be fully permeating the chorion. Therefore, for all these reasons, the chorion was left intact until the embryos naturally hatched for about half of the embryos tested, and it was removed at 24 hpf for the remaining half (referred to as dechorionating). In the latter case, the chorions were left in the dish in case there was any polymer stuck to them (e.g., see Figure 11; for more details see materials and methods). Embryos were not dechorionated before 24 hpf because it is possible to damage them if dechorionating before this time point. However, dechorionating embryos at 24 hpf or later stages has no effect on their development in normal conditions.

In the toxicity experiments, far fewer abnormal phenotypes were observed in embryos exposed to C. Starch than embryos exposed to CPAM. The most common phenotype observed in CPAM exposed embryos was a burst open yolk sac (the yolk sac provides food/nutrients during the early stages of life) (Figure 11). There are three relatively normal (RN) embryos, several where the yolk has burst and the head has become necrotic (NH), and some that have lost yolk, but the head has not yet become necrotic (OH). Chorions that were manually removed can be seen at the top, and some necrotic tissue (NT) from embryos that have already died and disintegrated can also be seen. If the embryos survived the initial burst yolk sac, necrosis (i.e. death of cells) began to appear shortly after, which ultimately killed the embryos (Figures 8 and 11).

In the C. Starch experiments, there was no statistically significant difference between the number of dechorionated embryos that died or had lethal phenotypes by 7 dpf when exposed to C. Starch compared to control embryos ($P= 0.731$, $n=14$ dishes and 412 embryos; Figure 12).

However, there was a statistically significant difference between the number of embryos that retained their chorions that died or had lethal phenotypes by 7 dpf compared to control embryos (P=1.6E-5, n=14 dishes and 418 embryos; Figure 13). This is presumably because there were two non-dechorionated dishes exposed to C. Starch that had higher levels of death than all the other dishes exposed to this chemical (S13 and S6 in Fig. 13). These higher values could be anomalies, as these results are not seen in the dechorionated embryos, which should be more sensitive to any toxic effects of the C. Starch. In contrast, exposure to CPAM resulted in a statistically significant increase in the number of embryos that died or had lethal phenotypes by 7 dpf compared to control embryos for both dechorionated embryos and embryos that retained their chorions (P=<2E-16, n=14 dishes and 419 embryos for embryos in their chorions and P=2.5E-16, n=14 dishes and 411 embryos for embryos dechorionated at 24 hpf; Figure 12 and 13).

CPAM affected the embryos relatively quickly, often causing fatalities and sometimes killing all 30 embryos in a dish within two hours of dechorionation. Most of the dechorionated embryos died by 2-3 dpf (Figures 8, 11, 12, 13, 14). In contrast, embryos that were left in their chorions usually survived to 4 dpf, and some survived until 7 dpf (Figure 7, 12, 13, 14). In addition, 100% +/- 1.10 of the dechorionated embryos exposed to CPAM were dead or dying by 7 dpf compared to 71.8% +/- 0.414 for non-dechorionated embryos (Figure 12 and 13). Taken together, these results suggest that the chorion has a partially protective effect against CPAM exposure.

Since 30 ppm of CPAM is a higher concentration than is likely to be released in to the environment during geotextile tube dewatering, the toxicity of 15 ppm of CPAM was also tested. The toxicity of C. Starch was not tested at lower concentrations because 30 ppm of that compound had no statistically significant effects on embryo survival, at least on dechorionated embryos. While the degree of lethality observed for 15 ppm CPAM was lower than for the higher dose of

30 ppm CPAM, there were still statistically significant increases in the number of embryos that were dead or dying after only 7 days of exposure to 15 ppm CPAM. As for 30 ppm, the effects were more pronounced when embryos were dechorionated. 34.0% +/- 0.331 of the dechorionated embryos exposed to 15 ppm CPAM were dead or dying by 7 dpf compared to 5.27% +/- 0.310 of dechorionated control embryos (P=2.5E-11, n=11 dishes and 326 embryos). 11.7% +/- 0.357 of the embryos in their chorions exposed to 15 ppm CPAM were dead or dying by 7 dpf compared to 3.75% +/- 0.323 of control embryos in their chorions (P=0.0020, n=14 dishes and 420 embryos).

In contrast to the 30 ppm exposure experiments, exposure to 15 ppm CPAM did not kill embryos as quickly. In two out of 11 dishes of dechorionated embryos, all the embryos died within 2 days of exposure (data not shown). For the rest of the dishes of dechorionated embryos and all the dishes of embryos that retained their chorions, less than 4 embryos died per dish over the 7 days of exposure (data not shown). However, many embryos had lethal phenotypes at 7 dpf. The most common lethal phenotype was a missing swim bladder (e.g., see Fig. 7) and in some cases this was coupled with spinal deformities.

5. CONCLUSIONS/FUTURE WORK

This paper provides evidence that C. Starch has the potential to be an effective alternative to CPAM in geotextile tube dewatering applications and is a more environmentally friendly option for dewatering. As previously reported, the data confirm that C. Starch requires a higher dose than CPAM to reach adequate flocculation, but it does not produce substantial residual concentrations in overdose situations. A 50% overdose of CPAM resulted in 17.4 ppm residual CPAM in the released liquid, whereas a 50% overdose of C. Starch only resulted in 3.8 ppm. The residual CPAM that may be released into the environment in overdose situations is concerning because the previously described toxicity experiments with zebrafish embryos suggest that CPAM is highly toxic.

The vast majority of zebrafish embryos exposed to 30 ppm CPAM for just 7 days died or had lethal phenotypes. In these studies, only visible morphological phenotypes were assayed, so many potentially lethal phenotypes were likely missed. In addition, embryos were only analyzed for 7 days, so there may be other phenotypes that only appear at later time points. Although 30 ppm is almost double the residual CPAM concentration that was obtained with the streaming current detection method, the severity of this toxic effect suggests that lower concentrations of CPAM are probably also toxic, especially over longer time periods. Consistent with this, a 7-day exposure to 15 ppm also significantly reduced embryo viability.

In the future, it will be interesting to test the toxicity of even lower CPAM concentrations. It could also be useful to determine the concentrations of residual CPAM after lower percent overdoses that are likely to occur in the field during geotextile tube dewatering. These experiments could help to identify a threshold CPAM concentration where adequate flocculation is usually achieved but a slight overdose is not overly toxic to aquatic life. However, to establish lack of

toxicity, studies would have to be conducted for a much longer time period and in much more detail. It is also important to test other flocculants used in geotextile tube dewatering for potential toxic effects on aquatic life. Based on the data obtained in this study, C. Starch is a more environmentally friendly alternative to CPAM and should be encouraged for use in geotextile tube dewatering applications despite its greater cost and more complex production process.

6. DATA AVAILABILITY STATEMENT

Some or all data, models, or code generated or used during the study are available from the corresponding author by request.

- Optimum dose results (plots of polymer added vs turbidity)
- Residual polymer results (calibration curves, plots of titrant added vs charge)
- Zebrafish embryo phenotype results (dead/dying vs unaffected for dechorionated and non-dechorionated embryos)

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FIGURE CAPTIONS

Fig. 1. Particle Size Distribution for Tully Coarse and Tully Fine sediments.

Fig. 2. AB wild-type (a) female and (b) male adult Danio Rerio (zebrafish).

Fig. 3. Polymer calibration curves using known concentrations of 1, 5, 25,50, and 100 ppm.

Fig. 4. (a) Settled slurry sample showing where supernatant sample was taken, then moved to the (b) streaming current detector to measure residual polymer.

Fig. 5. Experiment dish setup.

Fig. 6. Wild Type / normal dechorionated zebrafish embryos at 1, 3, 5, and 7 dpf. (e) shows wild type embryos left in chorions at 2 dpf. Scale bar is approximately 100 μM.

Fig. 7. Phenotypes observed in zebrafish embryos exposed to CPAM. Characteristic examples of (a–c) heart edema (HE), (b) trunk edema (TE), and (c) whole-body edema (BE) are shown in 3-4 dpf embryos. (d) shows 3-4 dpf embryos with necrotic tissue in the head and yolk regions (NT). (e) shows a relatively normal embryo and an embryo without a swim bladder. All (except the relatively normal embryo in E) are lethal phenotypes. Scale bar is approximately (a–c and e) 100 and (d) 50 μM.

Fig. 8. Embryos in their chorions at about 22 hpf that were exposed to 30 ppm CPAM: (a) stereomicroscope view of the central area of one dish of embryos; (b–e) magnified views of individual embryos. (b) has normal morphology, (c) has a small amount of necrosis (arrow) but is still alive, (d and e) have extensive necrosis and are dead. Embryos like (d) and (e) were removed from the dish as soon as they were discovered. Scale bar is (a) 500 and (b–e) 150 μM.

Fig. 9. (a) Original slurry, (b) slurry treated with C. Starch, and (c) slurry treated with CPAM.

Fig. 10. Change in turbidity due to the addition of C. Starch to the Tully Sand sediment sample.

Fig. 11. Embryos exposed to 30 ppm CPAM approximately 15 minutes after dechorionation at 24 hpf. Scale bar is approximately 350 μM.

Fig. 12. Percent of embryos in each dish that was dead or had a lethal phenotype by 7 dpf in the 30ppm experiments. All these embryos were dechorionated at 1 dpf.

Fig. 13. Percent of embryos in each dish that was dead or had a lethal phenotype by 7 dpf in the 30ppm experiments. These embryos retained their chorions until they hatched normally.

Fig. 14. Number of live embryos remaining in each dish exposed to CPAM over 7 days.

Table 1. Total number of embryos in their chorions and embryos dechorionated at 24 hpf exposed for each experimental condition.

EXPOSURE TYPE	DECHORIONATED EMBRYOS	EMBRYOS IN CHORION
Control (30 ppm exp.)	206	210
C. Starch (30 ppm)	206	208
CPAM (30 ppm)	205	209
Control (15 ppm exp.)	208	238
CPAM (15 ppm)	326	420

Table 2. Optimum dose of CPAM and C. Starch for the Tully Fine and Tully Sand soil samples.

SEDIMENT SAMPLE	FLOCCULANT	OPTIMUM DOSE
Tully Fines	CPAM	31 – 55 ppm
	C. Starch	220 – 240 ppm
Tully Sand	CPAM	1.1 – 2.0 ppm
	C. Starch	8.0 – 11.6 ppm

Table 3. Residual polymer concentration in samples treated with optimum dose and a 50% overdose of CPAM and C. Starch for Tully Fine.

POLYMER DOSE	RESIDUAL CPAM	RESIDUAL C. STARCH
Optimum	1.2 – 1.5 ppm	1.1 – 1.7 ppm
50% Overdose	1.5 – 17.4 ppm	1.7 – 3.8 ppm

Figure 1

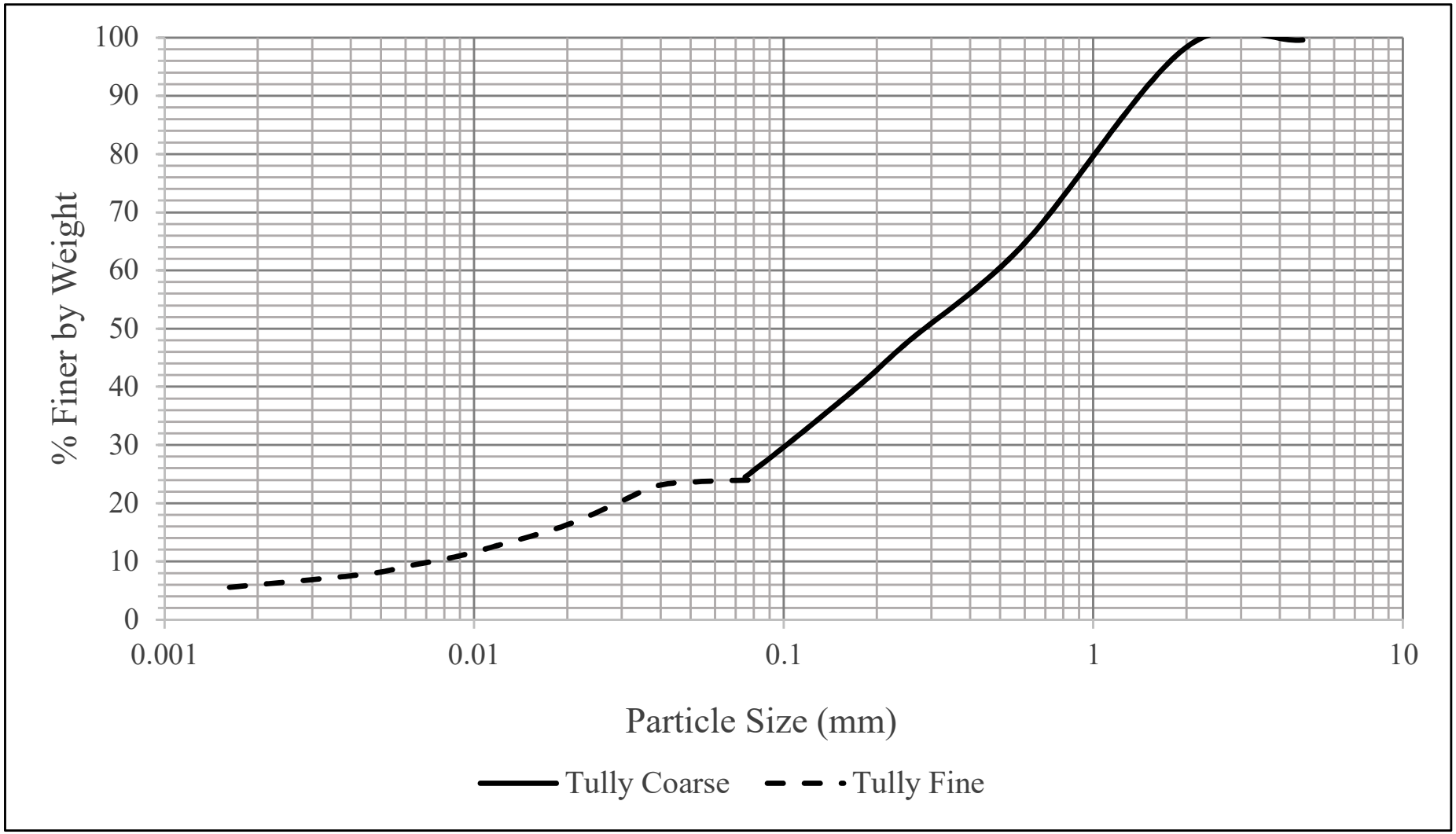

100
90
80
70
60
50
40
30
20
10
0
% Finer by Weight
0.001
0.01
0.1
1
10
Particle Size (mm)
Tully Coarse
Tully Fine

Figure 2

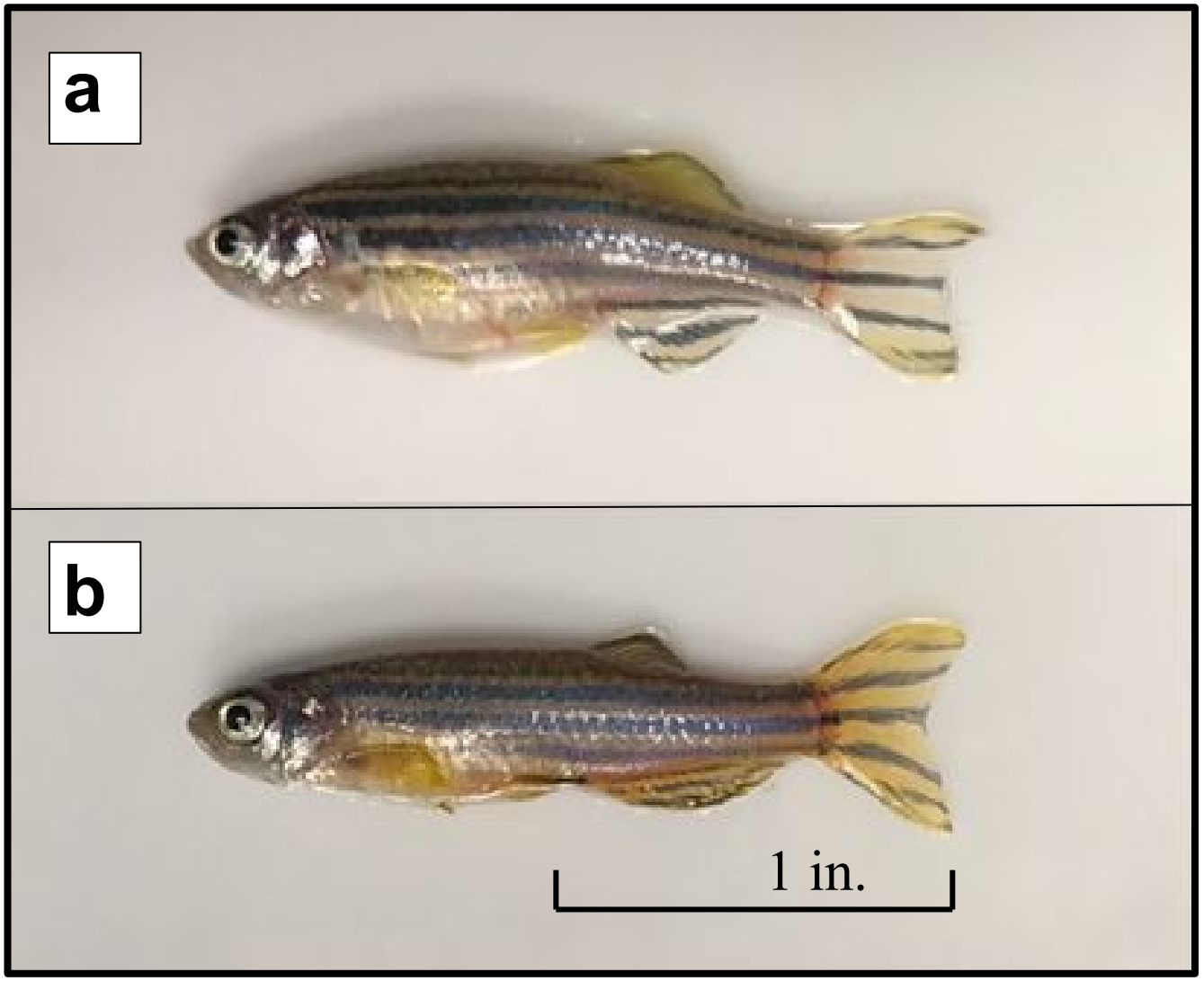


Figure 3

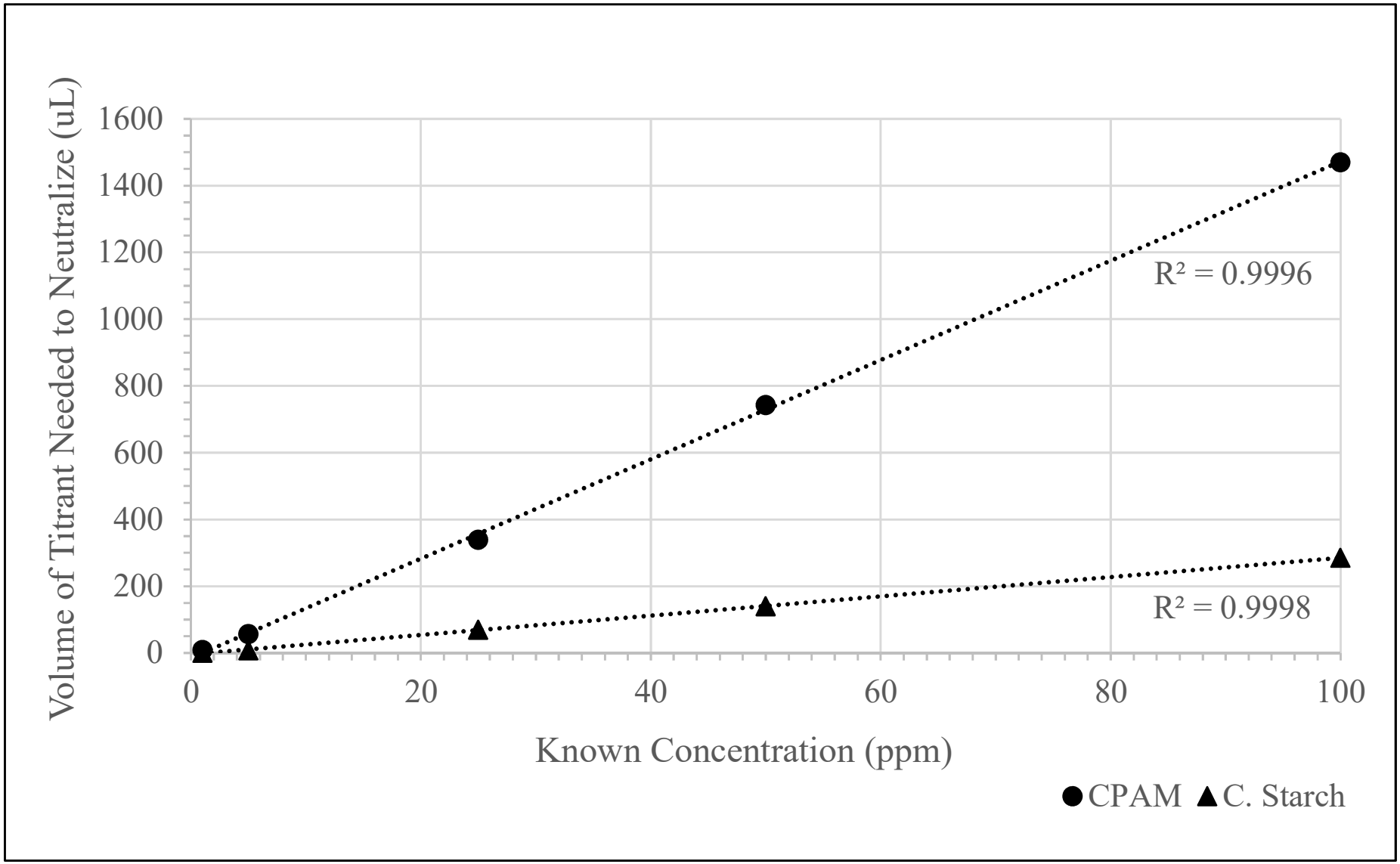

Volume of Titrant Needed to Neutralize (uL)
1600
1400
1200
1000
800
600
400
200
0
0
20
40
60
80
100
Known Concentration (ppm)
R² = 0.9996
R² = 0.9998
CPAM
C. Starch

Figure 4

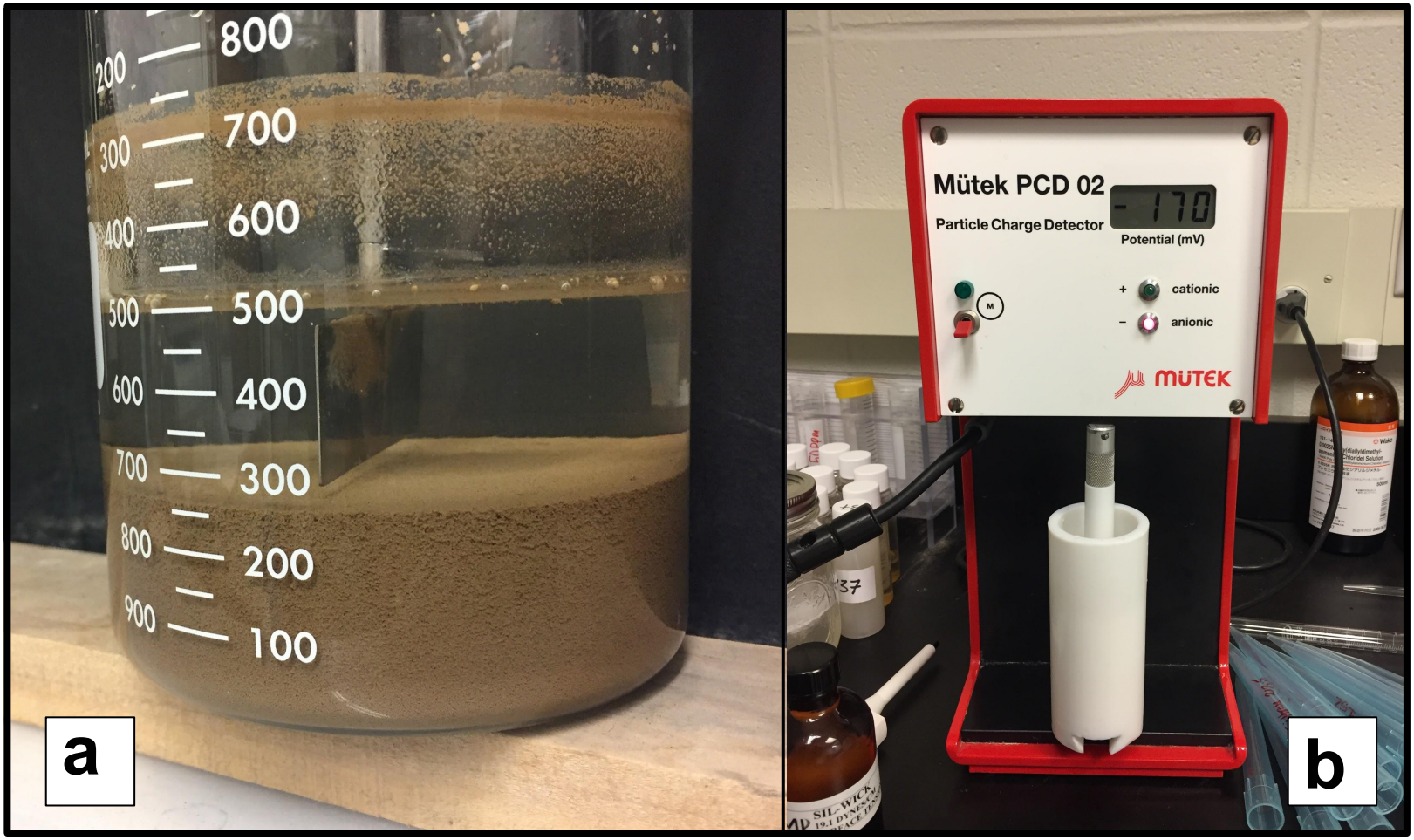

800
700
600
500
400
300
200
100
200
300
400
500
600
700
800
900
a
Mütek PCD 02
Particle Charge Detector
-170
Potential (mV)
+ cationic
- anionic
MÜTEK
b

Figure 5

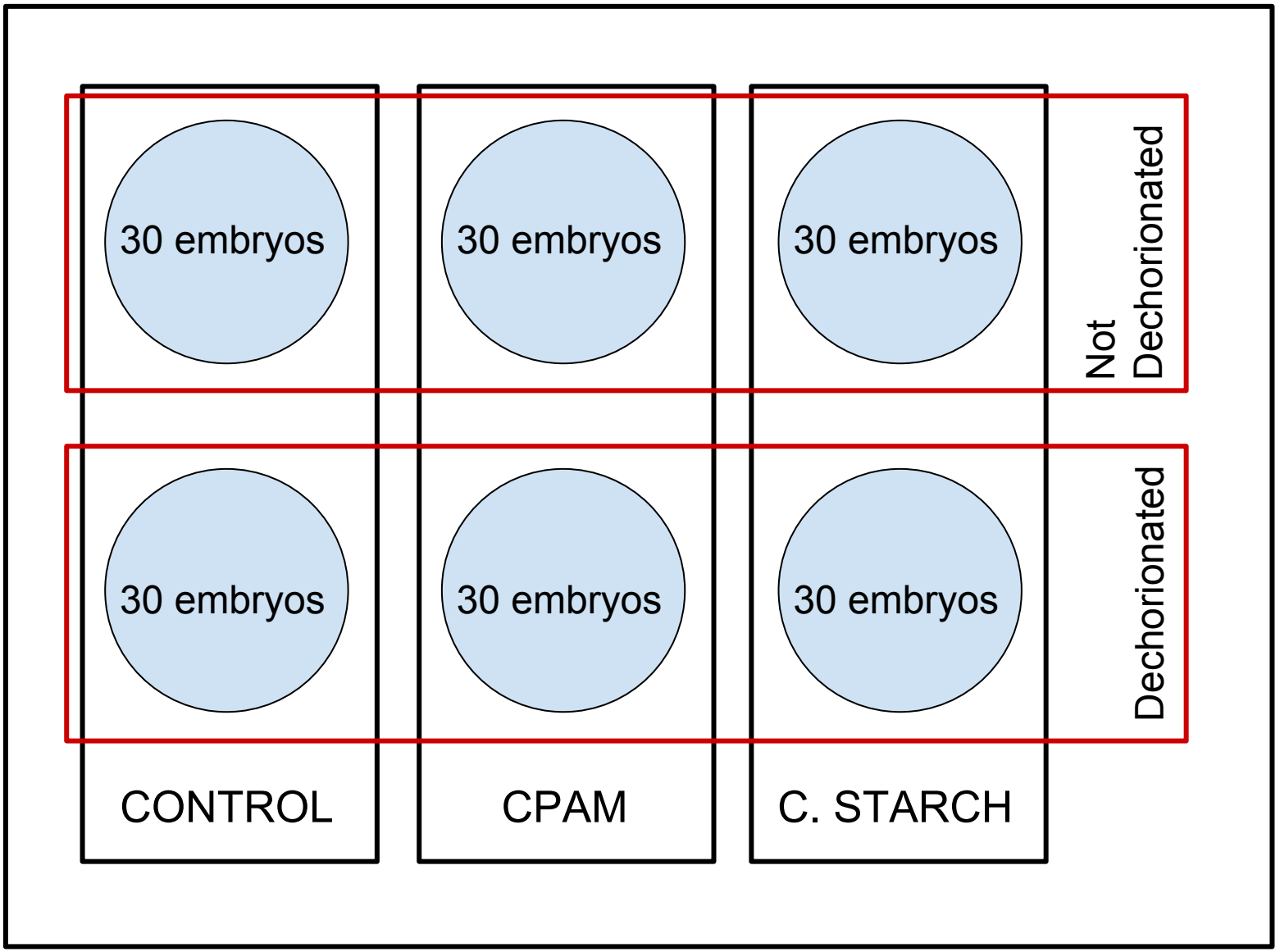

30 embryos
30 embryos
30 embryos
Not Dechorionated
30 embryos
30 embryos
30 embryos
Dechorionated
CONTROL
CPAM
C. STARCH

Figure 6

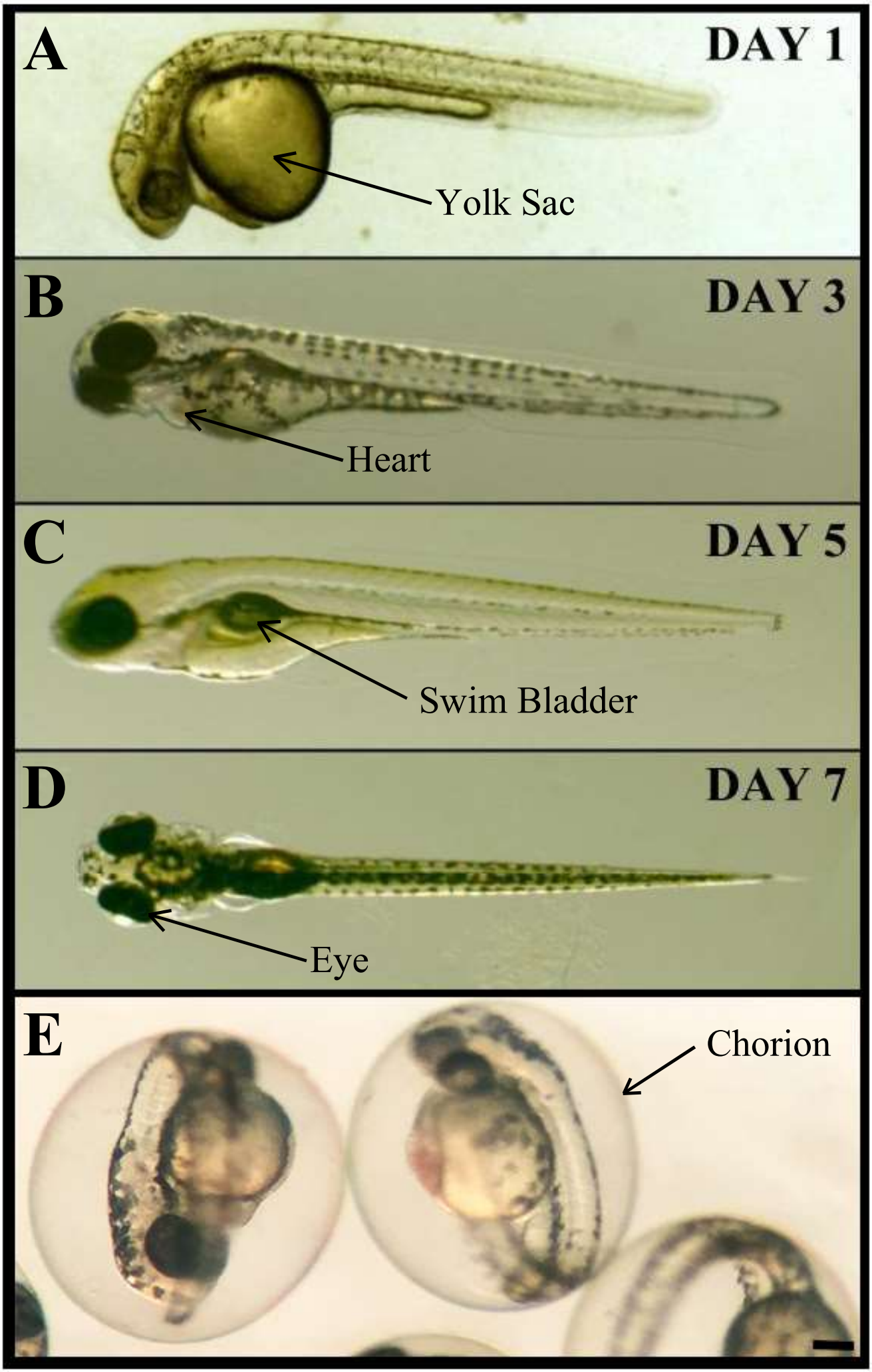

A
DAY 1
Yolk Sac
B
DAY 3
Heart
C
DAY 5
Swim Bladder
D
DAY 7
Eye
E
Chorion

Figure 7

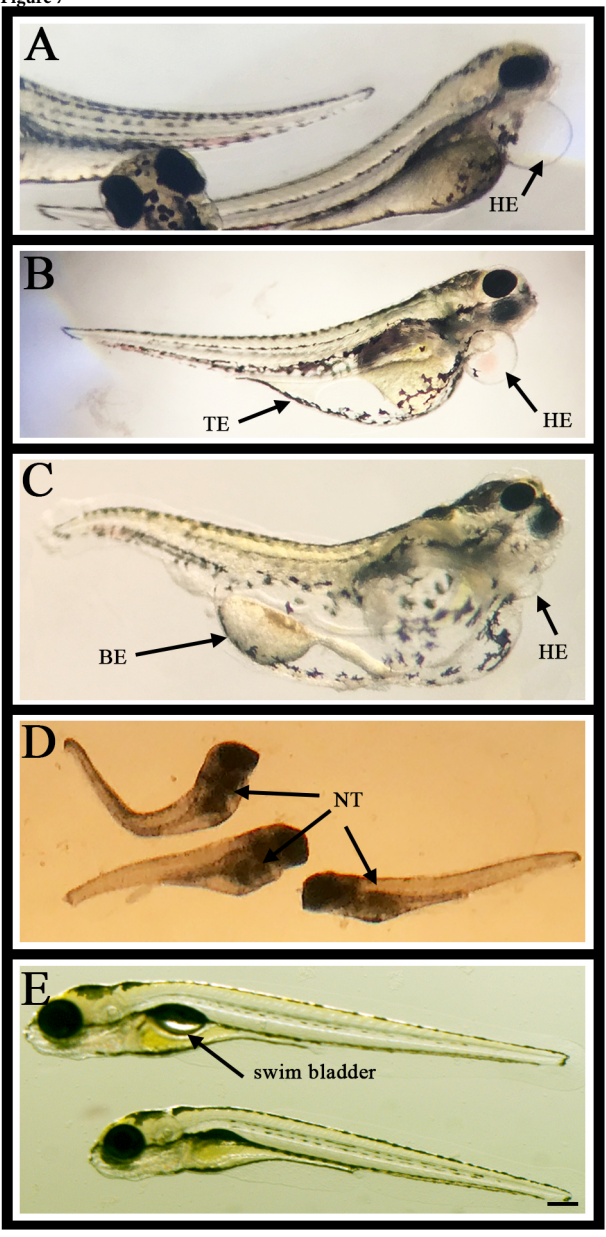


Figure 8

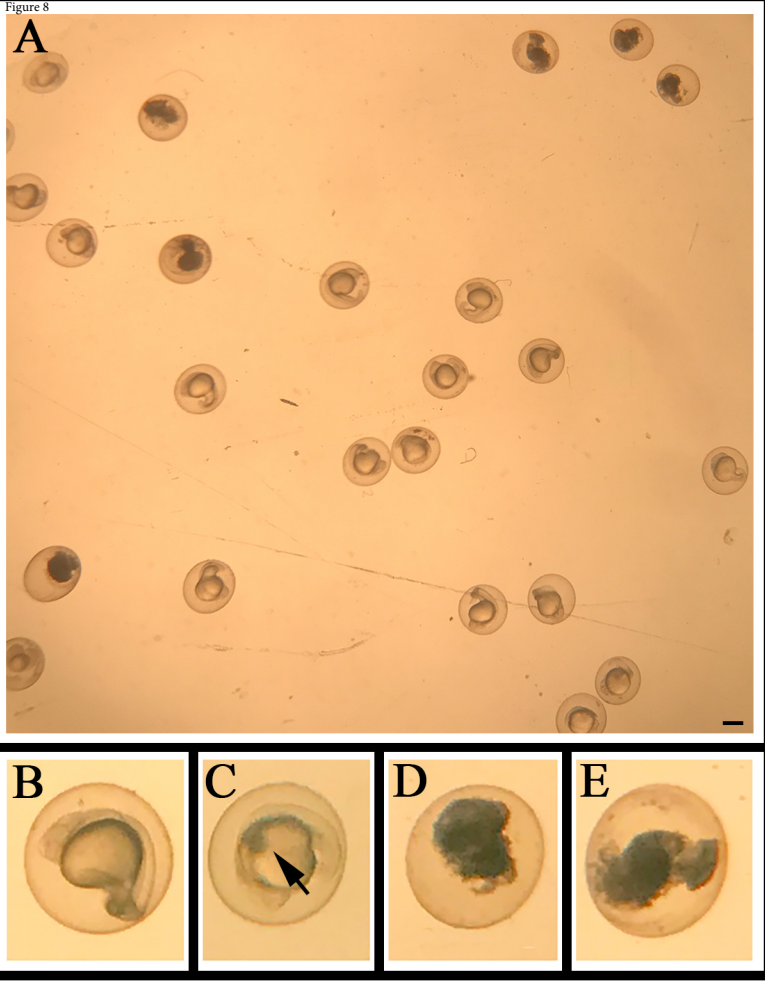


Figure 9

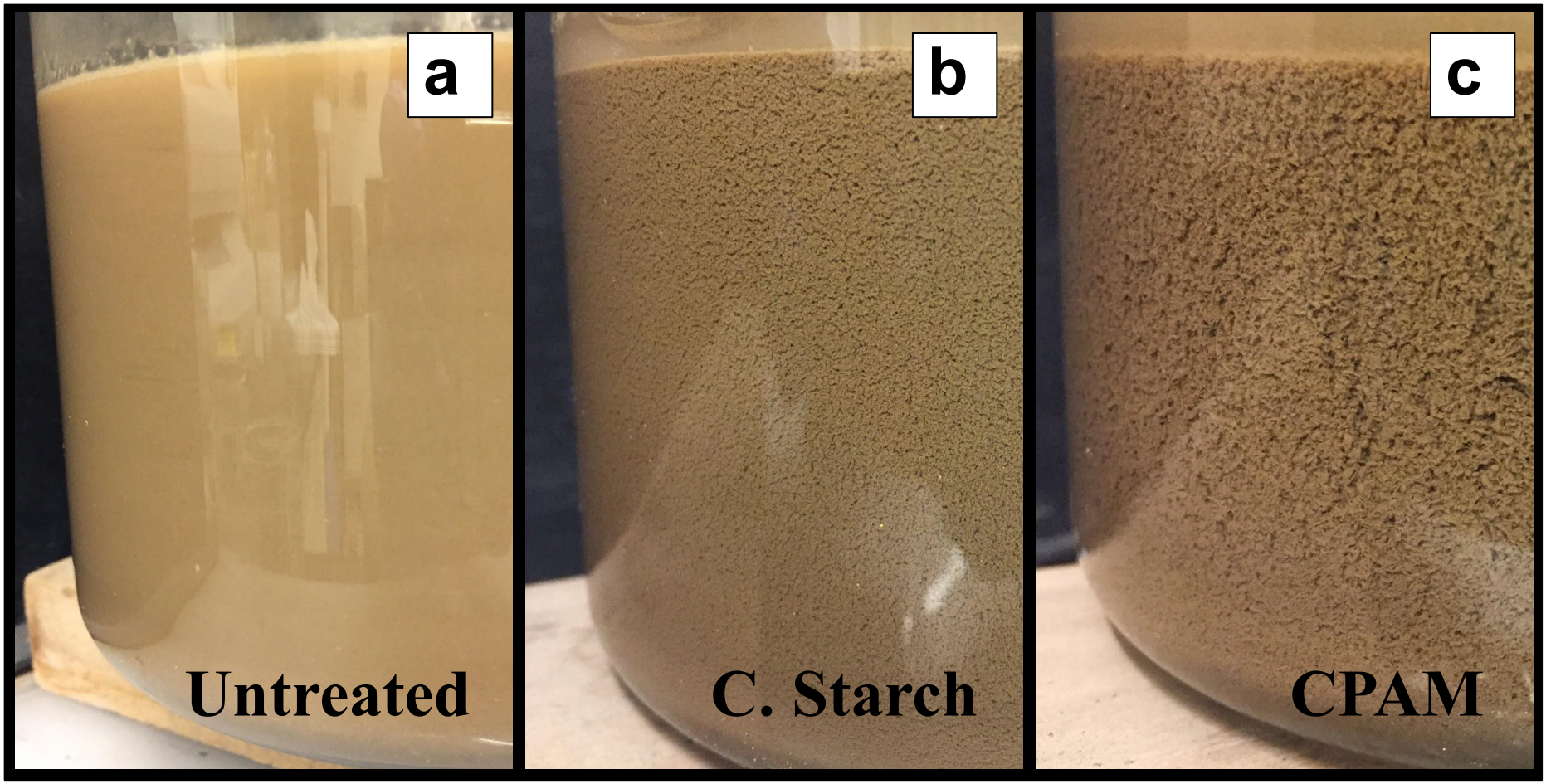


Figure 10

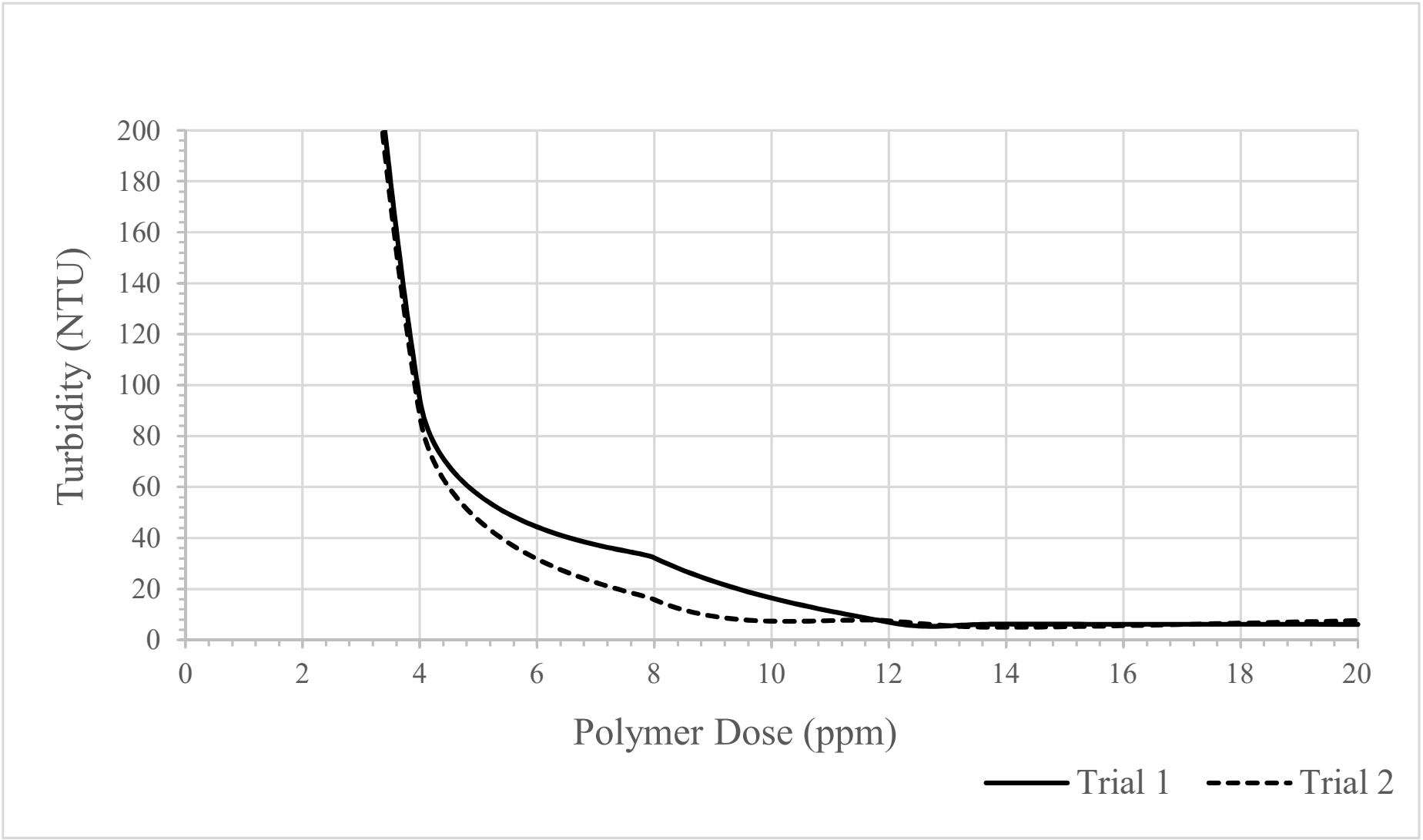

Turbidity (NTU)
200
180
160
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
Polymer Dose (ppm)
Trial 1
Trial 2

Figure 11

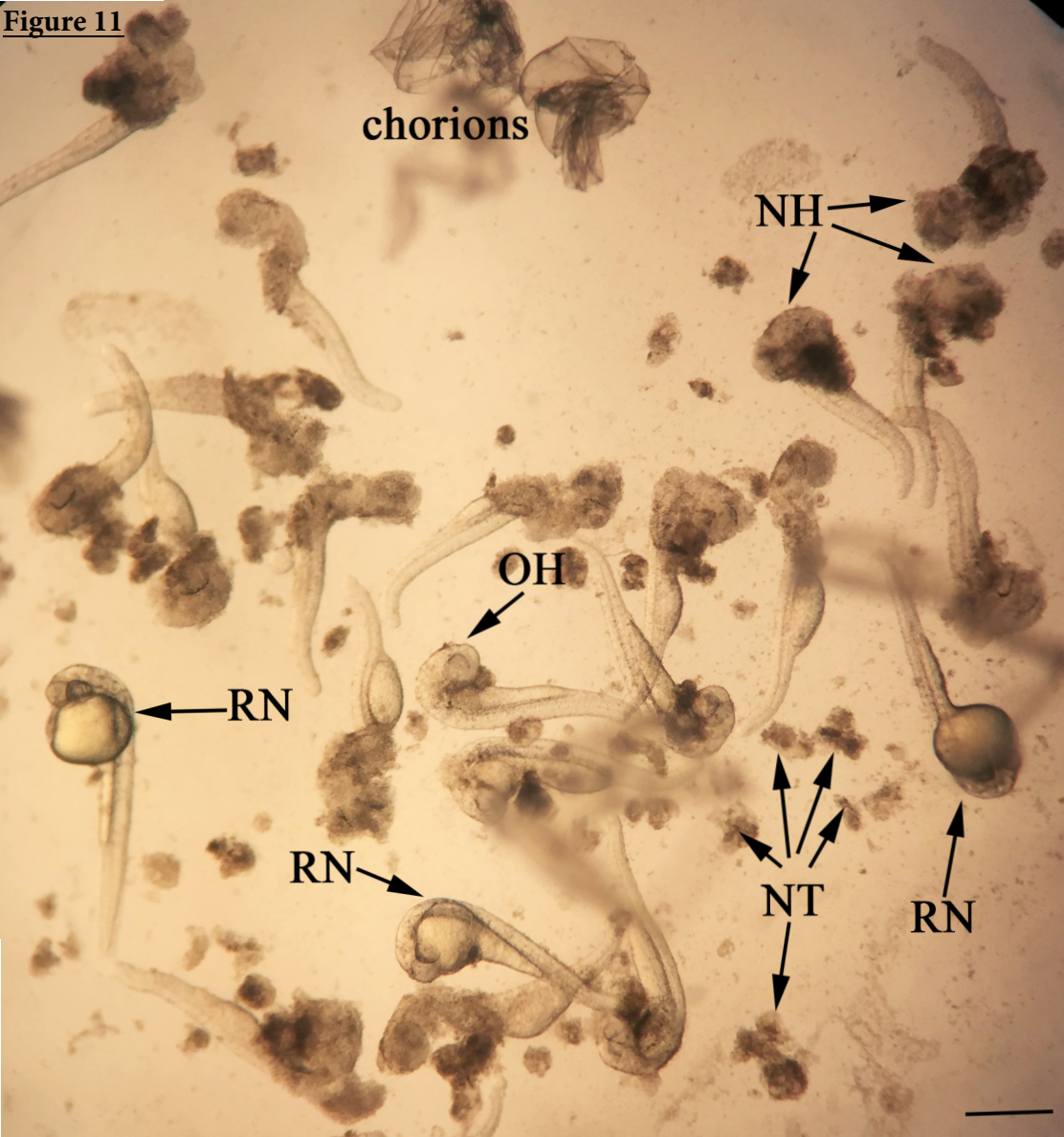


Figure 12

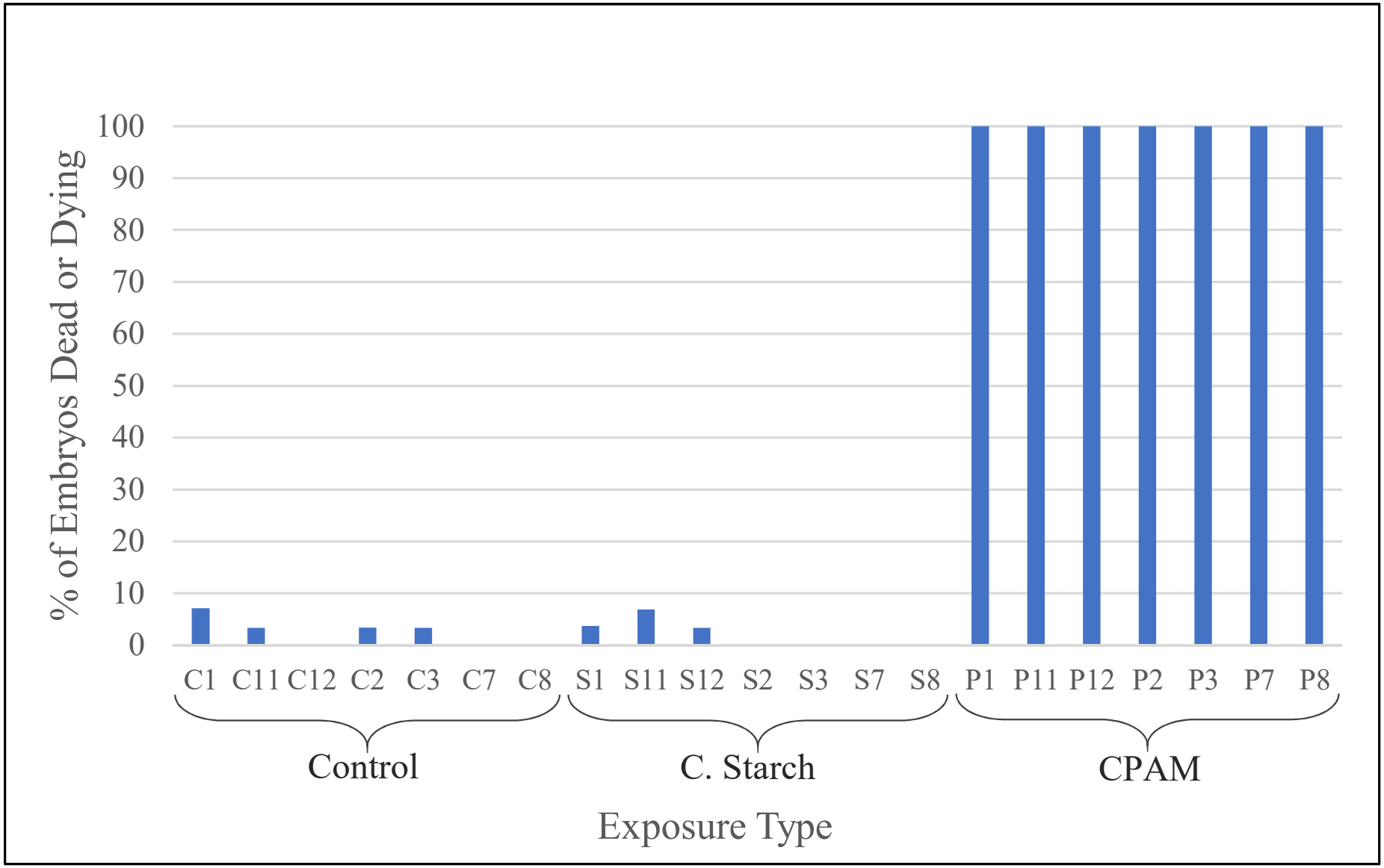

% of Embryos Dead or Dying
100
90
80
70
60
50
40
30
20
10
0
C1 C11 C12 C2 C3 C7 C8 S1 S11 S12 S2 S3 S7 S8 P1 P11 P12 P2 P3 P7 P8
Control
C. Starch
CPAM
Exposure Type

Figure 13

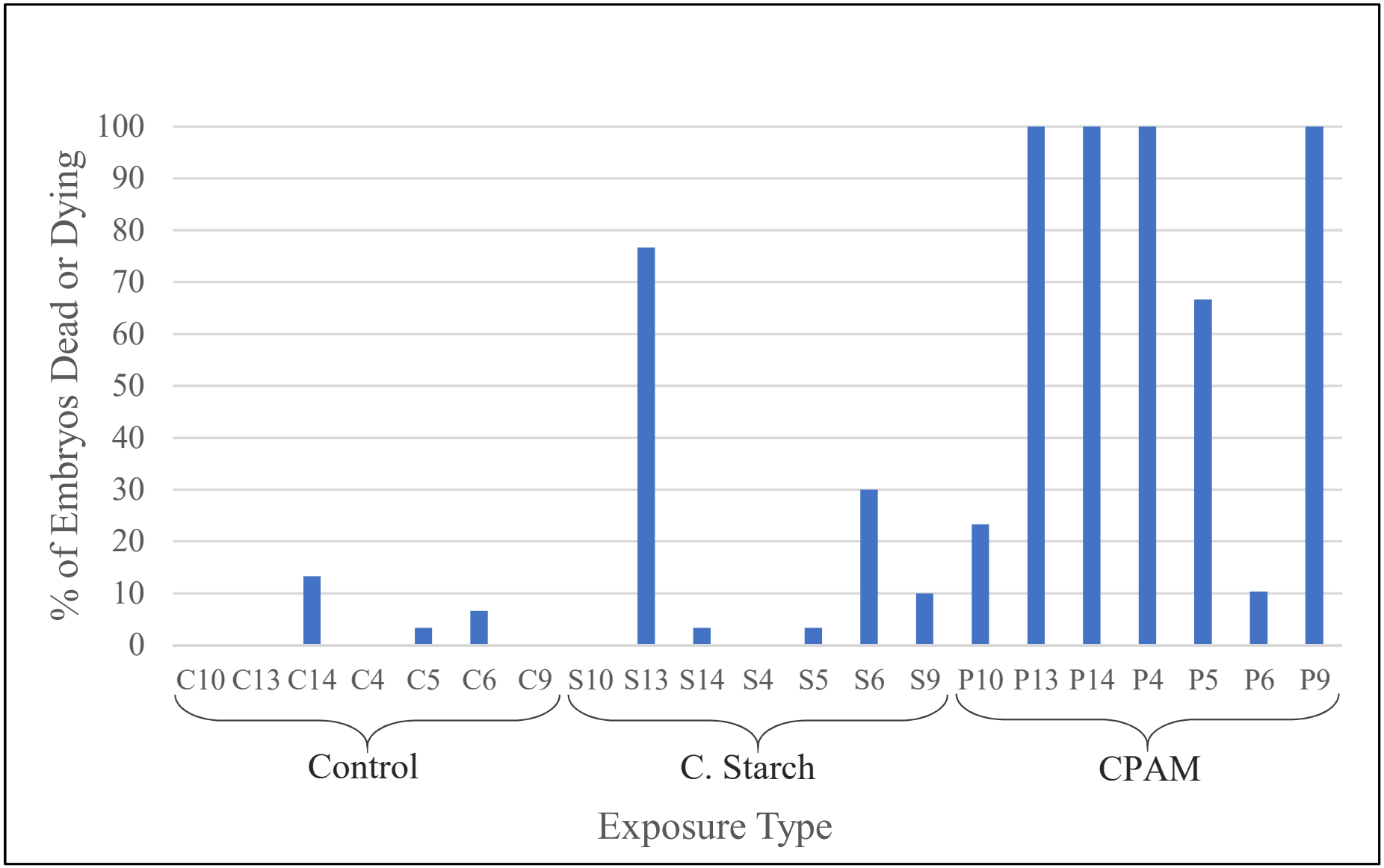
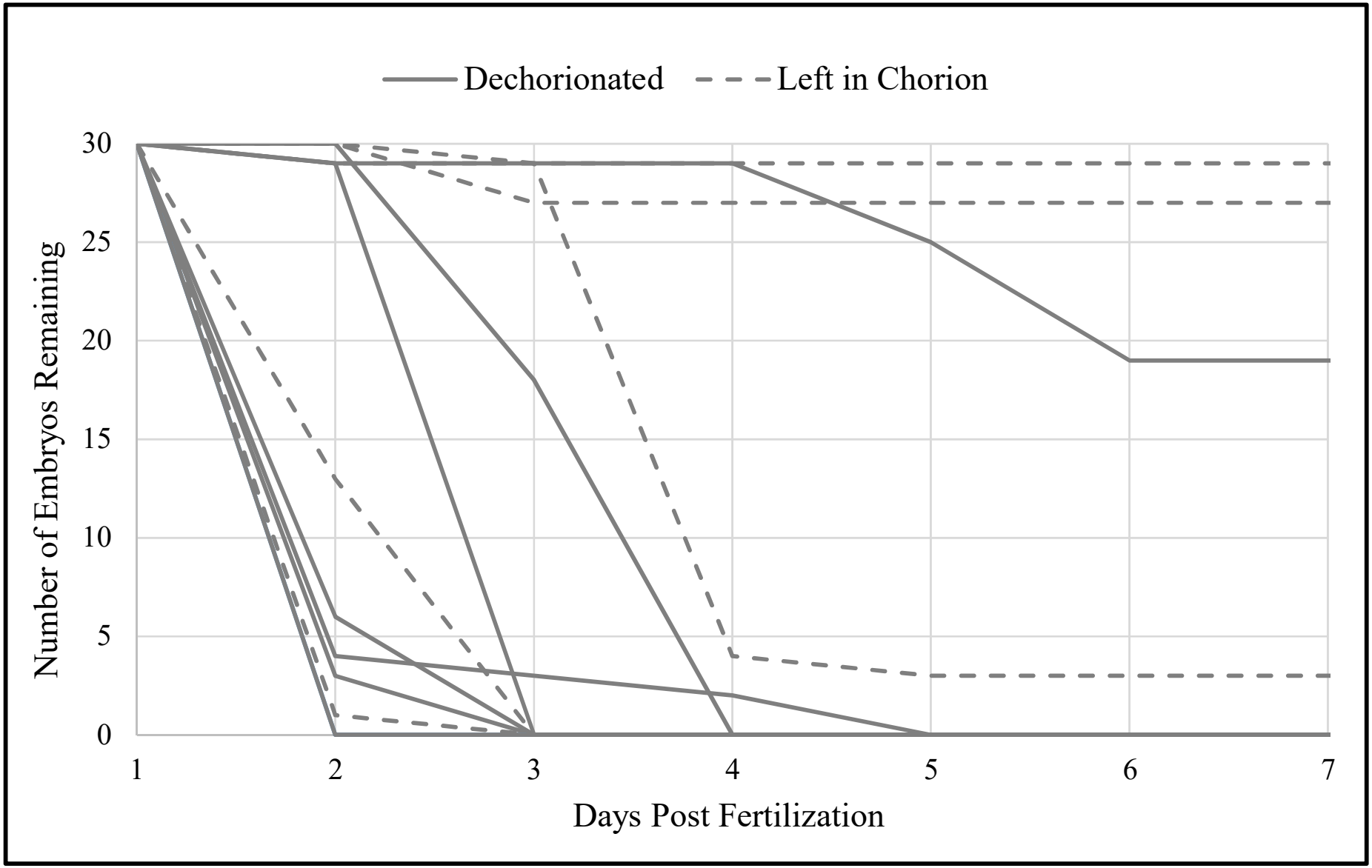

% of Embryos Dead or Dying
100
90
80
70
60
50
40
30
20
10
0
C10 C13 C14 C4 C5 C6 C9 S10 S13 S14 S4 S5 S6 S9 P10 P13 P14 P4 P5 P6 P9
Control
C. Starch
CPAM
Exposure Type

Figure 14


Dechorionated
Left in Chorion
Number of Embryos Remaining
30
25
20
15
10
5
0
1
2
3
4
5
6
7
Days Post Fertilization