



# Biological Effects of Inadvertent Perchlorate Releases During Launch Operations

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# Executive Summary

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## **Purpose of the this report**

Solid rocket motor fuel contains large quantities of ammonium perchlorate as the primary oxidant and this material has been shown to be toxic to life. In the event of an aborted rocket launch, it is possible that unspent propellant could be deposited near the launch site and coastal marine and terrestrial habitats could be adversely impacted. The purpose of this report is to present the results of a study of the effects of perchlorate on various ecosystem components expected to occur in the vicinity of launch sites. In particular, experiments were conducted on the effects of perchlorate on primary and secondary production in freshwater and marine water samples, decomposition processes in marine and freshwater sediments, freshwater wetland peat and upland soils, and effects on the behavior and growth of the fish species, the threespine stickleback. In addition, the potential bioaccumulation of perchlorate by freshwater and marine plankton and in the threespine stickleback was investigated.

## **General Methodology**

The effects of perchlorate on primary and secondary aquatic production was investigated in the laboratory by monitoring photosynthesis and the uptake of an amino acid, respectively, in freshwater and seawater samples in the presence of various concentrations of perchlorate. The rates of both processes were determined using a radiotracer methodology. The effects of perchlorate on decomposition processes in sediments, wetland peat and soil material were determined by measuring carbon dioxide, and in some cases methane formation, in laboratory incubation vessels supplemented with various concentrations of perchlorate. The effects of perchlorate on fish were determined in laboratory aquaria supplemented with various concentrations of perchlorate or with uniform pieces of solid rocket propellant. Fish mating and offspring rearing behavior were monitored as well as the growth of offspring. The accumulation of perchlorate in fish and plankton was also determined by extracting perchlorate using an automated extraction system followed by the removal of contaminating materials by ion exchange columns and the measurement of perchlorate by ion chromatography coupled to conductivity detection.

## **Summary of Results and Conclusions**

Aquatic primary production was only affected by perchlorate concentrations of 1000 ppm and this effect was minimal compared to control samples. There were no statistically significant decreases in production in the presence of either 10 or 100-ppm perchlorate compared to unamended controls or controls amended with 1000 ppm KCl (salt control). Hence, it is possible that perchlorate will affect photosynthesis in aquatic systems, but this effect appears to occur only when perchlorate levels are extremely high. The deleterious effect of perchlorate on photosynthesis tended to occur at light levels below maxima that occur at the surface of water bodies. Since most aquatic primary production occurs at light levels well below the maximum, it

is possible that any adverse effects of perchlorate on primary production would occur at water depths where the bulk of the depth integrated production occurs.

Bacterial production also was not adversely affected by the presence of perchlorate except at very high levels of perchlorate in seawater samples. Since coastal waters are constantly circulating, it is unlikely that phytoplankton or bacterioplankton will encounter such high levels of perchlorate for periods other than a few minutes.

Respiration in marine and freshwater sediments and wetland peat was not adversely affected by perchlorate concentrations as high as 1000 ppm. It is unlikely that concentrations exceeding this level would be encountered in sediments except in small regions in direct contact with solid propellant for extended periods of time. Perchlorate concentrations did not tend to decrease over time, at least during seven-day incubations. Anaerobic bacteria are capable of respiring perchlorate and this process has been observed in perchlorate-contaminated sediments. However, this ability tends to be associated with chronically contaminated environments and the samples used here had not been previously exposed to high levels of perchlorate and had not developed the capacity to degrade perchlorate. This is also the case in areas near launch sites, so it is unlikely that these sites would actively remove perchlorate anaerobically until they had been exposed for many months.

Soil samples exhibited significant decreases in respiration activity in the presence of 100 and 1000-ppm perchlorate. Therefore, it is possible that the deposition of perchlorate to coastal soils following a flight abortion could decrease the rate that material is decomposed in soil, which could adversely affect the recycling of nutrients and eventually plant growth. The extent of this effect will be controlled by the amount of propellant deposited, the average surface to volume ratio of deposited particles, and the number of particles deposited per area unit. Perchlorate dissolution data suggested that 100-ppm concentrations could be achieved in solutions in which volumes are restricted, and this may occur in soil water adjacent to dissolving propellant.

The presence of potassium perchlorate at concentrations up to 10 ppm and perchlorate concentrations nearing 30 ppm in aquaria containing solid propellant had no effect on stickleback mating or the birth and growth of fry. Fry mortality occurred in all treatments, but none were statistically different from controls. It is possible that fry will experience morphological or behavioral abnormalities, but these effects will not be detected until fry have grown further.

Perchlorate accumulated in both the algal/bacterial community and in fish in aquaria amended with perchlorate. The magnitude of the increase corresponded with the exposure concentration. It was unclear whether fish accumulated perchlorate by consuming contaminated algal material in aquaria or directly from dissolved perchlorate since fish food was not amended with perchlorate. Nonetheless, both the microbial and fish components accumulated significant levels of perchlorate that could be passed on to following trophic levels. Although this study did not detect severe effects of perchlorate stress, it is likely that the continued accumulation of perchlorate would lead to deleterious effects at some level.

# 1. Introduction

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Modern multi-stage rockets utilize solid rocket motors (SRMs) whose propellants contain components that are potentially harmful to ecosystems and managed species. These propellants contain three main components: ammonium perchlorate, aluminum, and a polymeric binder material such as cross-linked hydroxy-terminated polybutadiene. During flight, the solid rocket stages consume nearly all available fuel before they fall back to earth, and little to no fuel will reach sensitive habitats. However, if a failure occurs and the launch is prematurely aborted, debris containing intact propellant can be scattered around the launch site and in downrange areas. In most cases, launch trajectories are over the ocean so marine and coastal habitats are at most risk.

In addition to the direct impact of such debris, deleterious effects from launch failures could be associated with unspent SRM fuel, potentially creating a toxic condition due to leaching and dissolution of fuel components. It is expected that any toxic impact would be restricted to areas in the immediate vicinity of pieces of unspent fuel that failed to burn prior to impact. The ammonium perchlorate used as the oxidant in SRM fuel has the greatest potential to adversely affect coastal life. The perchlorate anion has been shown to affect thyroid activity in animals including humans (Brechner et al., 2000; Goleman et al., 2002a; Goleman et al., 2002b; Lamm et al., 1999; Manzon et al., 1998; Manzon et al., 2001; Riv'er and Flerov, 1975; Saito et al., 1998) and it affects cellular metabolism and growth (Stauber, 1998; Vijayalakshmi and Motlag, 1992). It also may bioaccumulate (Ellington et al., 2001; Smith et al., 2001; Susarla et al., 2000; Urbansky et al., 2000). However, little data exist on the concentrations of perchlorate that would be expected to occur via leaching and similar processes on pieces of unspent propellant.

This study focused on the effects of perchlorate on various ecosystem components and transformations such as bacterial and phytoplankton productivity, sediment and soil respiration, and effects on the ubiquitous fish, the threespine stickleback (*Gasterosteus aculeatus*). Sources of perchlorate included passively leached propellant and purposely added amounts of the pure reagent.

## 1.1. Objectives and Rationale

The purpose of this report is to describe the results of a study of the effects of SRM fuel on various components of the coastal ecosystem, especially plankton, depositional environments like sediments and soils, and a selected fish species. The primary objective was to ascertain the impact of the perchlorate anion on these ecosystem components since the perchlorate component of the fuel is probably the most serious environmental concern. The microbial portion of aquatic food webs generally constitutes the base of the ecosystem through primary and secondary production. Hence, a disruption of microbial species by SRM fuel components would affect all other aspects of the system that rely on this production including all higher forms of life. In addition, if the bioaccumulation of perchlorate occurs, it will begin at the primary and secondary production levels, i.e., phytoplankton and bacteria, and will be transmitted through the food web to each following trophic level, potentially impacting endangered animal species. We also chose

to concentrate efforts on the effects of perchlorate on degradation processes in sediments and soils since these transformations influence the recycling of materials back to primary producers such as phytoplankton and rooted plants, and disruption of decomposition could have far-reaching effects on ecosystem health. Finally, we chose to focus a portion of the study on the fish species the threespine stickleback. This species was chosen because of its cosmopolitan distribution, its occurrence in fresh and marine water systems throughout northern hemisphere temperate environments, including most areas where solid rocket fuel is used. Its behavior, ecology and physiology are well known. It is ideal for these studies since its small size is conducive to laboratory studies, and it also is easy to collect and to study in the wild. In addition, one variety is on the endangered species list and is found near Vandenberg Air Force Base in California where SRM rockets are launched. We chose ecosystem components of both the marine and terrestrial coastal environments that could be addressed in a finite time period and mostly in a laboratory setting to place tight control on experimentation.

## 1.2. Approach

The project was comprised of four main components:

### **1.2.1. Effects of perchlorate on primary and secondary production in fresh and marine waters.**

Experiments were conducted in which microbial activities were monitored in the presence of  $\text{ClO}_4^-$  concentrations covering three orders of magnitude. Primary production involved determination of rates of photosynthesis by phytoplankton in creek and coastal marine waters and at low, medium and high light regimes. Secondary production is the growth of bacteria using primarily dissolved organic matter that usually is derived from exudation by primary producing phytoplankton. Both forms of production are transferred to higher trophic levels through other plankton and eventually to fish and other animals.

### **1.2.2. Decomposition processes in depositional environments**

The effects of  $\text{ClO}_4^-$  on marine and freshwater sediments, wetland peats, and upland soils were studied in the laboratory. Since aborting the flights of errant rockets tends to occur soon after launch, it is possible that shallow marine waters and coastal terrestrial habitats will be affected. These environments rely on sediments and soils for the recycling of nutrients, unlike open ocean areas that recycle the majority of materials back to producers within surface waters. In addition, the perchlorate ion can serve as a bacterial electron acceptor (i.e., can be respired by bacteria) in anaerobic environments, so it is possible that perchlorate that is buried within anoxic sediments will be completely destroyed by bacterial metabolism. This potential loss does not occur in oxygen-containing habitats such as surficial soils and the water column. Incubated soils, sediments and peats were amended with a suite of perchlorate concentrations and their effects on respiration determined over time.

### **1.2.3. Effects of perchlorate on the behavior and growth of the threespine stickleback.**

Controlled laboratory experiments were conducted to monitor mating behavior, parental care, hatching success, and survival of fry in aquaria containing differing concentrations of perchlorate or pieces of SRM fuel.

### **1.2.4. Bioaccumulation of perchlorate in plankton and fish.**

Perchlorate concentrations were measured in phyto/zooplankton, bacteria, and sticklebacks after incubation with perchlorate to determine if perchlorate is bioaccumulated at various levels in the trophic hierarchy. This was achieved by developing and testing an automated tissue extraction system and measuring perchlorate using ion chromatography (IC).



## 2. Methods

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### 2.1. Aquatic Studies

Water samples were collected from a freshwater and a marine site. The freshwater site was Chester Creek on the University of Alaska Anchorage campus. Several liters of water were collected in a plastic carboy and were immediately transferred to five 2.6-liter Fernbach flasks. One flask was left un-amended, while three of the other four received potassium perchlorate ( $\text{KClO}_4^-$ ) at final concentrations of either 10, 100, or 1000 ppm. The fifth flask was amended with 1000 ppm of KCl to control for the effect of adding 1000 ppm of salt to the experimental flasks. The high level of  $\text{KClO}_4^-$  was a significant increase in the salt level in the freshwater samples, but added an insignificant increase in salt to the seawater sample that had a salinity greater than 30‰. Primary and secondary production were determined on subsamples from each flask at the beginning of the experiment and again after flasks were incubated in shaded light for one week.

#### 2.1.1. Primary production.

Photosynthesis was determined using a  $^{14}\text{C}$  method (Richardson, 1991). Subsamples (50ml) were removed from flasks and placed into polycarbonate rectangular bottles. Bottles were precleaned using 10% HCl, rinsed in 18 mega-ohm water, and left filled with ultrapure water for at least 24 hrs before use. Samples were split into groups of five bottles, each of which received 1.0  $\mu\text{Ci}$  of  $^{14}\text{C}$ -bicarbonate (New England Nuclear, Boston). The contents of one bottle (designated “time zero”) was immediately filtered through a 25 mm Whatman™ GF/F glass fiber filter which was added to a glass scintillation vial containing 250  $\mu\text{l}$  of 0.5N HCl. The vial was left open for 24 hr to facilitate the removal of unutilized  $^{14}\text{C}$ -bicarbonate after which scintillation fluid was added and radioactivity in vials counted using a scintillation counter. The other four bottles were incubated for approximately 6 hours. One bottle for each group of five was wrapped in aluminum foil to act as a dark control. The other three bottles were incubated in the light. At the end of the incubation, samples in each bottle were filtered and treated similarly to that described above for the “time zero” bottle. For each treatment, three sets of five bottles were used. One set was incubated at ambient light, the next at 50% light, and the third at 25% light. Light levels were attenuated using window screening. The amount of light passing through screens was determined using a light meter. One screen removed 50% of ambient light and two layers of screening removed almost exactly 75% of the light.

#### 2.1.2. Secondary production.

Bacterial production was determined using a tritiated leucine uptake technique (Kirchman, 2001). Triplicate 1.5 ml subsamples from each Fernbach flask were introduced into 2.0 ml micro-centrifuge tubes. Each tube received  $\sim 1.0 \mu\text{Ci}$  of  $^3\text{H}$ -leucine that was mixed with sufficient unlabeled leucine to bring each subsample to a final concentration of 20 nM leucine. Samples were incubated for one hour and then 75  $\mu\text{l}$  of 100% trichloroacetic acid (TCA) was added to each to stop the reaction and to precipitate proteins. Five minutes after addition of TCA, tubes were centrifuged at high speed for 10 minutes and the supernatant removed by pipette. One ml

of TCA was then added to each vial, vials were vortexed briefly, centrifuged, and the supernatant removed. This wash step was repeated and 1.0 ml of scintillation cocktail was added to each tube. Tubes were paced within scintillation vials and counted. Pre-killed controls consisted of duplicate vials for each treatment that were amended with 75  $\mu$ l of 100% TCA prior to the addition of the  $^3\text{H}$ -leucine and then treated identically to experimental vials.

## 2.2. Respiration in Depositional Environments

### 2.2.1. Sample sites and sampling protocols.

Samples were collected from four sites in Alaska: 1) freshwater sediments were collected in Chester Creek on the UAA campus (Hines et al., submitted); 2) bog peat was collected from Turnagain Bog in Anchorage (Hines et al., 2001); 3) marine sediment was collected on mudflats at Lowell Point just south of Seward Alaska, and: 4) soil samples were collected in a forested site on the UAA campus (Harper and Hines, submitted). Sediment and peat samples were collected by completely filling glass jars. Sediments in both cases consisted of sandy silts with some clay and appeared anoxic just below the surface as evidenced by black sulfidic layers. Bog peats were collected just below the surficial green *Sphagnum* moss layer and also were anoxic (gas ebullition). Sediment and peat samples were diluted 2:1 water:material using water from the site, and blended briefly. Slurries (40 ml) were added to 60 ml serum vials that were sealed with butyl rubber stoppers and then flushed with nitrogen. Diluent water was amended with  $\text{KClO}_4^-$  to achieve final concentrations of 10, 100, and 1000 ppm  $\text{ClO}_4^-$ . Samples were prepared in duplicate and one set of unamended vials acted as controls while another set of vials was amended with 1000 ppm KCl to control for salt effects. Soils were also slurried using tap water and placed into serum vials, but were not flushed with nitrogen prior to incubation and were not sealed with stoppers (i.e., were incubated aerobically).

### 2.2.2. Gas measurements.

Carbon dioxide was measured in each vial over time to determine the rate of sediment/soil respiration and any effects of  $\text{ClO}_4^-$  amendments. For all anaerobic incubations (sediments and peat), carbon dioxide was allowed to accumulate throughout the incubation period (~one week). Headspace gas samples were removed via syringe and 1.0 ml injected into a Shimadzu GC14A gas chromatograph via a sample loop. Carbon dioxide was separated from other gases using a Poropak Q packed column with helium as carrier gas. Detection was achieved using a thermal conductivity detector and the signal integrated via computer. Since soil slurries were incubated open to the air, carbon dioxide production was determined by sealing vials with stoppers for a few hours and measuring carbon dioxide at the beginning and end of each period. Methane was measured in the freshwater incubations (creek sediment and peat) by removing headspace gas via syringe and 1.0 ml injected through a sample loop into a Shimadzu GC9A gas chromatograph fitted with a Poropak Q-filled column with helium as carrier gas. Detection was achieved using a flame ionization detector and the signal integrated via computer.

### **2.2.3. Perchlorate.**

Perchlorate concentrations were determined in slurries at the beginning and end of incubations using a perchlorate ion specific electrode (Cole Parmer™). Electrodes were calibrated from standards prepared gravimetrically using either  $\text{KClO}_4^-$  or  $\text{NaClO}_4^-$  and a concentration range covering two orders of magnitude as suggested by the manufacturer.

## **2.3. Studies of the Effects of Perchlorate on the Threespine Stickleback**

### **2.3.1. Fish collection.**

Male and female threespine stickleback, were collected from Rabbit Slough in late May 2002 and again in mid June. All fish were kept in two large (approximately 400 liter) holding tanks for up to 11 days prior to experimentation. Males and females were held in separate tanks. Only males showing at least the initial stages of nuptial coloration (bluish tint on dorsal side of orbit) were retained for use in the experiment, while only the most gravid females were used. While in the holding tanks, the males lost their nuptial coloration, but began to regain it within 30 minutes after introduction into individual aquaria.

### **2.3.2. Experimental.**

Eighty-three aquaria containing approx 35 l of tap water were supplemented with 35 g aquarium salt producing a salinity of approximately 4‰ (slightly greater than 10‰ of the salinity found in seawater). Twenty aquaria, designated “low concentration,” were treated with 0.075 g  $\text{KClO}_4$ , resulting in a concentration of approximately 1.4 ppm  $\text{ClO}_4^-$ . Twenty aquaria designated “high concentration” were treated with approximately 0.53 g  $\text{KClO}_4$ , producing an average  $\text{ClO}_4^-$  concentration of 11.5 ppm. Twenty aquaria received approximately 1.1 grams of SRM propellant in pieces that were uniform in size and shape. The concentrations of  $\text{ClO}_4^-$  used in the “low” and “high” aquaria were intended to bracket a low and rather high level of  $\text{ClO}_4^-$  expected to occur after 20-30 days in the aquaria receiving SRM propellant. The levels chosen were based on data for the dissolution of  $\text{ClO}_4^-$  from similar size pieces of SRM fuel provided by scientists at Aerospace, Inc. in Los Angeles. Finally, 20 aquaria lacking  $\text{ClO}_4^-$  acted as controls.

In addition to the aquarium salt and  $\text{ClO}_4^-$  treatments, all 20 aquaria from each treatment received 20 g of nesting vegetation and one Petri dish full of sand. The remaining three aquaria, designated W1, W2, and W3, lacked vegetation, fish, or sand and only contained a low, high, or variable (solid propellant) treatment of  $\text{ClO}_4^-$ , respectively. When the  $\text{ClO}_4^-$  levels began to stabilize two days after the treatments were added, one adult male was added to each aquarium, except for aquaria W1-W3. Almost all males began constructing nests within 30 minutes of their introduction, and most had completed construction within 24 hours. Adult fish were fed frozen brine shrimp throughout the experiment.

Each fish was filmed for 10 minutes each day to record behavioral data. The order of filming was done randomly and changed daily to ensure that temporal changes in behavior would be excluded. After two days of baseline activity recording with only the males in the tanks, the first

females were introduced on the evening of 8 June. Females were left in aquaria for an average of 10 minutes before a spawning attempt was deemed unsuccessful, and the female was removed. Whenever a pair successfully spawned the female was removed, and the male's initial parental care activities were recorded for an extra 10 minutes (up to 20 minutes total per fish) on any given day. The female was removed as soon as it was determined that she had successfully spawned. Females were added to aquaria for the next seven days until each male had successfully spawned.

Males were left in aquaria to rear young, but were removed when fry were about 1/2 cm in length (about five weeks) to prevent the ingestion of fry by males. Males that successfully spawned and were removed from tanks were frozen immediately using liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . The  $\text{ClO}_4^-$  content of these fish was assayed (described below).

In addition to experiments using fish, bacterial production was measured weekly in three aquaria from each of the four treatments. Assays were identical to those described above for the aquatic studies (section 2.1.2). At the end of the experiment, water from these selected aquaria was filtered through  $1.2\ \mu\text{m}$  filters for later  $\text{ClO}_4^-$  analyses (see section 2.1.3) to assess the bioaccumulation of  $\text{ClO}_4^-$  in microscopic organisms over the several week incubation period. Finally, perchlorate concentrations were measured daily in each of the 80 aquaria using a perchlorate specific ion electrode system (Cole Parmer).

## 2.4. Bioaccumulation of Perchlorate

The bioaccumulation of  $\text{ClO}_4^-$  was determined in fish, and phyto/zooplankton. Male sticklebacks that were frozen were thawed, weighed, and half of each fish was homogenized by hand with a knife. The homogenate was mixed with Ottawa sand and extracted using ultrapure water in an automated extraction unit (ASE<sup>TM</sup>, Dionex Corp.) at  $100^{\circ}\text{C}$  and 1,500 psi. Extracts were passed through a strong cation exchange column to remove interfering ions and  $\text{ClO}_4^-$  concentrations determined using a Dionex DX600 ion chromatography system with a AS16 ion exchange column, and a conductivity detector and ion suppressor. Filters from aquaria were weighed, added directly to the ASE extraction unit, and analyzed identically to fish samples.

## 3. Results

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### 3.1. Aquatic Studies

#### 3.1.1. Freshwater.

Creek water samples exhibited significant rates of primary and secondary production (Figs 1 and 2). Both processes were calculated as a first order rate constant in which uptake rates are reported as the percentage of radiotracer consumed per hour. These data in ecological studies are usually transformed further to calculate a rate of carbon sequestration per volume per time, but this was not necessary in this case since effects of different treatments are being compared.

Primary production (photosynthesis) was most rapid at the 25% light level and least active in full sunlight. This is a common finding in water column samples since phytoplankton are photosensitive and maximum activities are generally restricted to water depths below the surface. In addition, photosynthetic activity was significantly lower at the end of the seven-day incubation than on the first day, indicating that phytoplankton probably died over time in the flasks. Data for each treatment were compared statistically using Analysis of Variance (ANOVA) and significant differences determined using a Tukey Test. In all cases, the samples amended with 1000 ppm  $\text{ClO}_4^-$  yielded statistically lower rates of photosynthesis than the unamended controls (Fig. 1). However, in five of the six cases, rates exhibited by the 1000-ppm samples were not significantly lower than those containing 1000 ppm KCl as opposed to 1000 ppm  $\text{ClO}_4^-$ . Hence, it appeared that much of the decrease in rate at the high levels of  $\text{ClO}_4^-$  was due to a salt effect. The rates in the 1000-ppm flasks were usually lower than those of the salt control, but only the 1000-ppm flask sampled on day one and incubated at 25% light displayed a photosynthetic rate that was significantly lower than both controls.

Bacterial production was also easily measured in creek water (Fig. 2). On the first day of the incubation, rates tended to decrease as  $\text{ClO}_4^-$  concentrations increased, but only the 1000-ppm sample was significantly lower than the unamended control. Since this sample was not significantly different from the salt control, it appeared that the decrease in rate was not due to  $\text{ClO}_4^-$ . Unlike photosynthetic activity, bacterial production at the end of the seven-day incubation increased slightly, but none of the flasks exhibited rates that were statistically different from each other.

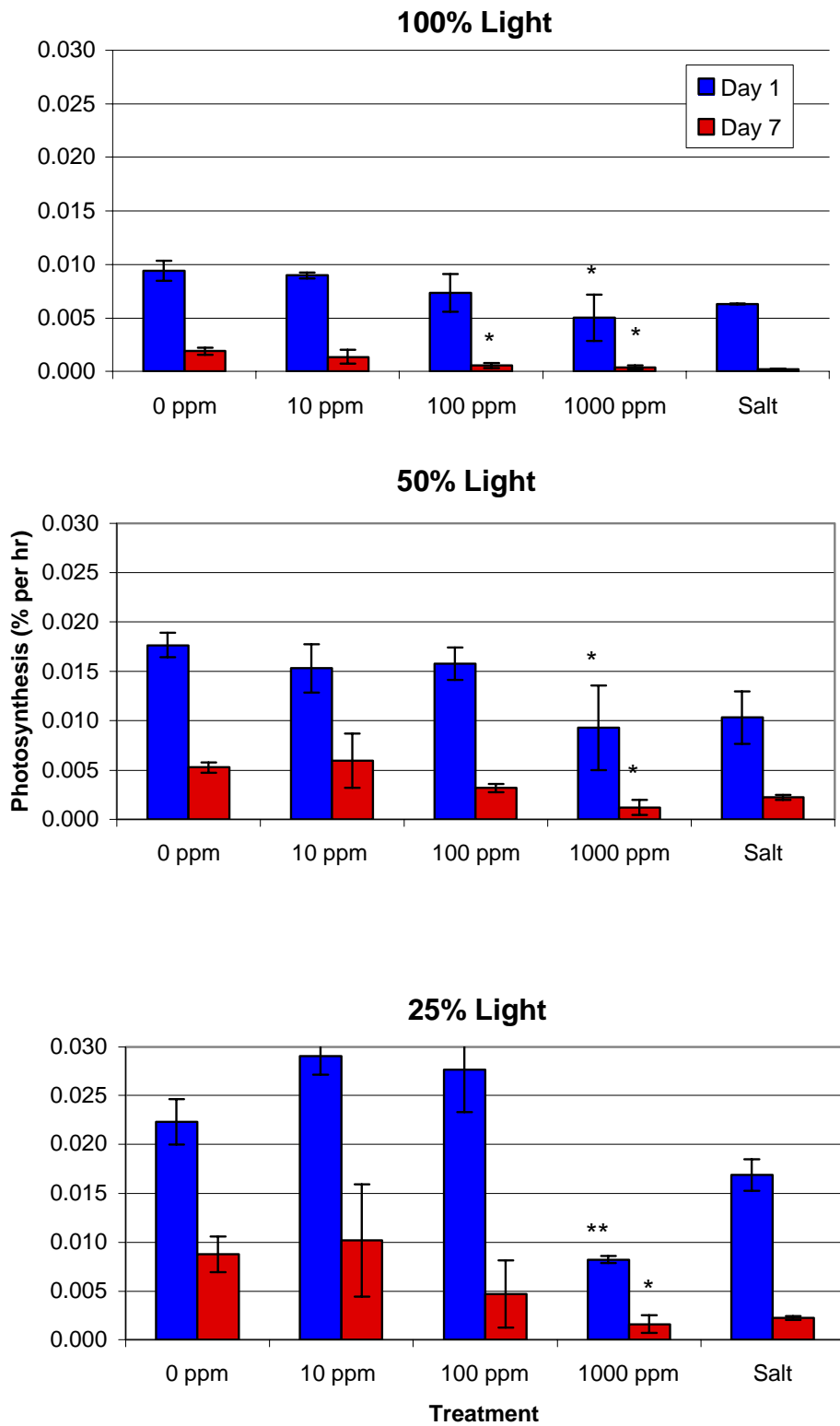


Figure 1. Photosynthesis in freshwater creek samples at the beginning and end of a seven-day incubation with different levels of  $\text{ClO}_4^-$ . \*statistically smaller than the unamended control (0 ppm) at the 95% confidence level. \*\*statistically smaller than 0 ppm and salt treatment.

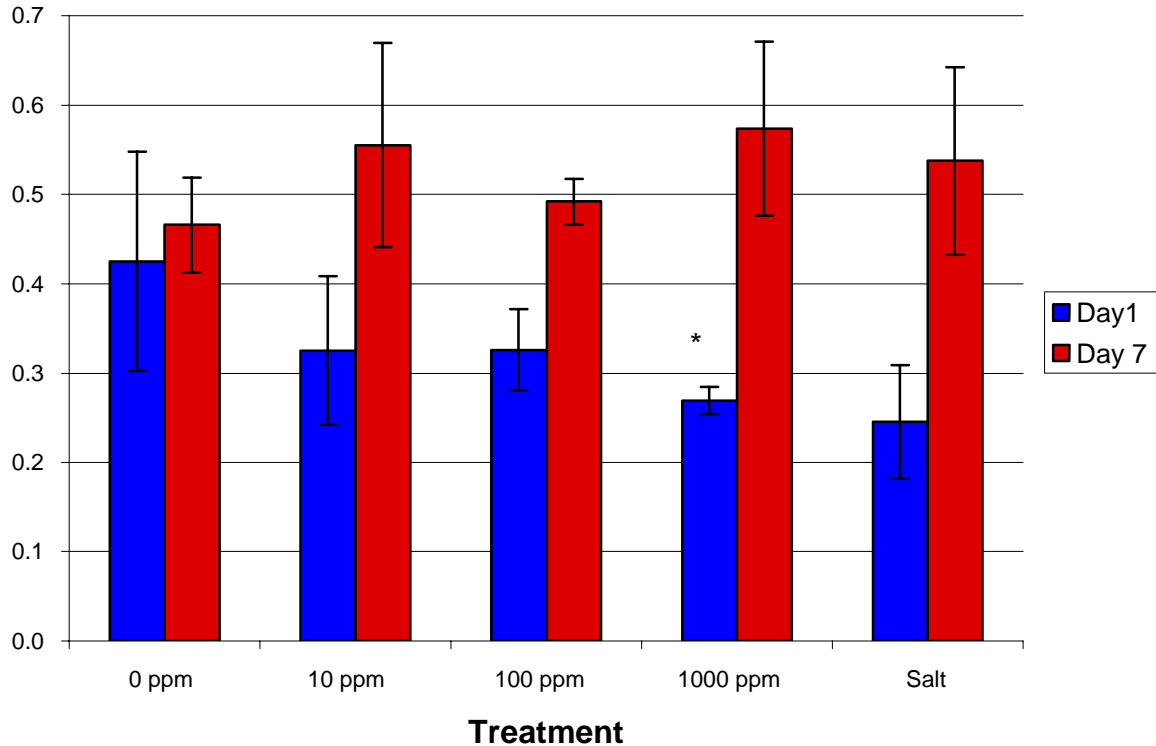


Figure 2. Bacterial production ( $^3\text{H}$ -leucine uptake) in freshwater creek samples at the beginning and end of a seven-day incubation with different levels of  $\text{ClO}_4^-$ . \*Statistically smaller than the unamended control (0 ppm) at the 95% confidence level. \*\*Statistically smaller than 0 ppm and salt treatment.

### 3.1.2. Seawater.

Photosynthesis in seawater samples displayed slower first order rate constants than freshwater samples (Fig. 3). However, it was anticipated that marine samples would exhibit less activity since seawater contains much higher levels of natural bicarbonate that dilutes the radiolabeled material and none of the rates were corrected for *in situ* concentrations of inorganic carbon. Photosynthesis was highest at the lowest light level, but the increase was not as dramatic as that noted for the freshwater samples. Similarly to freshwater, all of the 1000-ppm samples revealed slower photosynthetic rates than the unamended control, but one sample (1000 ppm at 50% light) was statistically lower than both the unamended and the salt controls. The 1000-ppm sample at 25% light was lower than the salt control, but was too variable to be statistically lower. The salt controls were statistically lower than the unamended samples in all six cases. This was surprising since the 1000-ppm KCl amended increased the salt content of the sample by only a few percent. This discrepancy was likely due to traces of trace metals present in these ultra pure salts. It is clear that trace metals adversely affect phytoplankton activity and this is especially true in seawater (Fitzwater et al., 1982). An examination of the chemical analysis of the salts printed on reagent bottle labels revealed that the KCl contained slightly higher concentrations of

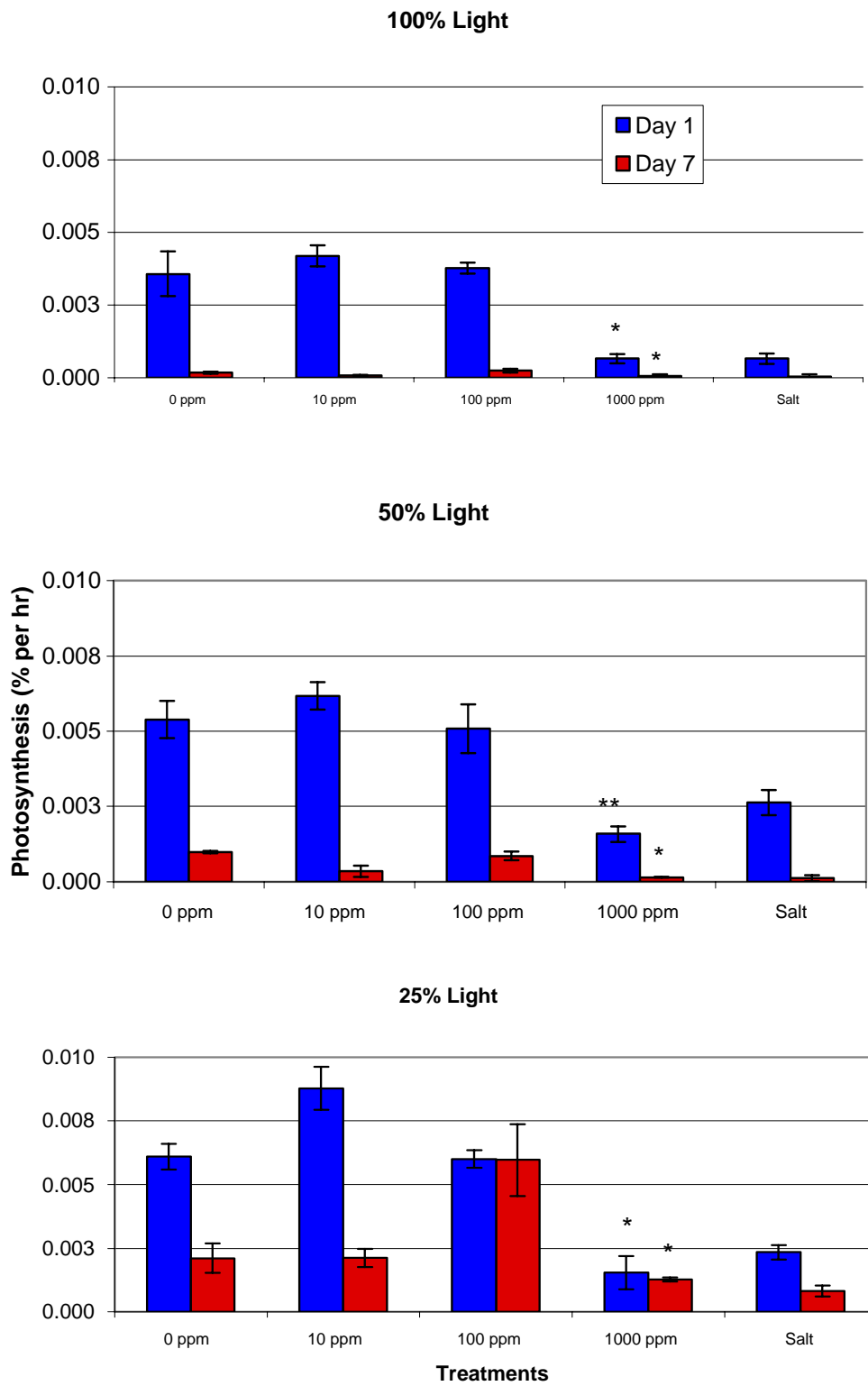


Figure 3. Photosynthesis in seawater samples at the beginning and end of a seven-day incubation with different levels of  $\text{ClO}_4^-$ . \*Statistically smaller than the unamended control (0 ppm) at the 95% confidence level. \*\*Statistically smaller than 0 ppm and salt treatment.



trace contaminants than did the  $\text{KClO}_4^-$ . Unlike the freshwater samples, the 10-ppm and sometimes the 100-ppm samples had higher rates of photosynthetic activity than did the unamended control (Fig. 3). This was most evident at the lowest light level where rates were most rapid. Photosynthesis in freshwater at 25% light also displayed an increase in rate in the presence of 10 and 100 ppm  $\text{ClO}_4^-$ , but this increase was not statistically significant. This stimulation was most likely also due to trace metals present in the  $\text{KClO}_4^-$ , but in this case the low levels of metals were stimulatory to phytoplankton, even though the higher levels were inhibitory. Even though purified reagents have relatively low levels of contaminating metals, the seawater samples used in this study were from very pristine waters and the perchlorate amendments were rather high. In addition, metals present in seawater samples are complexed as either ion pairs or with naturally occurring organic matter. Therefore, even small metal supplements from reagent grade salts could easily affect phytoplankton activity.

Bacterial production was also easily measured in seawater samples (Fig. 4). On the first day of the incubation, rates were nearly 20-fold higher than those in the freshwater samples (Fig. 2). Rates generally decreased with increasing  $\text{ClO}_4^-$  concentrations and rates in the 1000-ppm sample were significantly lower than the unamended control and the salt control. Unlike photosynthetic activity in freshwater, bacterial production at the end of the seven-day incubation decreased greatly to a level that was similar to freshwater. Activity at seven days again decreased relative to the unamended control, but not compared to the salt control.

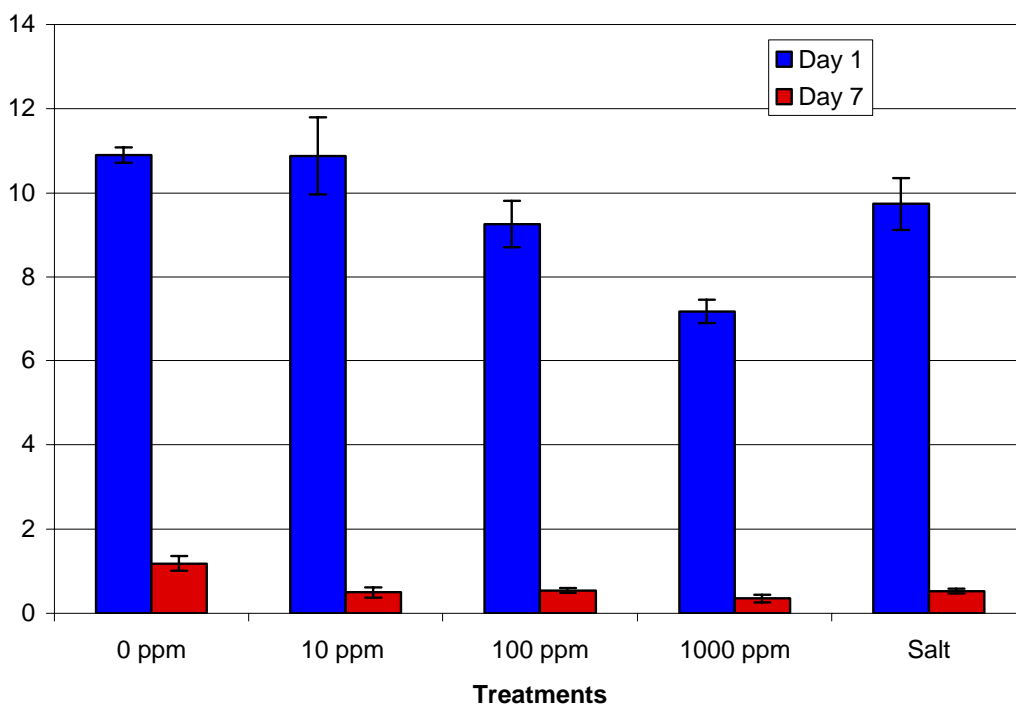


Figure 4. Bacterial production ( $^3\text{H}$ -leucine uptake) in a seawater sample at the beginning and end of a seven-day incubation with different levels of  $\text{ClO}_4^-$ . \*Statistically smaller than the unamended control (0 ppm) at the 95% confidence level. \*\*Statistically smaller than 0 ppm and salt treatment.

## 3.2. Depositional Environments

### 3.2.1. Freshwater sediments

Respiration in freshwater sediments incubated anaerobically was not affected adversely by the presence of  $\text{ClO}_4^-$  (Fig. 5). The 1000-ppm and salt controls exhibited the highest rates of carbon dioxide production, and this may have been due to trace elements contaminants in the ultrapure chemical preparations. Methanogenesis was also measured in these sediment slurries, but rates were quite low. However, methane concentrations at the end of the incubation were highest in the 1000-ppm and salt control vials, which mimicked the carbon dioxide data (Fig. 6).

Perchlorate concentrations in vials at the beginning and end of the incubation were measured using a  $\text{ClO}_4^-$  electrode to determine if  $\text{ClO}_4^-$  was consumed by sediments (Fig. 7). The  $\text{ClO}_4^-$  levels did not change in any of the vials during the incubation.

### 3.2.2. Marine sediments

Respiration in marine sediments incubated anaerobically was also not affected adversely by the presence of up to 1000 ppm  $\text{ClO}_4^-$  (Fig. 8). All vials containing  $\text{ClO}_4^-$  yielded slightly less carbon dioxide than the unamended control vials, but none were statistically lower. Since seawater contains large concentrations of inorganic carbon as normal salts, hydrochloric acid was added to each vial at the end of the weeklong incubation to drive all the inorganic carbon into the gas phase as carbon dioxide to account for any differences in the inorganic carbon content that might have occurred in different treatments over time. There were no significant differences in the final carbon dioxide concentration of any of the vials underscoring the lack of impact of  $\text{ClO}_4^-$  on these sediments (Fig. 9).

Perchlorate concentrations in marine sediment slurries did not vary from the beginning to the end of the incubation (Fig. 10).

### 3.2.3. Freshwater wetland peat

Respiration in bog peat material was also not affected by perchlorate amendments (Fig. 11). In this case, both carbon dioxide and methane accumulated rather linearly over time and there were no significant differences among the treatments. Perchlorate concentrations also did not vary throughout the incubation (Fig. 12).

### 3.2.4. Soil

Amendments of 100 and 1000 ppm  $\text{ClO}_4^-$  resulted in a statistically significant decrease in the rate of respiration in soil slurries compared to the unamended and KCl-containing slurries (Fig. 13). The addition of salt and 10 ppm  $\text{ClO}_4^-$  also resulted in a ~30% decrease in carbon dioxide production, but these decreases were not statistically significant from the 0 ppm control. Perchlorate levels did not decrease in any of the vials during the incubation (Fig. 14).

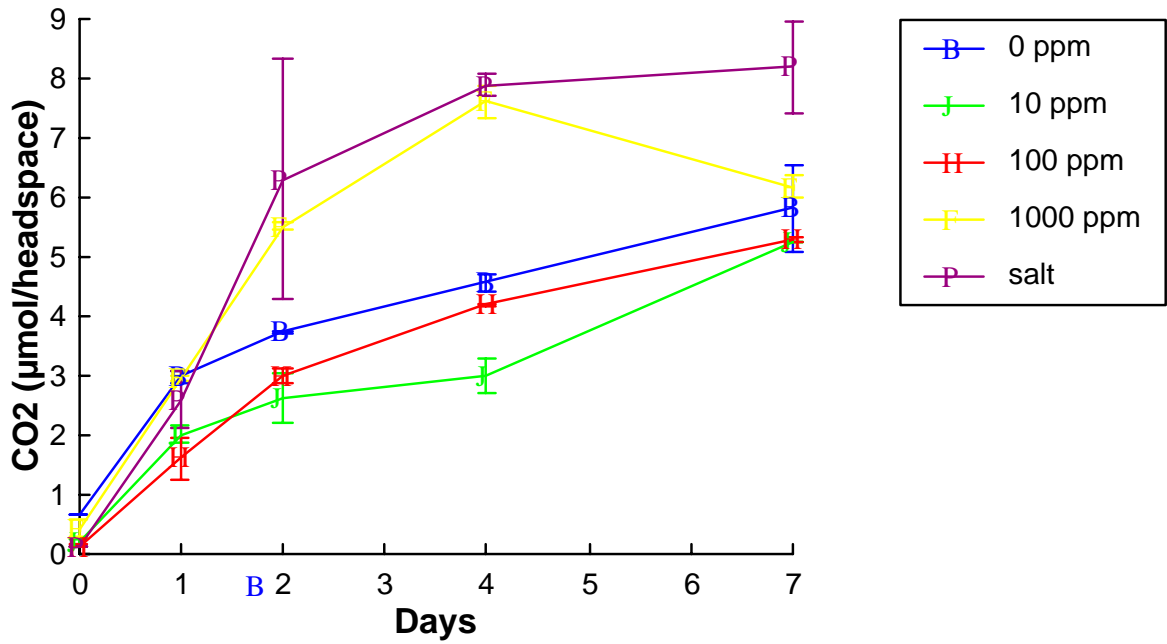


Figure 5. Effects of  $\text{KClO}_4^-$  at differing concentrations on respiration of freshwater sediment (creek mud) incubated anaerobically. Salt equals KCl concentration identical to the 1000 ppm  $\text{KClO}_4^-$  concentration.

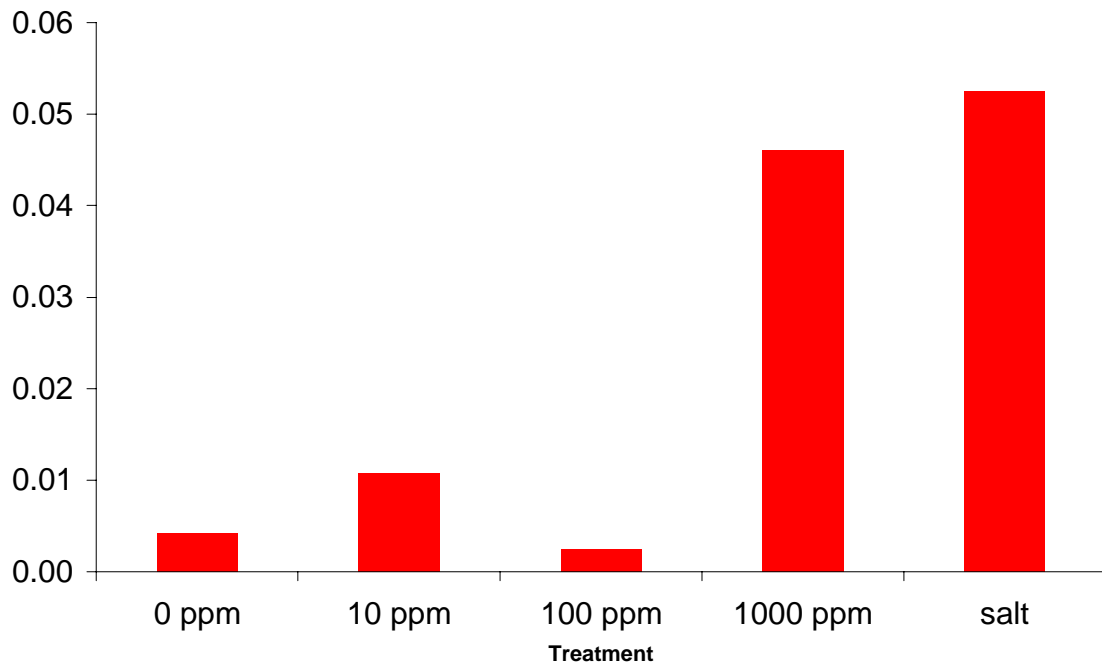


Figure 6. Methane concentrations in the headspace of vials of freshwater sediment slurries amended with differing concentrations of  $\text{KClO}_4^-$  after 8 days of incubation.

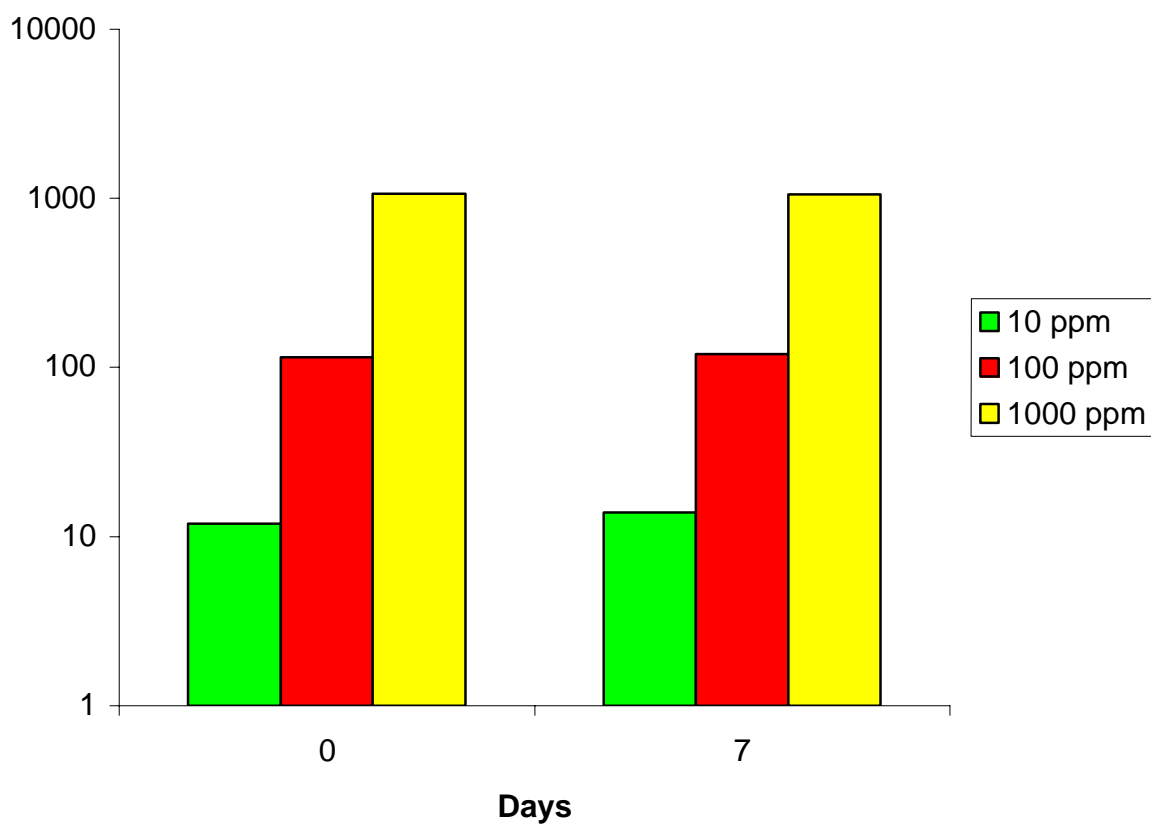


Figure 7. Perchlorate concentrations in freshwater sediment at the beginning and end of a weeklong incubation.

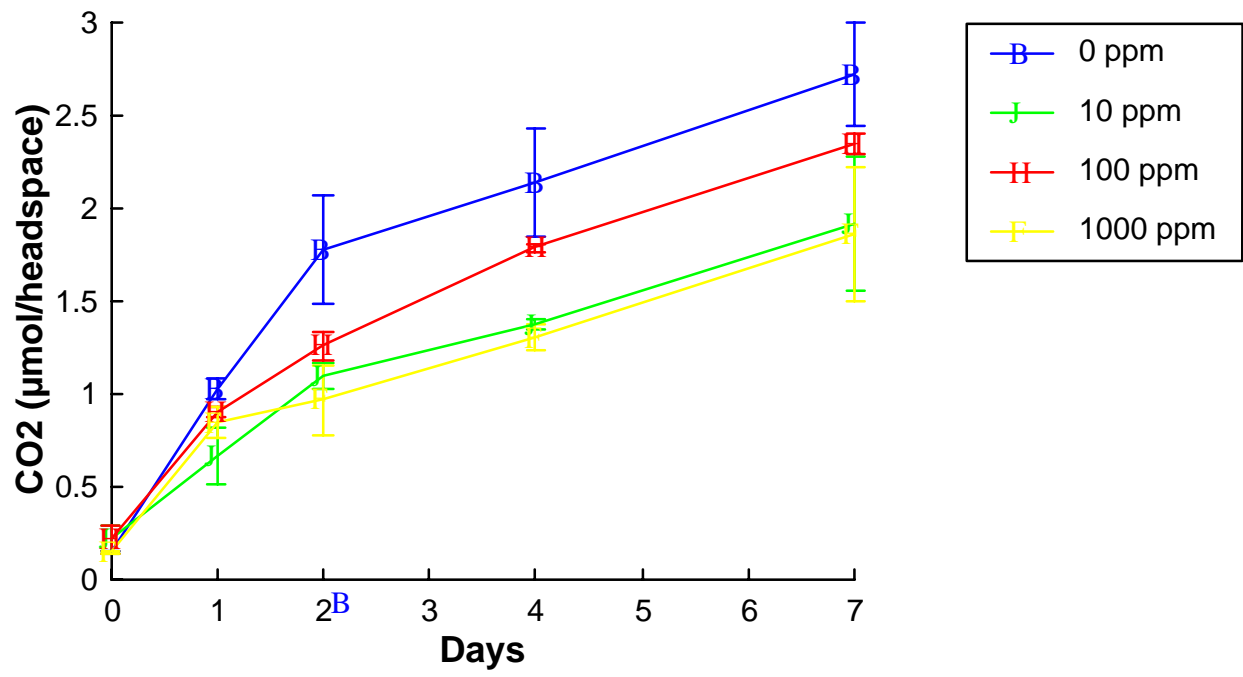


Figure 8. Effects of  $\text{KClO}_4^-$  at differing concentrations on respiration of marine sediment incubated anaerobically.

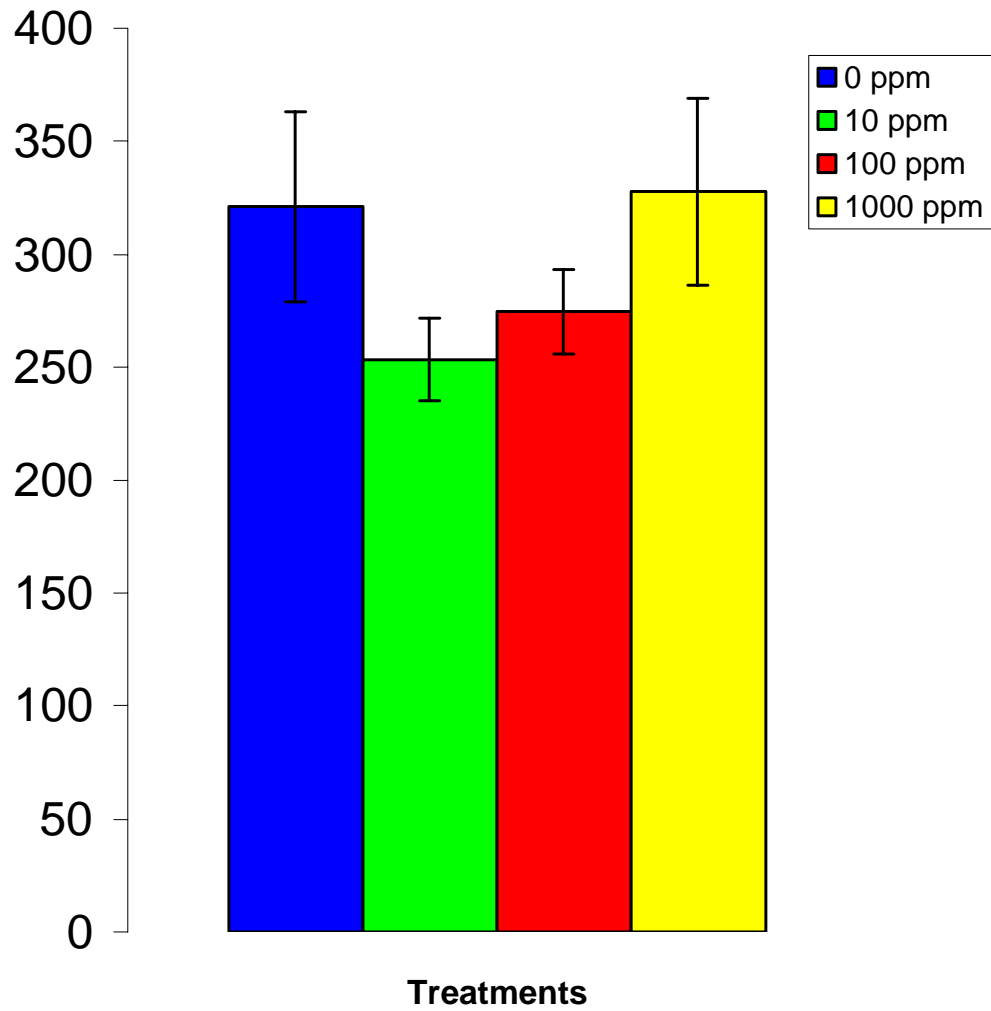


Figure 9. Concentrations of CO<sub>2</sub> in the headspace of vials incubated anaerobically with marine sediment after acidification.

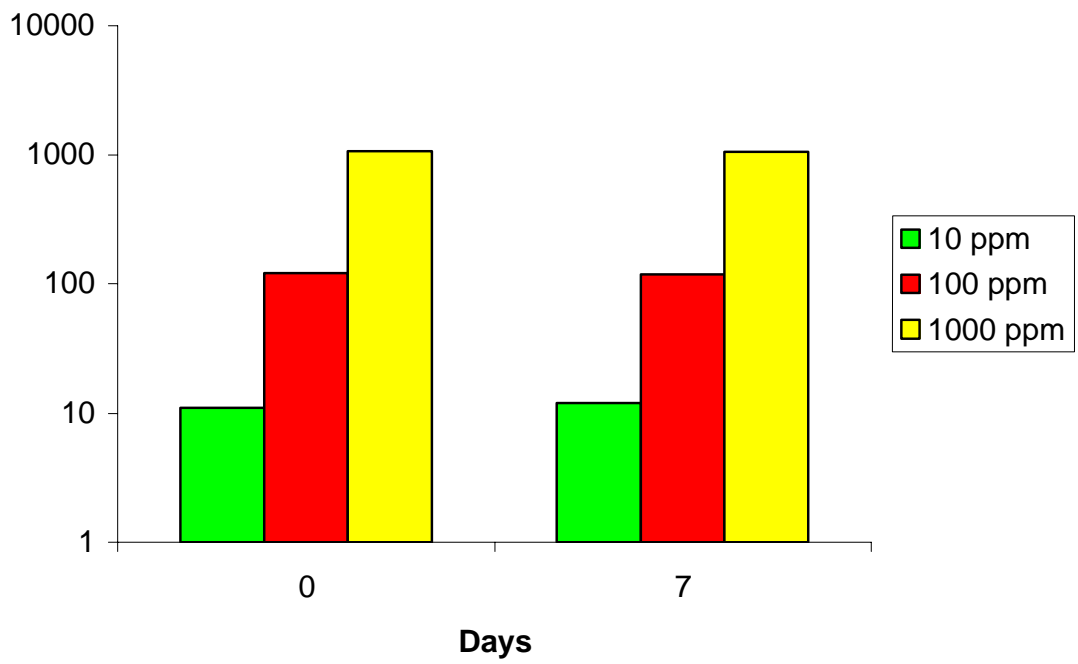


Figure 10. Perchlorate concentrations in marine sediment at the beginning and end of a weeklong incubation.



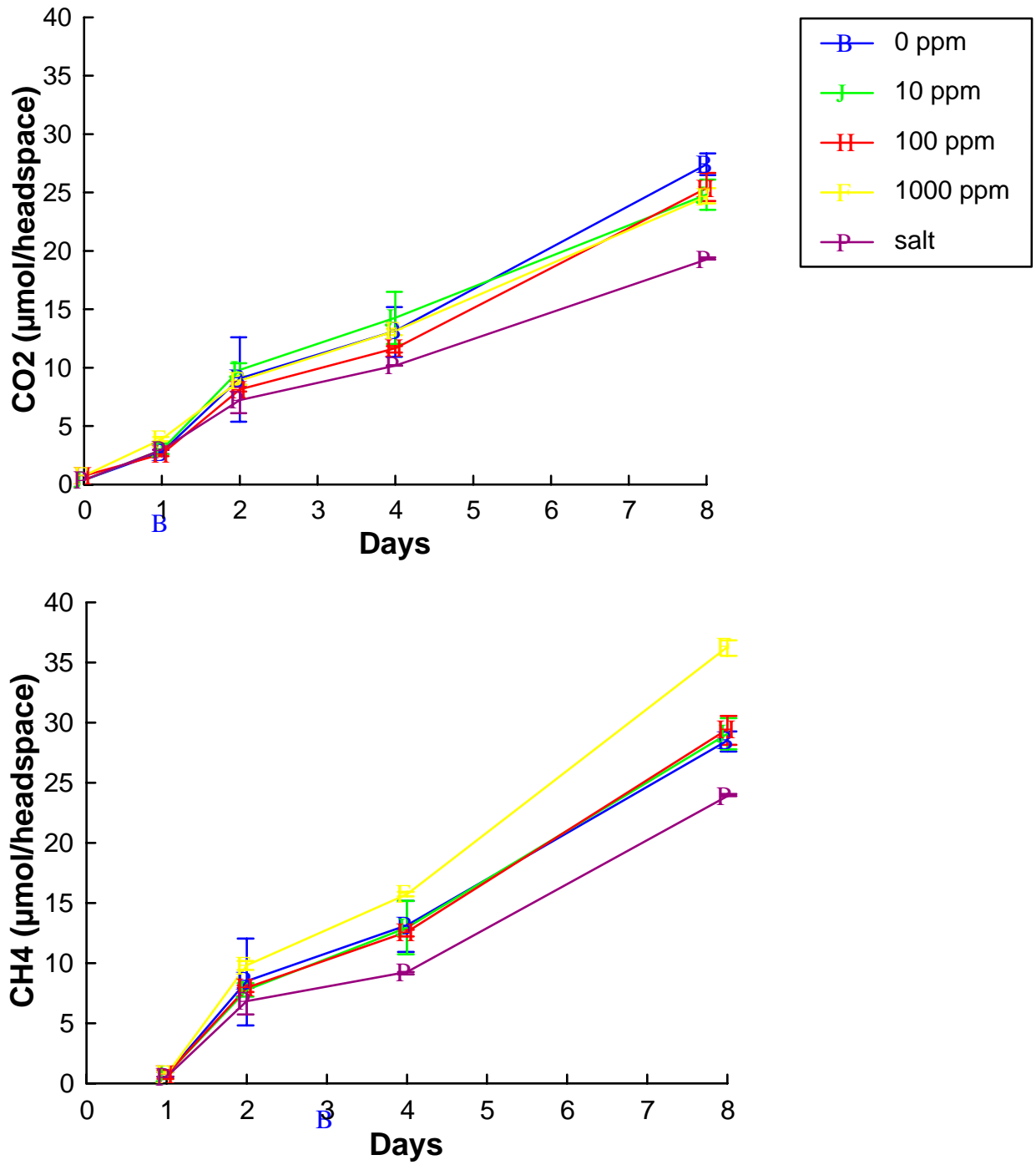


Figure 11. Effects of  $\text{KClO}_4^-$  at differing concentrations on respiration of bog peat incubated anaerobically. Salt equals KCl concentration identical to the 1000 ppm  $\text{KClO}_4^-$  concentration.

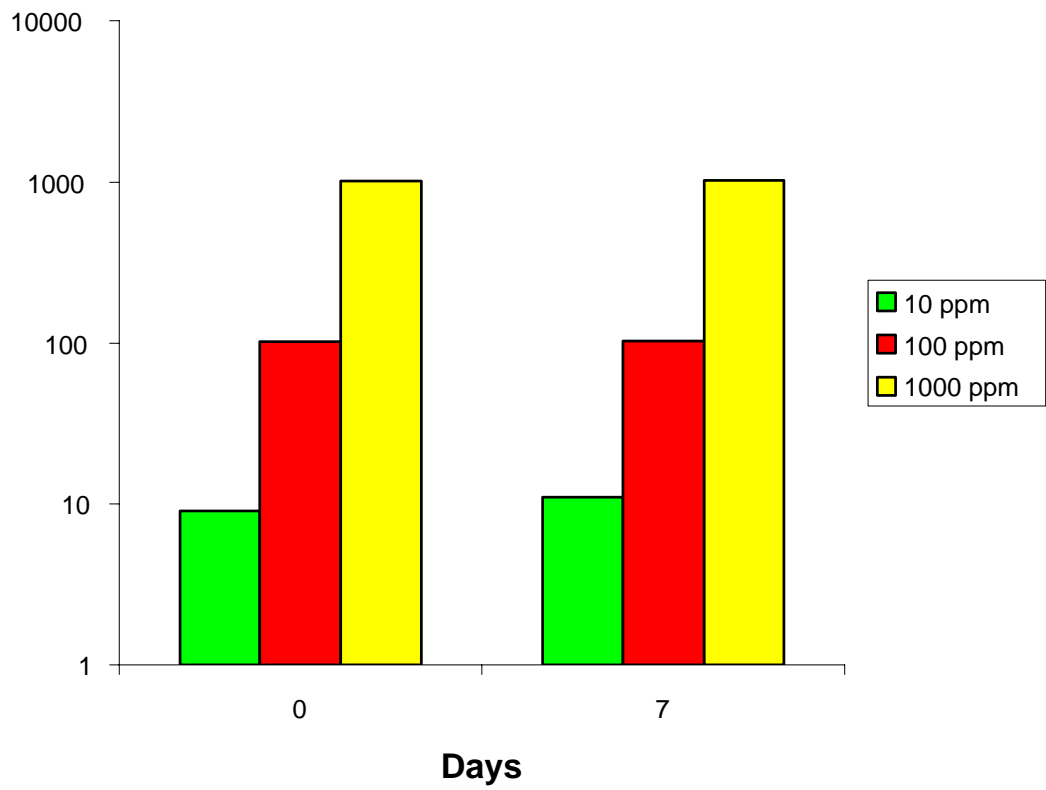


Figure 12. Perchlorate concentrations in bog peat at the beginning and end of a seven day incubation.

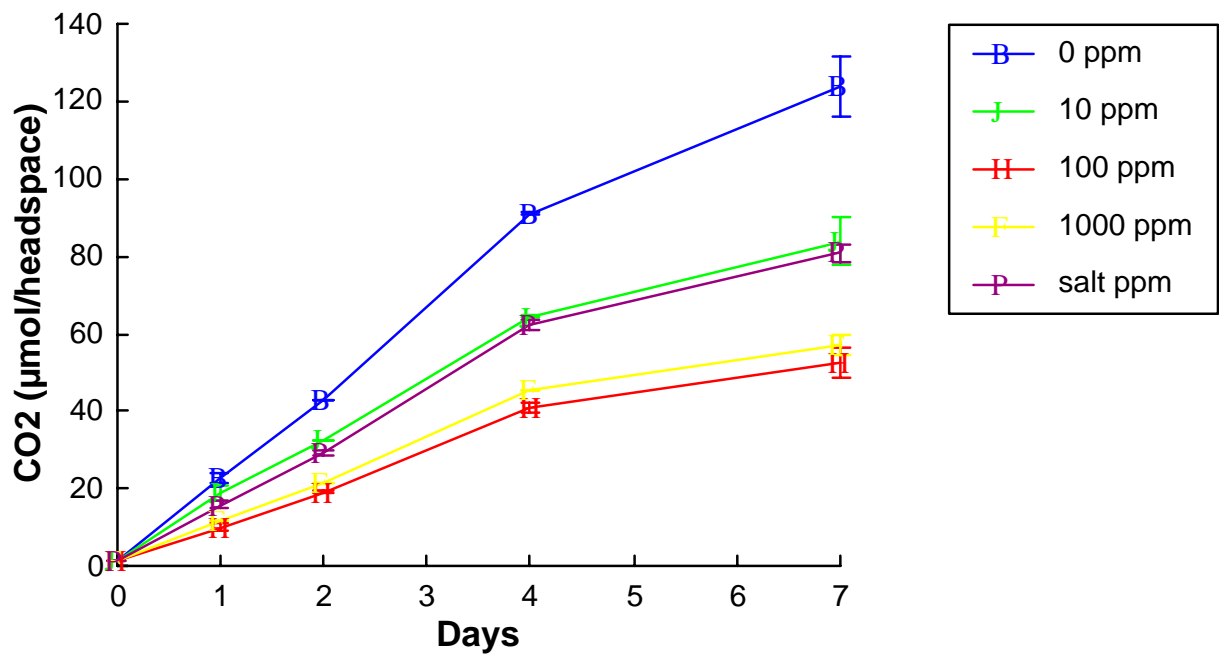


Figure 13. Effects of  $\text{KClO}_4^-$  at differing concentrations on respiration of upland soil incubated aerobically. Salt equals KCl concentration identical to the 1000 ppm  $\text{KClO}_4^-$  concentration.

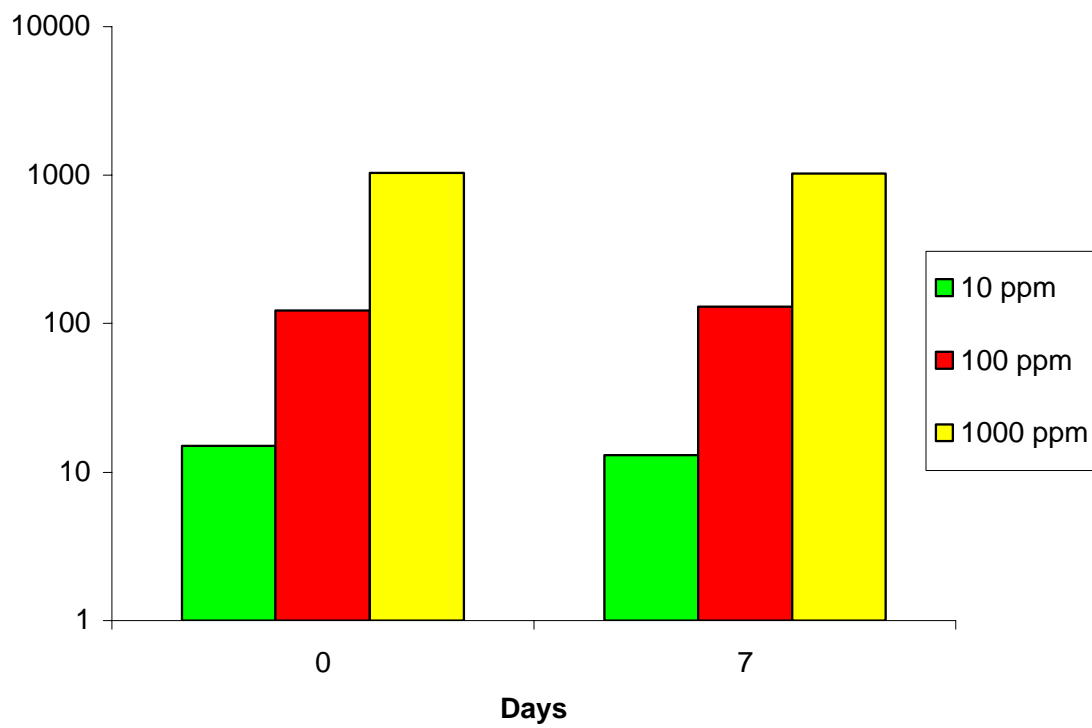


Figure 14. Perchlorate concentrations in upland soil at the beginning and end of a weeklong incubation.

## 3.3. Threespine Stickleback

### 3.3.1. Stickleback mating

Male sticklebacks tended to lose their red mating coloration after capture, but rapidly regained this trait when introduced to females (Fig. 15). Males were observed building nests, which included the secretion of a solution for “gluing” the nest together (Fig. 16). Females were observed entering the nest where they laid eggs (Fig. 17) and males were observed entering nests soon thereafter to fertilize these eggs (Fig. 18). The shortest time to successful spawning was one minute and three seconds and occurred on 13 June. The longest time to successful spawning was 11 minutes and occurred on 12 June. Based on initial data, the average time to spawning was approximately 6 minutes. Males averaged one failed spawning attempt each, prior to spawning successfully. The number of failed spawning attempts prior to success ranged from zero to three. Spawning success was not correlated with the type or level of  $\text{ClO}_4^-$  treatment. Male sticklebacks were replaced if they failed to conduct the normal behaviors associated with courtship or if they failed to produce a nest before or after their first introduction to a female. The first fry were noted on 14 June at 14:23. No adult males died prior to their removal from aquaria, and 90% successfully produced fry. Failure to produce fry was not correlated with exposure to  $\text{ClO}_4^-$  treatment.

Of the 80 aquaria that contained mating pairs, eight failed to hatch any fry. Of these, four failures were in control aquaria, one was in a 1.0-ppm aquarium, and three were in aquaria containing solid propellant. The males were removed from the aquaria when the fry had grown appreciably to prevent consumption of fry by males (~10 days). All the males were frozen and archived for later  $\text{ClO}_4^-$  analysis. Significant fry mortality occurred, which is common for stickleback fry raised in the lab. The loss was slightly over 50% with no fry remaining in 42 of the 80 aquaria. Of those 42 failures, 6 were controls, 7 occurred in 1.0-ppm aquaria, 10 in 10.0 ppm aquaria, and 12 in aquaria with propellant. Although these results suggested that the  $\text{ClO}_4^-$  was detrimental to fry, when aquaria with partial mortalities are included, there was no significant difference in mortalities for any treatment. For example, when aquaria containing two or fewer fry were considered as failures, the failure rate was 13, 12, 15, 16 aquaria for controls, 1 ppm, 10 ppm, and propellant, respectively. No morphological abnormalities were observed in adult male sticklebacks. Fry will not be large enough to analyze for morphological abnormalities until late October, 2002.

### 3.3.2. Perchlorate concentrations in aquaria

Perchlorate concentrations did not change appreciably in aquaria with single amendments of  $\text{ClO}_4^-$  at either the 1.0 or 10 ppm levels (Fig. 19). The perchlorate electrode yielded somewhat variable results despite the fact the calibration occurred daily. We noted about a 10% error in these measurements. Each data point in Figure 20 represents an average of results for all 20 replicate aquaria utilized. There was a slight decrease in the 10-ppm tanks, but this was not statistically significant. As expected,  $\text{ClO}_4^-$  levels increased continually and logarithmically over



Figure 15. Coloration of male threespine stickleback after exposure to a gravid female.



Figure 16. Male threespine stickleback (bottom) building a nest by adding glue while a female observes.



Figure 17. Female threespine stickleback entering the nest built by the male as the male observes just above.





Figure 18. The initiation of spawning. The female stickleback is just leaving the nest at the left while the male is preparing to enter the nest to fertilize the eggs.

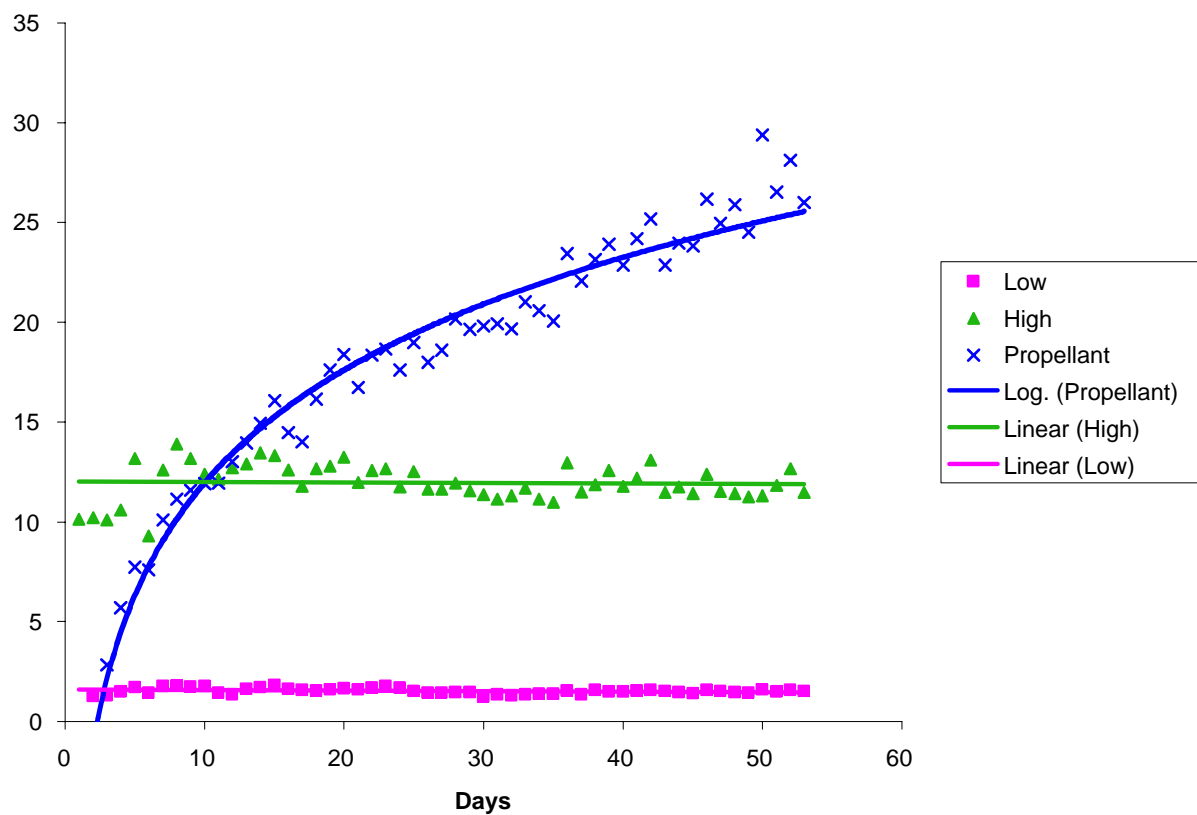


Figure 19. Perchlorate ( $\text{KClO}_4^-$ ) concentrations in fish aquaria amended with 1.0 (low), 10 ppm (high)  $\text{KClO}_4^-$ , or pieces of solid propellant. Data for low and high  $\text{KClO}_4^-$  levels are fitted to linear equations while data for tanks with solid propellant are fitted with a logarithmic equation. Numbers are averages of 20 aquaria for each treatment.

\time in aquaria containing solid propellant (Fig. 19). At the end of the experiment,  $\text{ClO}_4^-$  concentrations in these aquaria reached nearly 30 ppm.

### 3.3.3. Bacterial production in aquaria

Samples were collected from three aquaria within each of the four treatments on a weekly basis for six weeks. These samples were also split into duplicate subsamples to provide replicate analyses within each aquarium for a total of 24 measurements per week. Bacterial production was rapid in aquaria throughout the experiment and reached rates that were nearly twice as rapid as the highest rates recorded in natural samples (seawater samples), and over 10-fold higher than most of the natural samples (Fig. 20). Rates remained steady for about four weeks, but declined more than three-fold by the end of the experiment at six weeks. The variability in bacterial production rates for duplicate samples collected from individual aquaria was always smaller than the variability between aquaria. Hence, the error bars in Figure 17 are due to differences among aquaria not error due to the method itself. There were not differences in rates between any of the treatments throughout the experiment.

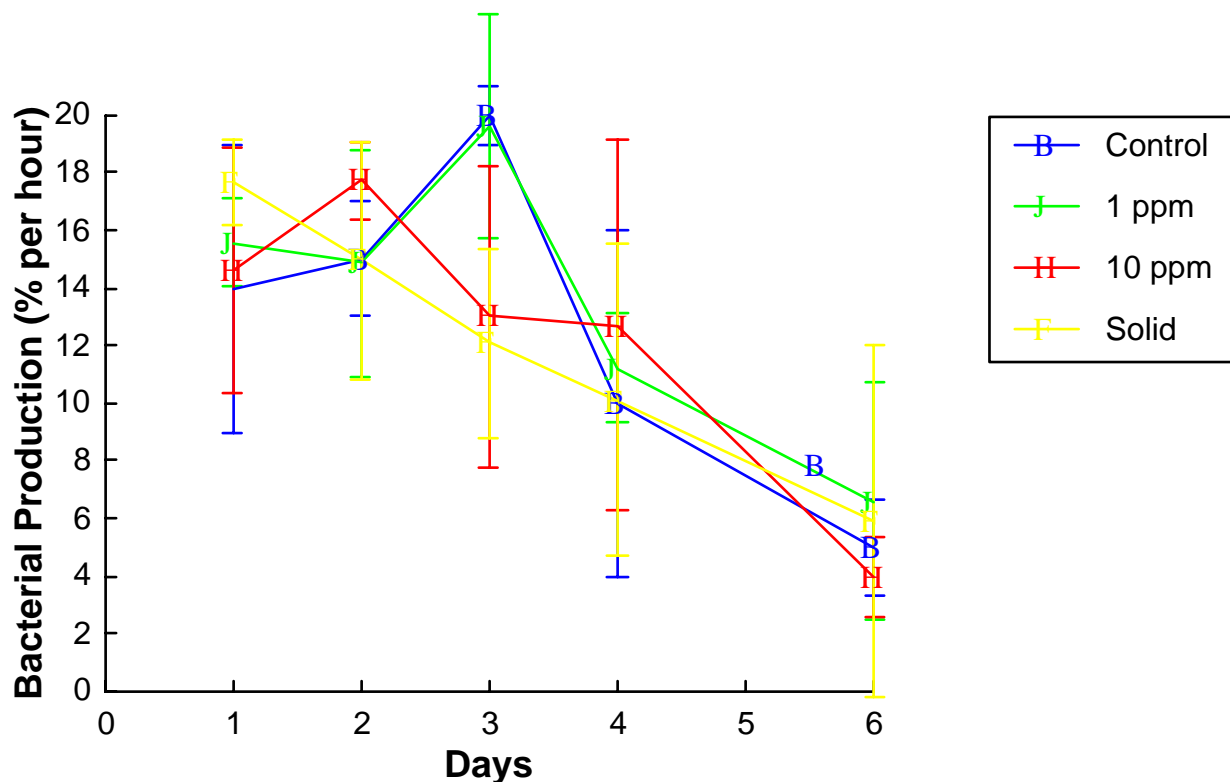


Figure 20. Secondary production (bacterial uptake of tritiated leucine) in fish aquaria over time amended with differing concentrations of  $\text{KClO}_4^-$  or solid rocket propellant.

### 3.4. Bioaccumulation of Perchlorate

Perchlorate concentrations were highest in fish removed from aquaria with the highest  $\text{ClO}_4^-$  levels (Table 1). Concentrations in fish at 1 ppm  $\text{ClO}_4^-$  were not higher than controls, but fish in the 100-ppm aquaria contained nearly 10-fold more  $\text{ClO}_4^-$  than the controls, whereas fish in aquaria containing SRM fuel displayed slightly higher  $\text{ClO}_4^-$ .

Table 1. concentrations of perchlorate in male fish and plankton in aquaria containing solid rocket motor fuel (propellant) or differing concentrations of  $\text{ClO}_4^-$ .

| Treatment  | n | $\text{ClO}_4^-$ , $\mu\text{g g}^{-1}$ ( $\pm$ SD) |
|------------|---|---|
| Male Fish  |   |   |
| 0 ppm      | 2 | $0.63 \pm 0.17$                                     |
| 1 ppm      | 3 | $0.54 \pm 0.15$                                     |
| 10 ppm     | 6 | $4.47 \pm 0.62$                                     |
| Propellant | 3 | $5.20 \pm 1.58$                                     |

Plankton tissue in aquaria amended with the highest levels of  $\text{ClO}_4^-$  contained the highest  $\text{ClO}_4^-$  concentrations (Table 1). Plankton cells were collected on glass-fiber filters, which are capable of adsorbing ions. To correct for this adsorption, solutions of  $\text{ClO}_4^-$  at different concentrations were prepared and run through filters that were analyzed identically to experimental samples. These data were subtracted from experimental values. Perchlorate was not detected in plankton filtered from the unamended

control aquaria (0 ppm). The detection limit for these analyses was approximately  $0.032 \mu\text{g g}^{-1}$  wet tissue. Similarly to fish data,  $\text{ClO}_4^-$  concentrations in plankton were nearly 10-fold higher in the 10 ppm aquaria than in the 1 ppm aquaria. Plankton  $\text{ClO}_4^-$  levels in the propellant-containing aquaria were higher still. Concentrations in general were similar to those noted in fish tissue.

## 4. Discussion

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### 4.1. Aquatic Studies

Perchlorate amendments had little impact on photosynthesis (primary production) or bacterial production (secondary production) in either freshwater samples or marine waters. However, of the three light levels used for the photosynthetic measurements, one each for freshwater and seawater exhibited a significant decrease in rate in the presence of 1000-ppm  $\text{ClO}_4^-$  compared to the 0-ppm and salt (KCl) controls indicating that  $\text{ClO}_4^-$  adversely affected primary production. The data were confounded by the effect of the salt alone on photosynthetic activity, which was most likely due to trace element effects common in photosynthetic measurements. However, decreases in rates relative to the salt control indicated the potential impact of  $\text{ClO}_4^-$  on phytoplankton. This effect was only significant at the 1000-ppm level, which is quite high and probably greatly exceeds levels that might occur in coastal aquatic habitats unless many small pieces of SRM fuel fall into a small water body with restricted circulation. The dissolution of SRM fuel in the fish aquaria revealed concentrations less than 35 ppm after 50 days in a relatively small volume of water. This concentration could be higher if the pieces of fuel are more numerous and the water volume is kept small. However, a larger piece of material has a smaller surface to volume ratio and would not release  $\text{ClO}_4^-$  at a rate directly proportional to weight alone. Therefore, the chances of achieving concentrations as high as 500-1000 ppm would be very remote.

Photosynthesis measurements were made using samples incubated in the presence of  $\text{ClO}_4^-$  for only one week. Previous studies have shown that  $\text{ClO}_4^-$  adversely affects marine microalgal activity (Stauber, 1998). It is certainly possible that photosynthesis might be inhibited at  $\text{ClO}_4^-$  concentrations less than 1000 ppm if samples could have been incubated for extended periods of time. This was not possible since the microbial communities within flasks displayed a sharp decrease in activity after the one-week incubation. However, the time frame of this experiment seems reasonable considering the fact that SRM fuel deposited in coastal waters would usually be subjected to continuous water currents that would move both  $\text{ClO}_4^-$  and plankton cells away from the dissolving fuel over time. Therefore, long-term exposure would probably be rare. This situation differs greatly from one in which  $\text{ClO}_4^-$  is entering a small water body for many years via polluted groundwater.

It was not entirely clear if changing light levels influenced the effect of  $\text{ClO}_4^-$  on phytoplankton activity. However, the lower light levels yielded the highest rates of activity and appeared to be most affected by the presence of  $\text{ClO}_4^-$ . One might expect that cells in full light would be more stressed than in lower light and also might be more susceptible to chemical stressors. However, this was not observed. It is disheartening that significant effects of  $\text{ClO}_4^-$  occurred at the lower light levels that generally support the bulk of planktonic primary production in aquatic environments, suggesting the effect of  $\text{ClO}_4^-$  may be more pronounced in regions supporting most of the primary production.

The day-one seawater samples incubated with 1000-ppm  $\text{ClO}_4^-$  did exhibit a significant decrease in bacterial production compared to both the unamended and salt controls. All of the  $\text{ClO}_4^-$ -containing samples decreased in activity after seven days, but none were statistically different from controls. However, it appears that  $\text{ClO}_4^-$ , at least at high levels, has an adverse effect on bacterial production as well as primary production, which could lead to additional ecosystem stress since both production processes supply energy and materials to following trophic levels. It should be borne in mind that these effects, at least in the short term, only occur at unusually high levels of  $\text{ClO}_4^-$ .

## 4.2. Depositional Environments

Perchlorate had little to no effect on decomposition rates (respiration) in the aquatic depositional environments studied. Increased  $\text{ClO}_4^-$  did not depress carbon dioxide production or methanogenesis in freshwater or marine sediments or in bog peat. There tended to be a slight decrease in respiration in marine sediment with increasing concentrations of  $\text{ClO}_4^-$ , but this decrease was not statistically significant. Respiration in freshwater sediment was actually stimulated by the addition of  $\text{ClO}_4^-$ , but the KCl controls also exhibited this increase indicating that trace elements within these salt preparations were somehow stimulating bacterial activity.

The lack of perchlorate loss in any of the sediment or peat incubations indicated that  $\text{ClO}_4^-$  was not being used as a terminal electron acceptor (TEA) by anaerobic bacteria. It has been demonstrated conclusively that some anaerobic bacteria will utilize  $\text{ClO}_4^-$  and chlorate as TEAs in lieu of other TEAs that have a lower reduction potential (Attaway and Smith, 1993; Coates et al., 1999; Herman and Frankenberger, 1999; Herman and Frankenberger, 1998; Kim and Logan, 2001; Wallace et al., 1998). The terminal step in the reduction of  $\text{ClO}_4^-$  by bacteria is the dismutation of the chlorite ion to chloride ion and oxygen gas. Hence, the anaerobic use of  $\text{ClO}_4^-$  adds  $\text{O}_2$  that can stimulate the degradation of organic matter enhancing sediment respiration (Logan et al., 2001). Perchlorate is not utilized by aerobic bacteria and persists in oxic environments (Logan, 1998; Logan et al., 2001). Since  $\text{ClO}_4^-$  has a relatively high reduction potential, we expected it to be a preferred TEA in both methanogenic and sulfate-reducing sediments such as freshwater peat and marine sediments, respectively. However, we noted no evidence for the use of  $\text{ClO}_4^-$  by bacteria in any of the samples. Anaerobic bacteria that respire  $\text{ClO}_4^-$  are rather diverse, but the trait is not widespread throughout the microbial world (Coates et al., 1999) and tends to be restricted to sites with chronic  $\text{ClO}_4^-$  exposure where bacteria have probably become adapted to its presence. That is not the case in the sediments that were used here and ambient microorganisms did not have the constitutive ability to utilize  $\text{ClO}_4^-$ . This is probably true for most natural habitats that surround launch sites, so these data suggest that  $\text{ClO}_4^-$  will not be consumed as it enters anoxic sediment unless  $\text{ClO}_4^-$  is present for extended periods of time, i.e., several months to years.

The soil samples studied exhibited a statistically significant decline in respiration in the presence of  $\text{ClO}_4^-$ , especially at the 100 and 1000-ppm levels. Salt at 1000 ppm also depressed respiration in soils, but not as much as that seen in the presence of even 100 ppm  $\text{ClO}_4^-$ . These experiments were conducted in soil slurries, i.e., saturated soils. It would have been difficult to conduct the study in such a way as to employ unsaturated soils. The distribution of  $\text{ClO}_4^-$  in unsaturated

soils would be extremely patchy, and solid propellant would have dissolved only when soils were wet. Hence, effects of deposited SRM fuel on soils would be restricted to soils directly in contact or near to pieces of fuel. However, the data here demonstrate that soils are susceptible to adverse impacts from SRM fuel. A depression in respiration would decrease the rate that nutrients are recycled in soils leading to a decrease in plant growth beyond what might occur simply due to any deleterious effects of the uptake of  $\text{ClO}_4^-$  directly by plants (Smith et al., 2001). Soils also did not consume  $\text{ClO}_4^-$  over time, but this was not surprising since incubations were conducted aerobically and  $\text{ClO}_4^-$ -reducing bacteria only respire  $\text{ClO}_4^-$  in the absence of oxygen.

### 4.3. Threespine Stickleback

The  $\text{ClO}_4^-$  did not appear to have any significant effect on fish for the duration of the experiment. Although a significant number of fish died during the experiment, this mortality is normal for sticklebacks in captivity and was not affected by the presence of  $\text{ClO}_4^-$  or the SRM fuel. We did not observe any abnormalities in the adult fish. However, males were exposed to  $\text{ClO}_4^-$  for only a few weeks and they were mature when added to aquaria. Fry were still young when this report was completed so it is too early to determine if any physical abnormalities will appear due to  $\text{ClO}_4^-$  exposure. It is likely that fry reared in the presence of  $\text{ClO}_4^-$  until maturity will exhibit some noticeable morphological or behavioral abnormalities, but this analysis will need to wait until the fry are grown. We also did not observe any unusual effects on the rate or success of stickleback mating or offspring hatching in  $\text{ClO}_4^-$ -containing aquaria. However, we still have hundreds of hours of videotape to analyze.

Higher concentrations than those used here in aquaria have been shown to cause abnormalities in animals. Exposure of frog eggs to ~200 ppm  $\text{ClO}_4^-$  for 70 days caused a 50% decrease in hatching success (Goleman et al., 2002b). Exposure to 500 ppm displayed a similar result in only five days. No developmental abnormalities were seen at 200 ppm, but metamorphosis was affected. Rates of water filtration by daphnia were depressed by exposure to >100 ppm  $\text{ClO}_4^-$ , but  $\text{ClO}_4^-$  concentrations of 2-20 ppm actually stimulated activity (Riv'er and Flerov, 1975). Sand dollar metamorphosis was impeded by  $\text{ClO}_4^-$  concentrations of 140 ppm (Saito et al., 1998). Terrestrial animals are also affected by  $\text{ClO}_4^-$  ingestion. Reproduction in rats fed  $\text{ClO}_4^-$  at up to 30 mg  $\text{kg}^{-1}$  day<sup>-1</sup> in drinking water was not affected, but thyroid glands were enlarged when exposed to  $\text{ClO}_4^-$  levels down to 30 mg  $\text{kg}^{-1}$  day<sup>-1</sup> (York et al., 2001).

The concentrations of  $\text{ClO}_4^-$  used here were intended to bracket levels expected from small pieces of propellant in relatively small confined water bodies. It is unlikely that higher concentrations would be sustained from falling debris. Obviously, if higher levels are encountered it is likely that effects would be more severe than those reported here. We may still observe abnormalities as data are examined further and fry are allowed to mature. The accumulation of  $\text{ClO}_4^-$  in fish and plankton (discussed below) is further evidence that fish may experience adverse effects due to  $\text{ClO}_4^-$  exposure during growth in the next several weeks.

### 4.4. Perchlorate Bioaccumulation

Both fish and plankton accumulated  $\text{ClO}_4^-$  to fairly high levels. The higher the concentration of  $\text{ClO}_4^-$  in the aquaria, the higher the tissue  $\text{ClO}_4^-$  level, and the magnitude of the increase corresponded well with the exposure concentration. Although  $\text{ClO}_4^-$  concentrations reached high levels in biota, it was still too low to significantly affect the aqueous concentrations of  $\text{ClO}_4^-$  in the aquaria. Hence, the dissolved  $\text{ClO}_4^-$  levels in aquaria did not decrease significantly over time despite the accumulation of  $\text{ClO}_4^-$  in biota tissue. It was not possible to ascertain whether  $\text{ClO}_4^-$  in fish was due to ingestion of plankton biota that was rich in  $\text{ClO}_4^-$  or due to exposure to dissolved  $\text{ClO}_4^-$ . Fish were fed brine shrimp that were not exposed to  $\text{ClO}_4^-$ , but it is possible that fish also consumed algae or other biota in aquaria. Nonetheless, it is clear that biota are capable of accumulating  $\text{ClO}_4^-$  and that this material can be passed onto succeeding trophic



levels. Smith et al. (2001) noted that aquatic organisms exposed to  $\text{ClO}_4^-$ -contaminated shallow groundwaters near an ammunition factory displayed elevated levels of  $\text{ClO}_4^-$  in tissues. Plants contained the highest levels at 0.5-5000 ppm, while insects, fish, and frogs exhibited up to 2, 0.2 and 0.2 ppm, respectively. They also noted that mammals in the area were contaminated with  $\text{ClO}_4^-$  indicating that  $\text{ClO}_4^-$  was passed to succeeding trophic levels since these animals were not exposed directly to  $\text{ClO}_4^-$ . Aquatic vascular plants can accumulate nearly 1000 ppm  $\text{ClO}_4^-$  even when exposed to  $\text{ClO}_4^-$  for durations of less than 11 days (Susarla et al., 2000). However, different species contained widely different  $\text{ClO}_4^-$  levels ranging from 0.001 to 981 ppm. Even dormant woody plants growing in  $\text{ClO}_4^-$ -contaminated water accumulate from 5-300 ppm  $\text{ClO}_4^-$  (Urbansky et al., 2000). In our study, both fish and a mixture of plankton (algae, zooplankton and bacteria) contained similar concentrations of  $\text{ClO}_4^-$  in tissues on a wet weight basis and this was directly correlated with the exposure concentration. Fish might have surpassed the planktonic  $\text{ClO}_4^-$  concentrations if they had remained in aquaria longer than a few weeks.

## 5. Conclusions

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- Primary production was not affected adversely by perchlorate exposure except at perchlorate concentrations up to 1000 ppm. Although this is a very high concentration of perchlorate, deleterious effects tended to occur at light levels that are most conducive to rapid photosynthesis. Hence, it is possible that a more chronic exposure that might occur after several weeks or months of propellant dissolution in a hydrologically restricted region could cause serious impact on plankton production. Since propellant deposition following a launch failure would occur primarily in coastal marine waters that are physically active, it is unlikely that adverse effects would be noted.
- Secondary, or bacteria production was also only affected at very high concentrations of perchlorate and it is unlikely that inadvertent deposition of propellant into aquatic systems would negatively impact bacterial activities in the water column.
- At levels up to 1000 ppm, perchlorate did not negatively impact respiration activity in freshwater, marine, or wetland sediments. It is possible that perchlorate concentrations as high as 1000 ppm could be achieved in depositional environments since pieces of propellant could be buried and diffusion of material restricted due to tortuosity. However, these high concentrations would be restricted to sediments immediately adjacent to propellant and in most likelihood would not affect large areas. Although the reduction of perchlorate was not observed, it is likely that bacteria adjacent to propellant particles would eventually be able to respire perchlorate and effectively remove it, at least within anaerobic environments. Therefore, the burial of propellant in sediments would be ideal for attenuating any deleterious effects of propellant on coastal biota.
- In contrast to sediments, respiration by upland soils was significantly affected by the presence of perchlorate and this effect was noted for concentrations as low as 100 ppm. Surficial soils rarely become anaerobic unless they are flooded for extended periods of time. Therefore, perchlorate would not be removed by bacterial respiration and would persist in the soil environment where it could adversely affect the ability of soils to support plant growth. Since plants also tend to bioaccumulate perchlorate, the effect of propellant deposition on soils may extend beyond plant growth alone. Fortunately, it is expected that the bulk of propellant debris will be deposited over oceanic regions that do not seem to be as sensitive to perchlorate as soils.
- Concentrations of perchlorate expected to occur in relatively small bodies of water following the deposition of propellant debris did not affect the mating or rearing activities of the threespine stickleback. Perchlorate also did not affect the mortality of fry.
- Microbial (algal and bacterial) and fish tissues accumulated perchlorate to high levels and the magnitude of accumulation corresponded well to exposure levels. Therefore, it is likely that even exposures to relatively low concentrations of perchlorate (<20 ppm) will result in the bioaccumulation of perchlorate throughout the food web.

## 6. References

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- Attaway, H. and Smith, M., 1993. Reduction of perchlorate by an anaerobic enrichment culture. *J. Ind. Microbiol.*, 12: 408-412.
- Brechner, R.J., Parkhurst, G.D., Humble, W.O., Brown, M.B. and Herman, W.H., 2000. Ammonium Perchlorate Contamination of Colorado River Drinking Water is Associated With Abnormal Thyroid Function in Newborns in Arizona. *Journal of Occupational and Environmental Medicine*, 42: 777-782.
- Coates, J.D., Michaelidou, U., Bruce, R.A., OConnor, S.M., Crespi, J.N. and Achenbach, L.A., 1999. Ubiquity and diversity of dissimilatory (Per)chlorate-reducing bacteria. *Appl Environ Microbiol*, 65: 5234-5241.
- Ellington, J.J., Wolfe, N.L., Garrison, A.W., Evans, J.J., Avants, J.K. and Teng, Q., 2001. Determination of Perchlorate in Tobacco Plants and Tobacco Products. *Environmental Science & Technology*, 35: 3213-3218.
- Fitzwater, S.E., Knauer, G.A. and Martin, J.H., 1982. Metal contamination and its effects on primary production measurements. *Limnol. Oceanogr.*, 27: 544-551.
- Goleman, W.L., Carr, J.A. and Anderson, T.A., 2002a. Environmentally Relevant Concentrations of Ammonium Perchlorate Inhibit Thyroid Function and Alter Sex Ratios in Developing *Xenopus laevis*. *ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY*, 21: 590-597.
- Goleman, W.L., Urquidi, L.J., Anderson, T.A., Smith, E.E., Kendall, R.J. and Carr, J.A., 2002b. Environmentally Relevant Concentrations of Ammonium Perchlorate Inhibit Development and Metamorphosis In *Xenopus laevis*. *ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY*, 21: 424-430.
- Harper, M.D. and Hines, M.E., submitted. Carbonyl sulfide uptake by boreal forest soil. *Atmos. Environ.*
- Herman, D.C. and Frankenberger, W.T., Jr., 1999. Bacterial Reduction of Perchlorate and Nitrate in Water. *Journal of Environmental Quality*, 28: 1018-1024.
- Herman, D.C. and Frankenberger, W.T.J., 1998. Microbial-Mediated reduction of perchlorate in groundwater. *Journal of Environmental Quality*, 27: 750-754.
- Hines, M.E., Duddleston, K.N. and Kiene, R.P., 2001. Carbon flow to acetate and C<sub>1</sub> compounds in northern wetlands. *Geophys. Res. Lett.*, 28: 4251-4254.
- Hines, M.E., Duddleston, K.N., Reich, R.N. and Kiene, R.P., submitted. Inhibition of the anaerobic decomposition of dimethylsulfide in bog peat. *Environ. Microbiol.*
- Kim, K. and Logan, B.E., 2001. Microbial reduction of perchlorate in pure and mixed culture packed-bed bioreactors. *Water Research*, 35: 3071-3076.
- Kirchman, D., 2001. Measuring bacterial biomass production and growth rates from leucine incorporation in natural aquatic environments. In: J.H. Paul (Editor), *Methods In Microbiology*, Vol. *METHODS IN MICROBIOLOGY*. Academic Press Inc, 525 B Street, Suite 1900, San Diego, CA 92101-4495, USA, pp. 227-237.
- Lamm, S.H., Braverman, L.E., Li, F.X., Richman, K., Pino, S. and Howearth, G., 1999. Thyroid health status of ammonium perchlorate workers: A cross-sectional occupational health study. *Journal of Occupational and Environmental Medicine*, 41: 248-260.

- Logan, B.E., 1998. A Review of Chlorate- and Perchlorate-Respiring Microorganisms. *Bioremediation Journal*, 2: 69-79.
- Logan, B.E., Zhang, H.S., Mulvaney, P., Milner, M.G., Head, I.M. and Unz, R.F., 2001. Kinetics of perchlorate- and chlorate-respiring bacteria. *Appl Environ Microbiol*, 67: 2499-2506.
- Manzon, R.G., Eales, J.G. and Youson, J.H., 1998. Blocking of KClO<sub>3</sub>-Induced Metamorphosis in Premetamorphic Sea Lampreys by Exogenous Thyroid Hormones (TH); Effects of KClO<sub>3</sub> and TH on Serum TH Concentrations and Intestinal Thyroxine Outer-Ring Deiodination. *General and Comparative Endocrinology*, 112: 54-62.
- Manzon, R.G., Holmes, J.A. and Youson, J.H., 2001. Variable effects of goitrogens in inducing precocious metamorphosis in sea lampreys (*Petromyzon marinus*). *Journal of Experimental Zoology*, 289: 290-303.
- Richardson, L.L., 1991. Comparison of <sup>14</sup>C primary production determinations made by different laboratories. *Mar. Ecol.-Progr. Ser.*, 72: 189-201.
- Riv'er, I.K. and Flerov, B.A., 1975. (An experimental study of the toxic effects of phenol, ammonium perchlorate and polychlorpinene on *Daphnia pulex* (De Geer)). Nauka, Leningrad (USSR).
- Saito, M., Seki, M., Amemiya, S., Yamasu, K., Suyemitsu, T. and Ishihara, K., 1998. Induction of metamorphosis in the sand dollar *Peronella japonica* by thyroid hormones. *Development Growth & Differentiation*, 40: 307-312.
- Smith, P.N., Theodorakis, C.W., Anderson, T.A. and Kendall, R.J., 2001. Preliminary Assessment of Perchlorate in Ecological Receptors at the Longhorn Army Ammunition Plant (LHAAP), Karnack, Texas. *Ecotoxicology*, 10: 305-313.
- Stauber, J.L., 1998. Toxicity of chlorate to marine microalgae. *Aquatic Toxicology*, 41: 213-227.
- Susarla, S., Bacchus, S.T., Harvey, G. and McCutcheon, S.C., 2000. Phytotransformations of perchlorate contaminated waters. *Environmental Technology*, 21: 1055-1065.
- Urbansky, E.T., Magnuson, M.L., Kelty, C.A. and Brown, S.K., 2000. Perchlorate uptake by salt cedar (*Tamarix ramosissima*) in the Las Vegas Wash riparian ecosystem. *Science of the Total Environment*, 256: 227-232.
- Vijayalakshmi, K. and Motlag, D.B., 1992. Biochemical changes in mitochondria during chronic perchlorate toxicity. *Journal of Environmental Biology*, 13: 149-158.
- Wallace, W., Beshear, S., Williams, D., Hospadar, S. and Owens, M., 1998. Perchlorate reduction by a mixed culture in an up-flow anaerobic fixed bed reactor. *Journal of Industrial Microbiology & Biotechnology*, 20: 126-131.
- York, R.G., Brown, W.R., Girard, M.F. and Dollarhide, J.S., 2001. Two-Generation Reproduction Study of Ammonium Perchlorate in Drinking Water in Rats Evaluates Thyroid Toxicity. *International Journal of Toxicology*, 20: 183-197.