

EFFECTS OF TRICLOSAN ON A DETRITAL-BASED, AQUATIC FOOD WEB

by

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Abstract

The effect of triclosan, a commonly used antimicrobial agent, on various components of a freshwater food web was investigated. A mesocosm experiment was conducted over an 8 week long period, with six treatments comprising a gradient of triclosan concentrations (control, 2.3 $\mu\text{g L}^{-1}$, 11.5 $\mu\text{g L}^{-1}$, 23.0 $\mu\text{g L}^{-1}$, 115.0 $\mu\text{g L}^{-1}$ and 230.0 $\mu\text{g L}^{-1}$). These values were based on the maximum environmental concentration detected in a 2000 survey of streams in the United States, which was 2.3 $\mu\text{g L}^{-1}$. It was found that triclosan had a significantly negative impact on algal growth and leaf decomposition rate. No significant relationship was found between exposure to TCS and microbial metabolism. Similarly, no significant relationship was found between TCS exposure and caddisfly (*Lepidostoma unicolor*) larval growth and development despite the demonstrated reduction of leaf decomposition by microorganisms. As *L. unicolor* is a detritivore, the breakdown of organic matter by microbes serves as an important food source. It is possible that any negative effects of TCS on *L. unicolor*, either directly acting on the larvae or indirectly through their food source, were compensated for by the abundance of food provided. The results from this study suggest that TCS has the potential to influence aquatic ecosystem functions, particularly those involving basic trophic levels, at sufficiently high concentrations. However, there may be subtler effects of TCS not detected here, such as changes in microbial population densities and composition, that may have long-term implications.

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INTRODUCTION

Pharmaceuticals and personal care products (PPCPs) have become ubiquitous in the environment due to increasing use and unregulated discharge. PPCPs comprise a large number of compounds with many potential origins, from prescription or non-prescription medications used in human and in veterinary applications to detergents, hormones, fungicides, and disinfectants used domestically, industrially, or agriculturally (Kolpin *et al.*, 2002). More than 80 compounds, pharmaceuticals, and drug metabolites have been detected in the aquatic environment in investigations carried out in Australia, Europe, and North America (Heberer, 2002; Ying & Kookana, 2007). These surveys have only included a small subset of the many compounds used by humans and merely represent a starting point for investigations on the transport of organic wastewater contaminants in water bodies (Kolpin *et al.*, 2002). As a result, there are concerns that PPCPs may negatively affect water quality and ecosystem and human health (Kolpin *et al.*, 2002).

Triclosan (TCS; $C_{12}H_7Cl_2O_2$) is an antimicrobial agent commonly used in consumer products, with a typical concentration of 0.1-0.3% in cosmetics (Sabaliunas *et al.*, 2003). In addition to personal care products, TCS is also found in acrylic, plastic, and textile items (Oliveira *et al.*, 2009). At least in the United States (US), TCS is among the most widely distributed environmental contaminants and was among the seven most frequently detected compounds in a national survey of streams (Kolpin *et al.*, 2002). In that survey, TCS was detected in 57.6% of streams sampled, with a maximum environmental concentration of $2.3 \mu\text{g L}^{-1}$ and a median concentration of $0.14 \mu\text{g L}^{-1}$ (Kolpin *et al.*, 2002). A survey of five rivers downstream from wastewater treatment plants (WWTP) in Australia detected TCS at concentrations up to 75 ng L^{-1} in surface waters (Ying & Kookana, 2007).

Typically, PPCPs, including TCS, are non-point pollutants that become components of domestic wastewater after disposal and enter the environment through the discharge of effluent from WWTPs and disposal of sludge on land (McAvoy *et al.*, 2002). The concentration and distribution of TCS in the aquatic environment depend on its usage by consumers, removal during wastewater treatment, partitioning through sorption and ionization and degradation in surface waters, particularly as a result of exposure to sunlight

(Sabaliunas *et al.*, 2003). Although biodegradation and sorption remove TCS from the aqueous phase during wastewater treatment, it is still found in low but detectable levels in effluent water (McAvoy *et al.*, 2002). Depending on the specific conditions, TCS is typically reduced by 96% using activated-sludge treatment and 58-86% using trickling-filter treatment, with final effluent values varying from $<0.5 \mu\text{g L}^{-1}$ for the former and 1.6 to $2.7 \mu\text{g L}^{-1}$ for the latter (McAvoy *et al.*, 2002). The removal rates for TCS in five selected waste water treatment plants in Australia were found to range between 72% and 93%, with aqueous concentrations of TCS ranging from 23 ng L^{-1} to 434 ng L^{-1} in effluents with a median concentration of 108 ng L^{-1} (Ying and Kookana, 2007).

The uses of TCS have been approved by the regulatory bodies responsible for the safety of consumer products in many countries, including Canada and the US (Oliveira *et al.*, 2009). However, after being commonly used for over 30 years, there is growing concern over TCS due to reported adverse effects on aquatic organisms (see Table 1). A majority of the work done on TCS has focused on short term effects, such as toxicity assays, on individual species over time periods of a few hours to several days. For instance, Orvos *et al.* (2002) found a half maximal effective concentration¹ (EC_{50}) of $62.5 \mu\text{g L}^{-1}$ for reduced biomass growth in *Lemna gibba* over an exposure period of 4 hours. TCS has also been found to be toxic to the phytoplankton *Dunna tertiolecta*, with an EC_{50} of $3.55 \mu\text{g L}^{-1}$ over a 96 hour test period (DeLorenzo and Fleming, 2008). These effects are of particular concern due to past research indicating that some of the earliest responses to anthropogenic stress in aquatic ecosystems include the loss of sensitive species or changes in the population structures of small, rapidly reproducing species with strong dispersal capabilities like phytoplankton (Schindler, 1987). TCS was found to be toxic to the freshwater invertebrate *Hyalella azteca* with a median lethal dose² (LC_{50}) of $0.2 \mu\text{g L}^{-1}$ over a period of 10 days (Dussault *et al.*, 2008). This may be of particular interest, because some benthic invertebrates are among the most sensitive 'higher' organisms to pollution stress, and they may serve as good indicators of environmental damage (Schindler, 1987).

In addition to toxic effects, TCS has been found to induce physiological and behavioural abnormalities. The effects of Oliveira *et al.* (2009) found that *Danio rerio* larvae

¹ EC_{50} is a commonly used measure of drug potency, giving the concentration of a compound that results in a response halfway between the baseline and the maximum

² LC_{50} is the dosage required to kill half the individuals of a tested population after a specified test duration

exposed to 0.5 mg L^{-1} of TCS for 96 hours had severe developmental deformities, including spine malformations and pericardial oedema. Similarly, adult rainbow trout were found to display sublethal effects of TCS at $4.3 \text{ } \mu\text{g L}^{-1}$, including loss of equilibrium, erratic swimming, and spinal curvature (Orvos *et al.*, 2002). With exposure to $2.3 \text{ } \mu\text{g L}^{-1}$, *Bufo americanus* tadpoles were found to experience earlier and higher rates of mortality (Smith and Burgett, 2005). At $0.23 \text{ } \mu\text{g L}^{-1}$, *Rana pipiens* tadpoles displayed reduced activity - lowered swimming and feeding rates (Fraker and Smith, 2004). As indicated by the sampling of existing research, TCS has a wide range of effects on organisms from different taxa and theoretically could affect all the components of an aquatic food web, from primary producers to primary consumers and so on.

Table 1. Brief summary of TCS effects on various organisms.

Organism	Minimum Concentration	Effects	Reference
<i>Anabaena flosaquae</i> (cyanobacteria)	EC50 (4 h): 0.97 µg L ⁻¹	Biomass growth rate reduction	Orvos <i>et al.</i> , 2002
<i>Bufo americanus</i> tadpoles	2.3 µg L ⁻¹	Earlier and higher mortality	Smith & Burgett, 2005
<i>Ceriodaphnia dubia</i> (water flea)	EC50 (48 h): 130 µg L ⁻¹	Increased mortality	Orvos <i>et al.</i> , 2002
<i>Chironomus tentans</i> (midge)	LC50 (10 d): 0.4 µg L ⁻¹	Toxicity	Dussault <i>et al.</i> , 2008
<i>Danio rerio</i> larvae (zebrafish)	0.5 mg/L (96 h)	Severe developmental effects; spine malformations, pericardial oedema, undersized	Oliveira <i>et al.</i> , 2009
<i>Danio rerio</i> adults	Seen at the various levels, but especially 0.9 mg L ⁻¹	Abnormal behaviors exhibited (erratic swimming, equilibrium loss, unusual operculum movement)	Oliveira <i>et al.</i> , 2009
<i>Daphnia magna</i> (water flea)	LOEC (21 days) 200 µg L ⁻¹	Reproduction reduced	Orvos <i>et al.</i> , 2002
<i>Dunna tertiolecta</i> (phytoplankton)	EC50 (96 h): 3.55 µg L ⁻¹	Toxicity	DeLorenzo & Fleming, 2008
<i>Hyaella azteca</i> (amphipod)	LC50 (10 d): 0.2 µg L ⁻¹	Toxicity	Dussault <i>et al.</i> , 2008
<i>Lemna gibba</i> (duckweed)	EC50 (4 h): 62.5 µg L ⁻¹	Biomass growth reduction	Orvos <i>et al.</i> , 2002
<i>Lepomis macrochirus</i> (Bluegill Sunfish)	LC50 (96 h): 370 µg L ⁻¹	Toxicity	Orvos <i>et al.</i> , 2002
<i>Navicula pelliculosa</i>	EC50 (4 h): 19.1 µg L ⁻¹	Biomass growth reduction	Orvos <i>et al.</i> , 2002

(diatom)				
<i>Oryzias latipes</i> (Killifish)	LC50 (24 h): 0.47 mg L ⁻¹	Toxicity		Kim <i>et al.</i> , 2009
<i>Palaemonetes pugio</i> (Grass Shrimp)	LC50 (96 h): 305 µg L ⁻¹	Toxicity		DeLorenzo <i>et al.</i> , 2007
<i>Pseudokirchneriella subcapitata</i> (cyanobacteria)	EC50 (4 h): 4.46 µg L ⁻¹	Biomass growth reduction		Orvos <i>et al.</i> , 2002
Rainbow trout fry	71.3 µg L ⁻¹	Statistically significant reduction in 35-d post hatch survival		Orvos <i>et al.</i> , 2002
Rainbow trout	4.3 µg L ⁻¹	Sublethal effects include loss of equilibrium, fish jaw locked open, erratic swimming, spinal curvature, and quiescence		Orvos <i>et al.</i> , 2002
<i>Rana pipiens</i> tadpoles	0.23 µg L ⁻¹	Reduced activity (moving through the water and feeding)		Fraker & Smith, 2004
<i>Scenedesmus subspicatus</i> (green alga)	LOEC (72 h): 1.2 µg L ⁻¹	Reduced biomass endpoint and growth rate endpoint		Orvos <i>et al.</i> , 2002
<i>Skeletonema costatum</i> (diatom)	EC50 (4 h): 66 µg L ⁻¹	Biomass growth reduction		Orvos <i>et al.</i> , 2002
<i>Tetrahymena pyriformis</i> (Protozoa)	EC50 (96 h): 0.21 µg L ⁻¹	Toxicity		Harada <i>et al.</i> , 2008
<i>Thamnocephalus platyurus</i> (freshwater crustacean)	LC50 (96 h): 0.60 mg L ⁻¹	Toxicity		Kim <i>et al.</i> , 2009
<i>Vibro fischeri</i> (bacteria)	EC50 (15 min): 0.52 µg L ⁻¹	Toxicity		Harada <i>et al.</i> , 2008
<i>Xenopus laevis</i> (South African Clawed Frog)	LC50 (96 h): 0.82 µg L ⁻¹	Toxicity		Harada <i>et al.</i> , 2008

At present, there is need for work on the potential long-term effects of TCS exposure at ecological levels beyond individual species. Previous researchers have recommended further study on the potential chronic and sublethal effects of TCS (DeLorenzo *et al.*, 2008; Kim *et al.*, 2009). Due to the consistent and unrestricted release of TCS into the environment and its potentially decades-long environmental half life (Miller *et al.*, 2008), non-target organisms may be exposed to low levels for their entire lives and over several generations (Daughton and Ternes, 1999; Chalew and Halden, 2009). The possibility of chronic effects on aquatic organisms is particularly important as effects could accumulate so slowly that major change remains undetected until a point of irreversibility is reached (Daughton and Ternes, 1999). Oliveira *et al.* (2009) proposed that continuous exposure to chemicals like TCS could result in unnatural adaptation or selection, with particular risk for algal communities located downstream of wastewater treatment plants. Dussault *et al.* (2008) also emphasized the need for chronic toxicity effects to be studied. One of the previous studies with the longest durations was conducted by Orvos *et al.* (2002) to determine the effect of TCS on *Daphnia magna* reproduction and survival over 21 days. Another relatively long-term study researched the effects of TCS exposure on *Oryzias latipes* reproduction (Ishibashi *et al.*, 2004). In a two part study, the hatchability and incubation period of fertilized eggs were investigated over a 14 day period while the reproduction of mating pairs was determined for a 21 day period (Ishibashi *et al.*, 2004).

Effects on a Detrital-Based Aquatic Food Web

Previous research has not directly investigated the impact of TCS on basic trophic levels, but evidence of negative effects on primary producers and decomposers has been found. As a result, it is possible that TCS may affect aquatic food webs, such as those including the detritivorous caddisfly *Lepidostoma unicolor*.

Primary productivity, such as biofilm development and riparian vegetation inputs, like leaf litter, serve as basic trophic levels and are vital to the ecology of most stream ecosystems (Going and Dudley, 2008; Harada *et al.*, 2008). Vannote *et al.* (1980) proposed that these energy sources, together with organic matter transport, storage, and usage by macroinvertebrates and geomorphic processes in the water bodies, are integral to the structure and function of aquatic ecosystems. Subsequent studies have supported this theory,

including work by Irons *et al.* (1988) that found that leaf litter was differentially preferred by the stream shredder *Hydatophylax variabilis* depending on its species and nutrient status. It was suggested that as a result, riparian vegetation may strongly influence detrital food webs in streams (Irons *et al.*, 1988). Biofilm consists of an aggregate of microorganisms which may include algae, bacteria, and fungi (O'Toole *et al.* 2000); it serves as a food source for *L. unicolor* larvae and other organisms. However, allochthonous sources of organic carbon, such as leaf litter, often exceed what is provided by autochthonous primary production in aquatic ecosystems (Wallace and Webster, 1996; Wallace *et al.*, 1997; Dodds and Cole, 2007). Additionally, although leaf litter is an important source of organic matter, it is not directly fed upon by many organisms and must first be broken down by microorganisms (Marks *et al.*, 2009). The processing of such allochthonous detritus is considered vital for energy flow and nutrient cycling in headwater streams (Wallace and Webster, 1996; Wallace *et al.*, 1997). In general, the processing of detritus in streams occurs in a three step process as described by Petersen and Cummins (1974). First, approximately 15% of the leaf litter mass is lost through leaching; in streams, these leached compounds are rapidly converted to biomass and carbon dioxide (CO₂). After leaching, colonization and conditioning by microorganisms takes place; the leaves are either broken down and converted to biomass through microbial growth or lost through microbial respiration. Following microbial colonization, which converts materials like cellulose into forms that can be assimilated by detritivores, animal processing and continued microbial action take up most of the remaining litter.

TCS is a broad-spectrum antimicrobial agent, bacteriostatic at low concentrations and bactericidal at higher concentrations (Russell, 2004), that inhibits fatty acid synthesis in bacteria, which is necessary for building cell membranes and reproduction (DeLorenzo *et al.*, 2008). TCS is effective against gram positive and gram negative bacteria and is also bacteriostatic for molds, fungi, and yeast (McAvoy *et al.*, 2002). Past research has demonstrated the negative impacts of TCS on microorganisms. Harada *et al.* (2008) found that the EC₅₀ (15 min) for toxicity in *Vibrio fischeri* was 0.52 µg L⁻¹. DeLorenzo & Fleming (2008) found that sufficient concentrations of TCS can result in a significant decrease in *Dunaliella tertiolecta* (phytoplankton) populations. These findings were similar to growth rate sensitivities exhibited by the fresh water algae *Scenedesmus subspicatus*. Being primary

producers, these organisms serve vital ecosystem functions, such as providing food, cycling nutrients, and producing oxygen. Most telling of this research is that growth rate effects were seen at ecologically relevant levels of TCS ($EC_{50} = 2.8 \mu\text{g L}^{-1}$ for *S. subspicatus*, while the maximum reported environmental concentration is $2.3 \mu\text{g L}^{-1}$). Previous work by Tabak *et al.* (2009) also found that TCS reduced the number of viable *Salmonella enterica* serovar Typhimurium cells in biofilm. Additionally, Orvos *et al.* (2002) found that TCS hindered the growth of *S. subspicatus* and *Anabaena flos-aquae*, but did not seem to kill cells.

Present Study

In keeping with the suggestions of past research, the work conducted here investigated whether long term exposure to TCS could affect various components of an aquatic food web, including leaf decomposition, biofilm development, microbial populations, and *L. unicolor* (caddisfly) development. By examining these variables, in particular the effect on primary producers and decomposition, it could be inferred whether or not TCS had the capacity to impact ecosystems on a whole through cascading effects. In order to understand how TCS may affect ecosystems overall, mesocosms that simulated a freshwater ecosystem were created and maintained for a period of eight weeks. Mesocosm experiments are considered useful for addressing chemical related problems with a larger scope than individual species assays (Schindler, 1987).

The previously demonstrated negative impacts of TCS on microorganisms and invertebrates suggest that increased concentrations of TCS should reduce biofilm development and rates of organic matter decomposition, if TCS does affect these factors. As a result of reduced microbial conversion of leaf litter with higher TCS concentrations, the growth rate of the detritivorous caddisfly *L. unicolor* might also decline through reduction of its food source. It was predicted that TCS would hinder biofilm development and leaf litter decomposition by interfering with microbial activity. Due to the direct physiological effects seen in other organisms, including many invertebrates, it was also possible that TCS had direct negative impacts on *L. unicolor*.

Test Consumer

L. unicolor (order Trichoptera: Lepidostomatidae) undergoes a four-stage life cycle

(egg, larva, pupa, and adult). Once the larvae hatch from egg masses laid in the water, they begin to build cases, which function in respiration and as ballast, camouflage, and protection from predators (Peckarsky *et al.*, 1990). Within the case, the larvae grow, continuing to remake or enlarge their protective shell several times before developing into pupae. The larvae and pupae of all caddisfly species are aquatic (Thorp and Covich, 1991). *L. unicolor* is found in lake and stream habitats ranging from British Columbia south to California and New Mexico and east to Quebec.

As benthic invertebrates, caddisflies play an important role in aquatic food webs and may be vulnerable due to the potential of sediments to act as a repository for anthropogenic contaminants such as TCS (Dussault *et al.*, 2008). Although the larvae are not typically predated upon due to their protective casings, the pupa and adult stages are important food sources for higher trophic levels, such as fish and amphibians (Thorp and Covich, 1991). In some streams, caddisflies make up a large proportion of the available plant biomass (Thorp and Covich, 1991).

Caddisflies are able to survive in a variety of water conditions, but prefer shallow, cool, well oxygenated waters. *L. unicolor* feed on algae and organic plant and animal materials that have settled to the bottom of the water body; in this way, they are important in processing this source of organic matter and transferring energy through the aquatic food web (Going and Dudley, 2008). Caddisflies have been identified as important biological monitoring species due to their abundance and diversity in streams, and varying tolerance levels for pollution (Thorp & Covich, 1991).

METHODS

Collection and Husbandry of Test Consumer

Approximately 225 *L. unicolor* larvae were collected from the Malcolm Knapp Research Forest near the Coast Mountains by Maple Ridge (Fig. 1) and transported back to facilities at the University of British Columbia (UBC) in plastic containers filled with natal pond water and placed in a cooler. The larvae were housed in a refrigerator for two weeks,

with alder leaves being added each week to provide food. After this period, 40 were euthanized and dried to establish an average mass for the initial sample collected and the others were placed in the prepared mesocosms.

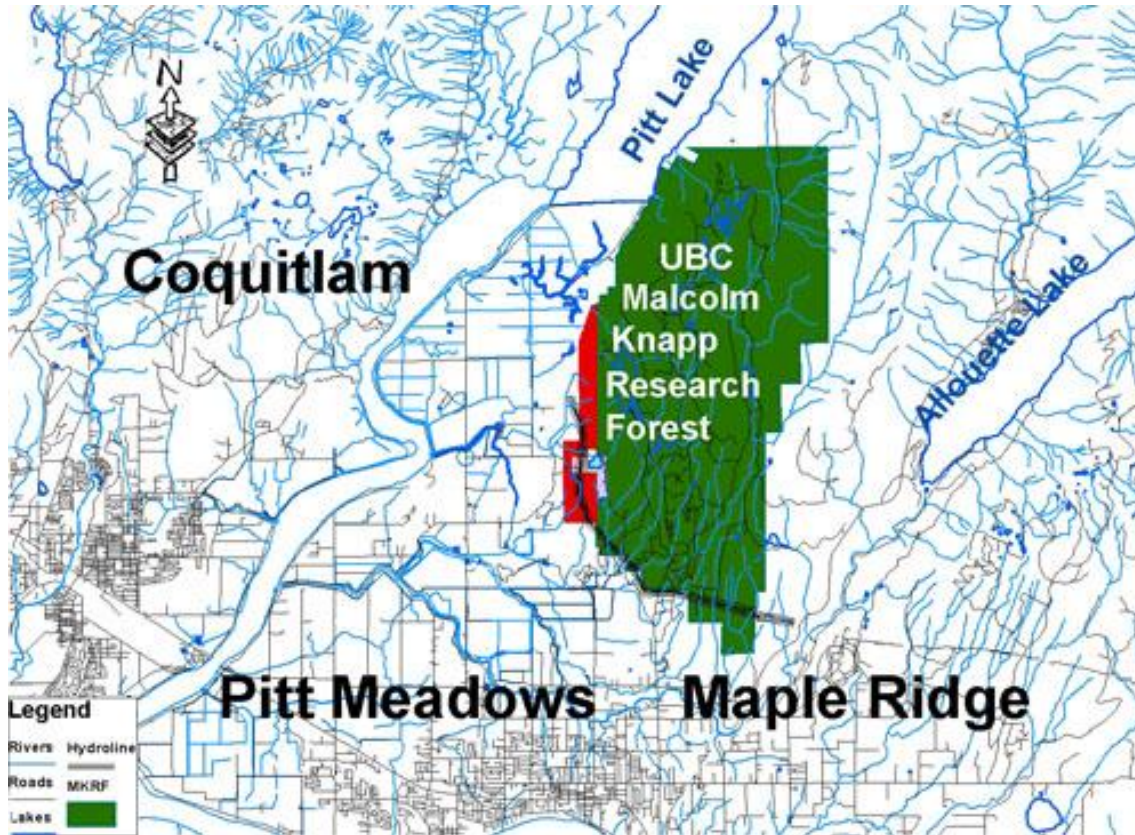


Figure 1. Map of the Malcolm Knapp Research Forest, where the *L. unicolor* larvae were collected (University of British Columbia, 2010).

Mesocosm Design

Prior to beginning the experiment, 20 L buckets (0.3 m in diameter and 0.5 m in height) were washed with Tergazyme©, filled with municipal tap water, and allowed to stand for a period of two weeks to allow any chemicals and/or toxins to leach out. The buckets were filled with 1 L of pond water to provide a colonizing community of zooplankton and phytoplankton and 9 L of municipal tap water. The pond water was filtered through a 63 µm sieve to prevent colonization by large predaceous invertebrates. Each bucket was given approximately 5.0 g of dried *Alnus rubra* leaf litter to provide substrate and nutrients to support periphyton development. The leaf litter had previously been soaked in water for two days and then dried in order to allow some of the organic matter to leach out. Two grow

lights were hung 85 cm above the buckets and placed on a 10:14 light-dark cycle to support primary production (Fig. 2). Because *L. unicolor* creates its casings using vegetation, Douglas-fir needles were included to provide the larvae with materials for case construction. Double distilled water was added as necessary to maintain a constant 10 L volume for the 8 week long duration of the experiment.

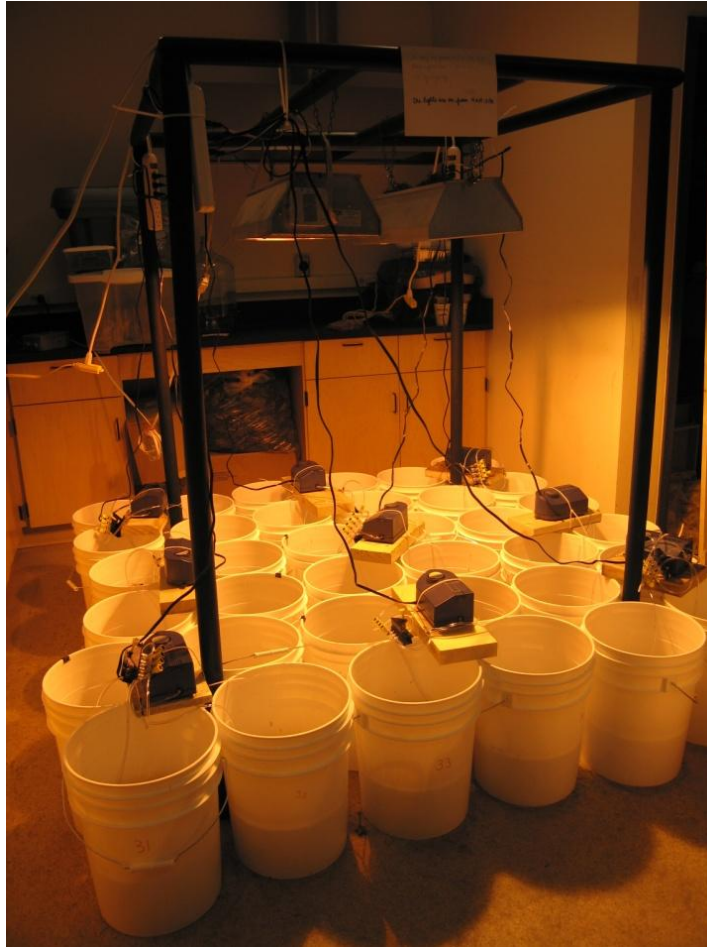


Figure 2. Experimental Setup. Shown here are the 36 mesocosms, with an aerator shared between every four buckets. The grow lights were suspended above on a steel frame.

Temperature loggers were placed in six randomly chosen buckets to record the temperatures of the mesocosms and to track any fluctuations with time. For each of the buckets, there was a daily cycle of temperature rise and decline associated with the light-dark cycle of the grow lights. There were slight variations between the average temperatures of the buckets (Table 2), with a minimum of 19.91 °C and a maximum of 20.95 °C. However, these differences should have been random with respect to treatments (Fig. 3).

Table 2. Average Temperatures for Buckets

Bucket	Average Temperature (°C)
2	20.49
6	20.21
15	20.94
20	20.38
25	19.91
27	20.95

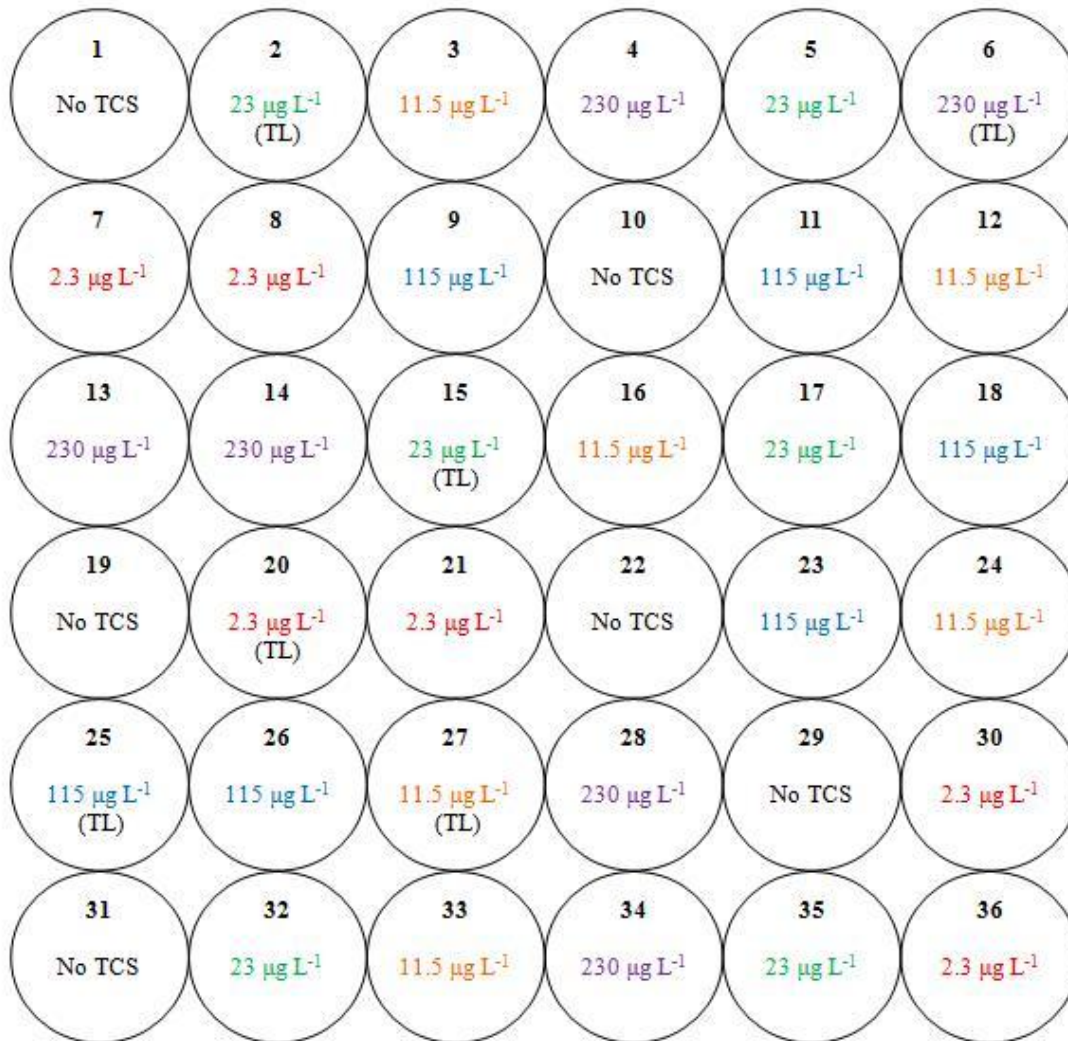


Figure 3. Experimental layout of TCS treatments (colour coding indicates 0x, 1x, 5x, 10x, 50x, 100x the maximum environmental concentration detected in US streams) and position of temperature loggers (TL).

The experiment consisted of six TCS concentration treatments (control, 2.3, 11.5, 23, 115, and 230 $\mu\text{g L}^{-1}$; Fig. 3). The TCS levels were chosen to create a gradient of 1x, 5x, 10x, 50x and 100x the maximum observed levels of TCS found in the environment by Kolpin *et al.* (2002). An aerator tube was placed in each of the buckets to maintain sufficiently high oxygen levels in the water; every four buckets had an aerator. Each treatment included six larvae and there were six replicates of each TCS concentration, for a total of 36 mesocosms. At the end of the experiment the larvae were euthanized. The developmental stages of the larvae were noted (larva, pre-pupa, pupa) and they were then dried to constant mass.

Biofilm development experiment

Two 5 cm x 5 cm ceramic tiles were included in each of the mesocosms, elevated on a petri dish to reduce the accessibility of the larvae, to measure the impact of TCS on biofilm development, in particular algae growth. One set of ceramic tiles was removed from the buckets midway through the experiment (4 weeks) and the second set was removed at the end (8 weeks). The tiles were sampled for chlorophyll-*a* (Chl-*a*). The periphyton was scraped off the tiles, rinsed into a holding container with distilled water, and then measured for Chl-*a* concentration. Each sample was filtered onto an un-ashed Whatman fine glass fibre filter, from which the Chl-*a* was extracted using 90% acetone. The samples were covered and refrigerated for 24 hours to ensure proper extraction and then measured for Chl-*a* using a Turner TD-700 Fluorometer. To correct for the presence of phaeophytin, a few drops of 1% hydrochloric acid (HCl) was added to each sample, allowed to sit for approximately 30 minutes in the dark, and then measured again in the fluorometer. The Chl-*a* concentrations used for results was the difference between these two values.

Decomposition experiment

2.0 g amounts of dried leaf litter were packaged in 250 μm mesh bags. As with the ceramic tiles, two bags were included in each mesocosm for measurement of leaf decomposition halfway through and at the end of the experiment. To ensure that the change in leaf mass was due to decomposition through microbial action, the mesh used was fine enough to exclude and prevent feeding by the caddisfly larvae. At 4 weeks and at 8 weeks, mesh bags were removed from the mesocosms. To measure the change in leaf mass, the

leaves were removed, rinsed and placed in a drying oven (60°C) for a minimum of 24 hours. The leaves were then weighed on a precision scale. Each sample of leaves was then ashed for 4 hours at 500°C to determine the ash-free dry mass (AFDM), which removed inorganic matter.

Microbial respiration experiment

6 weeks into the experiment, measurements of microbial respiration were taken. Alder leaves were removed from each of the buckets, and from these ten 17 mm disks were cut out. Both autotrophic and heterotrophic respiration were measured; in order to do this, 5 disks were placed into separately labeled tubes for each sample and then filled with distilled water. Periodically, the dissolved oxygen and temperature of the distilled water used to fill the tubes was measured. For each sample, the time was carefully recorded. The tubes used for measuring heterotrophic respiration were allowed to sit in the dark for approximately 24 hours at room temperature while the ones that also included autotrophic respiration were allowed to sit exposed to light over the same period of time. After incubation was done, a dissolved oxygen reading was taken for each sample by placing the oxygen probe directly into the tube. The leaf disks were then removed, placed into labeled aluminum tins, and placed in the drying oven (60°C). After 24 hours, the dry mass of these disks were determined. The disks were then ashed in the muffle furnace at 500°C for 2 hours to determine the AFDM of the disks.

Statistical analysis

Analyses were conducted using the SAS system (ver. 9.1, SAS Inc, Cary, NC). Repeated measures (2 sample periods) Analyses of Covariance (RM-ANCOVA) were conducted for the leaf decomposition and biofilm development data (concentration as a covariate, $\log(x+0.01)$ -transformed). This was done to see if the effect of a certain TCS treatment changed the variable tested - either leaf decomposition or biofilm development - over different lengths of time, as measurements were taken at the midway and end points. Analyses of Covariance (ANCOVA) were conducted for the caddisfly final mass and microbial respiration measures. This was appropriate as the data were only collected once over the duration of the experiment.

RESULTS

Algal Development

For both the midway and final measurements of biofilm development, there were significant negative relations between concentration of Chl-*a* and concentration of TCS (RM ANCOVA, $P = 0.013$, $F_{1,34} = 16.85$, Fig. 4). Between the control and the maximum TCS concentration treatment, there was a 56.16% difference in algal biomass at the 4 week sampling period and a 65.56% difference at the 8 week mark.

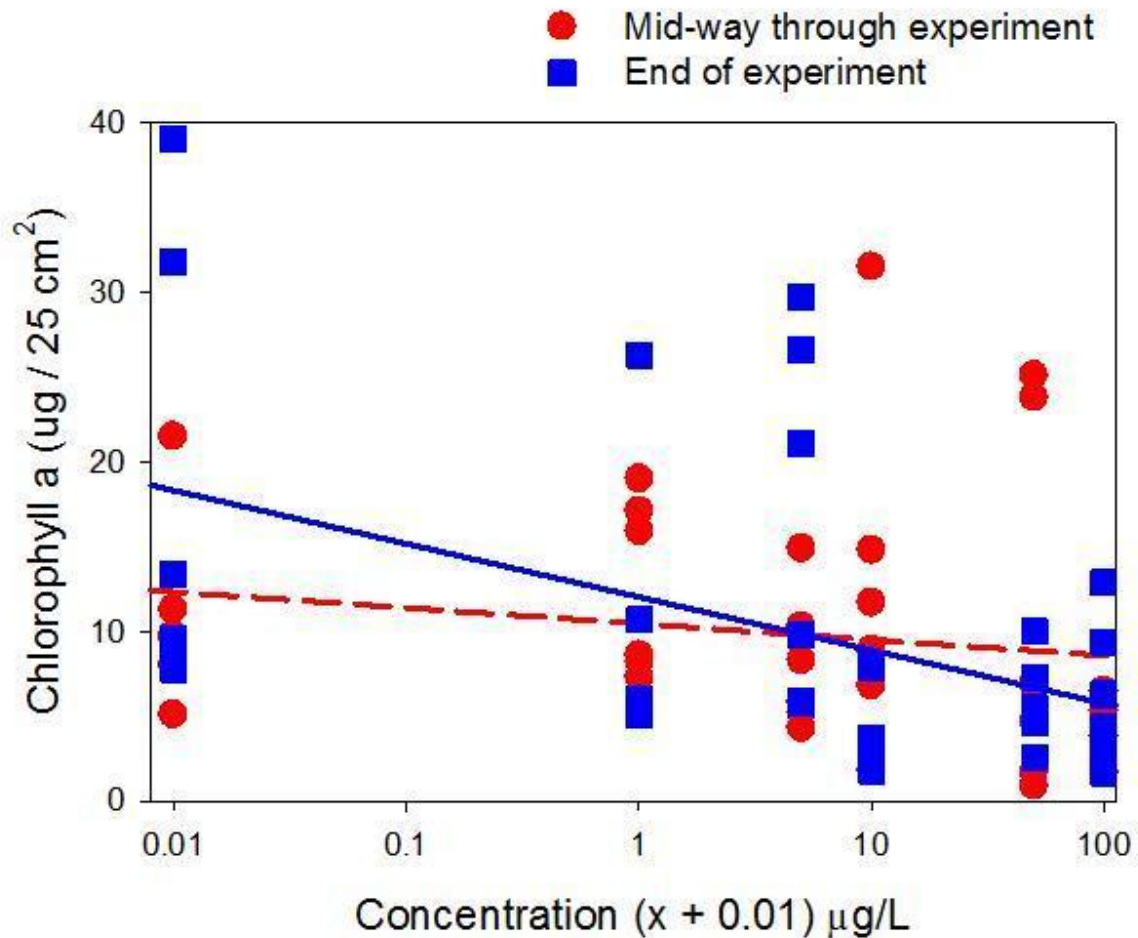


Figure 4. Biofilm development on ceramic tiles (measured as Chl-*a* concentrations) at different TCS concentrations on two sampling dates (4 weeks and 8 weeks).

Leaf Decomposition

Leaf decomposition was shown to have a statistically significant negative relation with increasing concentrations of TCS (RM-ANCOVA, $P < 0.0001$, $F_{1,34} = 19.75$, Fig. 5). The regressions for the midway and final data are near parallel (Fig. 4), indicating that relative decomposition rates remained steady for the different TCS treatments. Between the control and the maximum $230.0 \mu\text{L}^{-1}$ treatment, there was a 35.15% difference in leaf decomposition at the midway point of the experiment and a 16.49% difference at the end.

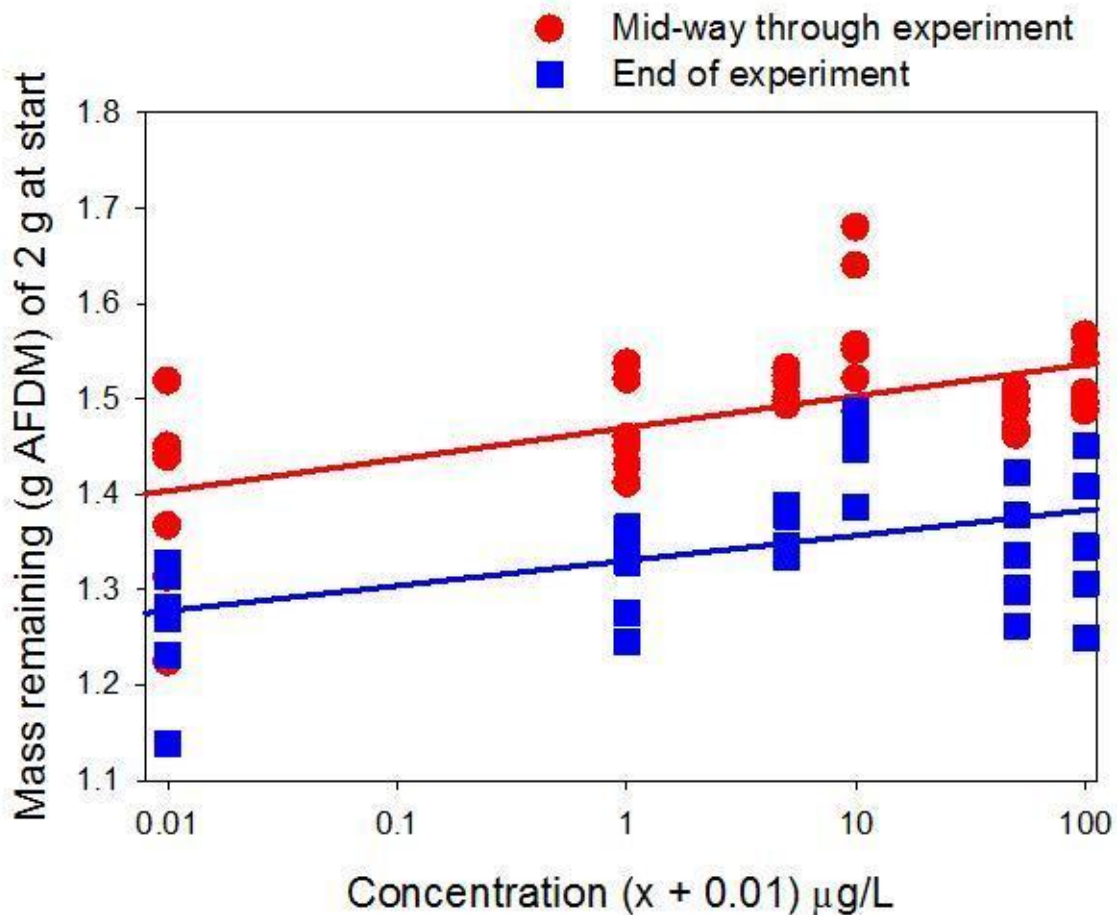


Figure 5. Leaf decomposition at different TCS concentrations. Figure shows the AFDM of alder leaves remaining from the original 2 g.

Caddisfly Growth

The average mass of the larvae pre-treatment, as determined by a sample of 40 larvae, was 2.079 g (standard deviation = 1.026). Across the treatments, the caddisfly larvae grew a considerable amount, with the final average masses being 8.406 g (control; standard error of

the mean = 0.266), 7.958 g (1 x; standard error of the mean = 0.380), 9.153 g (5x; standard error of the mean = 0.601), 8.599 g (10x; standard error of the mean = 0.255), 8.548 g (50x; standard error of the mean = 0.312), and 7.954 g (100x; standard error of the mean = 0.244). There was no statistically significant relation between the final mass of the caddisflies and the TCS treatment, with the average mass of the caddisflies being similar for all the treatments ($P = 0.533$; Fig. 6).

Over the course of the experiment, some of the caddisfly larvae reached the pre-pupae and pupae stages of development. These were removed for the average mass measurements, as caddisflies in the pre-pupae and pupae stages have sealed their cases and stopped feeding, resulting in a loss of mass. An examination of the caddisfly life stages – particularly the proportion that had started pupation - did not suggest that any of the TCS treatments resulted in faster or slower development.

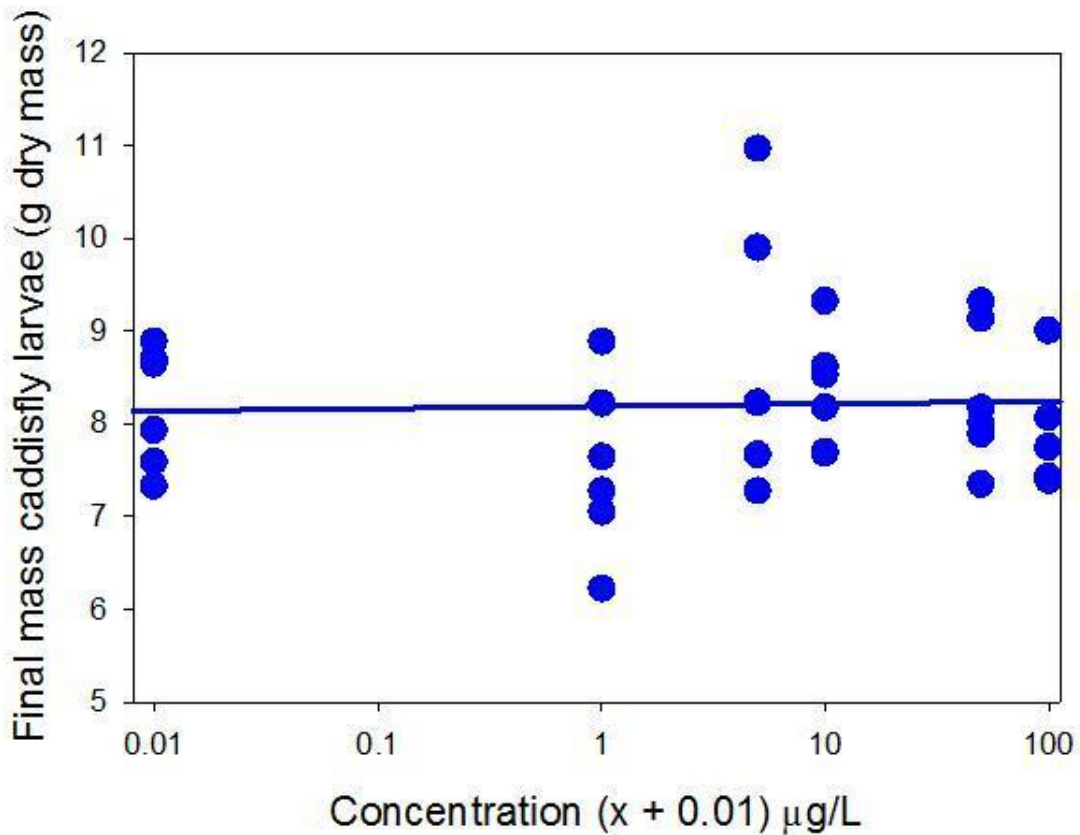


Figure 6. *Lepidostoma unicolor* (Trichoptera) larval masses at the end of the experiment with respect to TCS concentrations.

Microbial Respiration

For both autotrophic and heterotrophic respiration, there were no statistically significant relationships with increasing concentrations of TCS (Figs. 7a and 7b). A negative trend in oxygen consumption with increasing TCS concentrations was observed, however. Overall, the average oxygen consumption was higher for the samples exposed to light over the 24 hour test period.

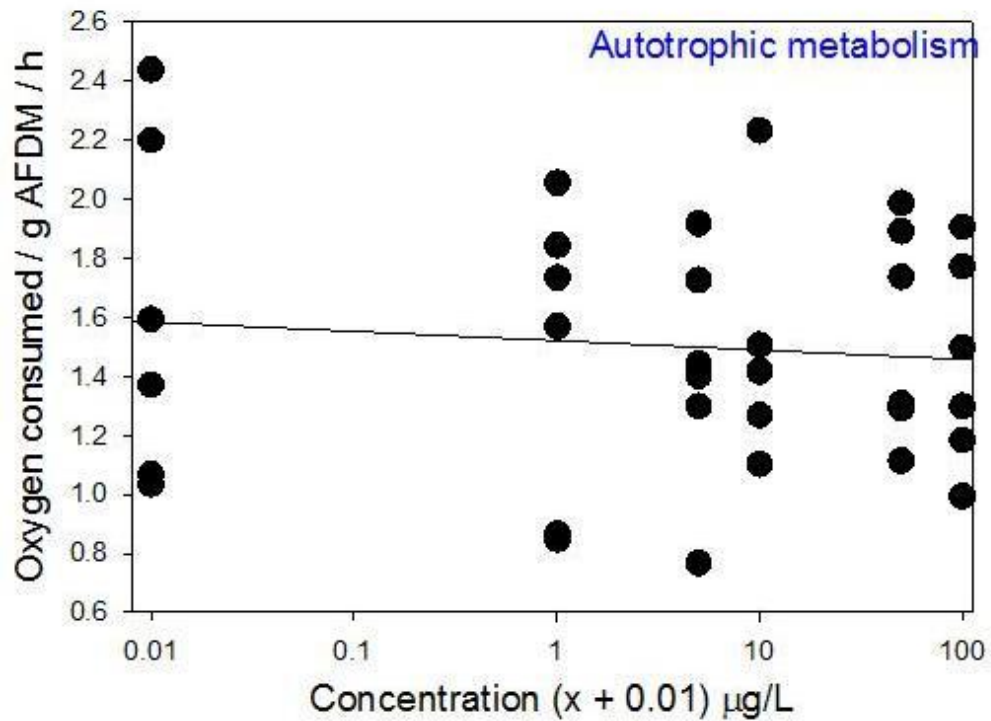


Figure 7a. Oxygen consumption by autotrophs and heterotrophs measured over a 24 hour period for different TCS concentrations. Samples were uncovered so that photosynthesis could occur with light exposure.

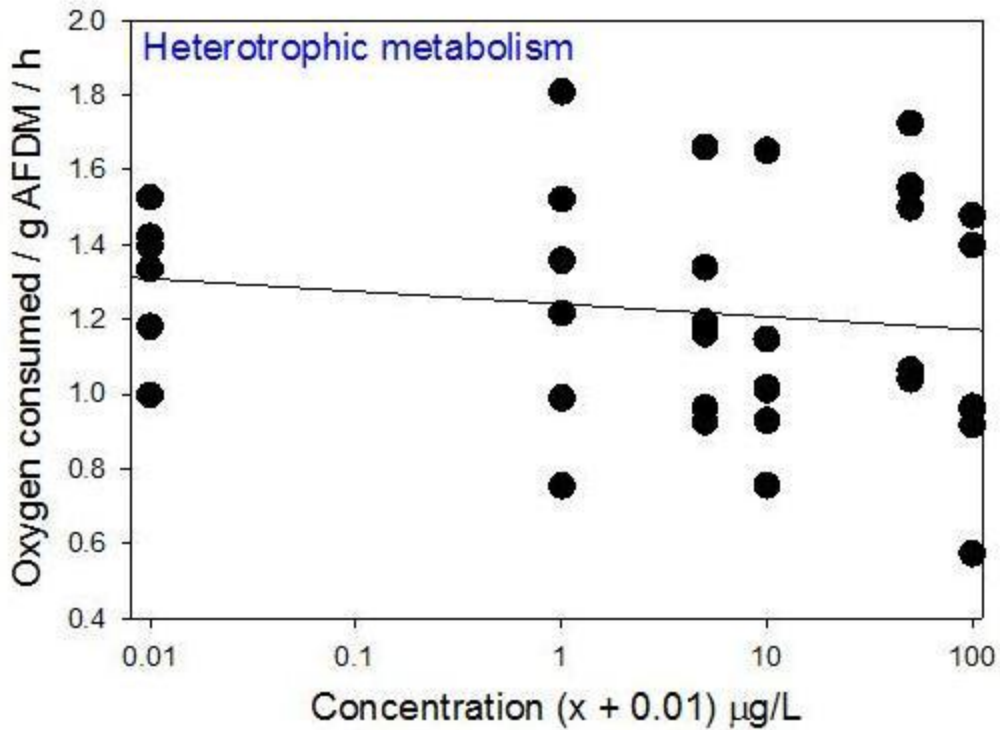


Figure 7b. Oxygen consumption by heterotrophs measured over a 24 hour period for different TCS concentrations. Samples were covered to prevent light exposure and thus primary productivity.

DISCUSSION

The experiment found that increasing levels of TCS had significantly negative effects on algal growth and leaf decomposition. For the algal growth, the regressions for the midway and final concentrations of Chl-*a* were not parallel. The experiment-wise mean Chl-*a* concentrations were relatively similar for both the midway and end points of the experiment, suggesting that additional time [in this case, four weeks] did not allow for much more biofilm development. It may be possible that there is a threshold for the growth of microorganisms, beyond which development is inhibited by TCS. This would be in keeping with the bacteriostatic properties of TCS. However, it is notable that there was much variation within each of the treatments. The results for leaf decomposition were also in keeping with the bacteriostatic qualities of TCS at low concentrations for bacteria and fungi, because the

decomposition of organic matter in freshwater ecosystems is mostly carried out by such organisms. Although uncertain in this case, at the higher concentrations TCS may have been bacteriocidal and killed microorganisms instead of simply inhibiting their growth and action. It is important to note that, while the TCS concentrations of the highest treatments may not be found in the environment, the negative relationship suggests that ecologically relevant concentrations may still result in reduced algal growth and litter decomposition to a lesser extent.

No statistically significant relationship was found between TCS exposure and microbial respiration, but a negative trend was observed. Interestingly, the autotrophic metabolism measurement resulted in higher oxygen consumption for almost all of the samples; this was counterintuitive, as autotrophic activity was expected to produce oxygen through photosynthesis. No explanation has been determined for this pattern.

Over all the TCS treatments, larval mass gain and development were very similar, and no statistically significant relationship was found. This suggests that TCS had no direct physiological effect on the larvae. However, because the caddisflies had an abundance of food available to them, it is possible that this may have counteracted any negative impacts of TCS on larval growth and development. In the natural environment, sufficiently high levels of TCS may reduce the amount of food available to caddisflies and other benthic invertebrates that feed on biofilm and degrading matter. It has been suggested that detritivores may compensate for lower-quality foods by consuming them at higher rates (Eggert and Wallace, 2007), so if the quality of the leaf litter was reduced by the presence of TCS the larvae may have simply eaten more of it. Additionally, TCS may still pose threats to aquatic ecosystems through bioaccumulation in tissue, which may lead to chemical biomagnification through the food chain in aquatic and terrestrial ecosystems (Chalew and Halden, 2009).

The mesocosms created for this experiment provided insights into the potential impacts of TCS in natural freshwater ecosystems. The significant declines in leaf litter decomposition and biofilm development with increasing levels of TCS provided evidence that TCS release into the environment may have significant implications for food webs and ecosystems, as they comprise the most basic trophic levels. The 56.16% and 65.56% reductions in algal development between the control and maximum TCS treatment ($230.0 \mu\text{g L}^{-1}$) at the midway and end points of the experiment suggest that sufficiently high

environmental concentrations of TCS could significantly reduce primary productivity within bodies of water. This was in keeping with the findings of Tatarazako *et al.* (2004) that TCS had a significant negative effect on algae. Besides a loss of or reduction in a food source for organisms, TCS may reduce the ability of an ecosystem to contain and remove environmental contaminants. It has been suggested that reduction in periphyton growth may result in further transport of contaminants in stream ecosystems, as the periphyton may remove some compounds from the water through uptake (Hill *et al.*, 2010). Due to this effect on primary producers, it may be predicted that ecosystem dynamics could be significantly altered if TCS was discharged into the environment at high levels.

It has been suggested that several generations of consumers are necessary in order to detect responses to changes in detritus (Wallace *et al.*, 1997), so the effects of TCS on natural ecosystems may be gradually building up. Although no statistically significant relation was found between microbial metabolism and TCS exposure, the negative trend suggested the potential of a relationship. If TCS has the ability to alter the composition or density of microbial populations, it may result in differential colonization of invertebrates on plant litter; there has been evidence suggesting that benthic invertebrates demonstrate a preference for non-sterile leaves over sterile leaves of the same species (Petersen and Cummins, 1974). Similarly, large particle feeders, including caddisflies, have been shown to prefer species of leaves that are easier and faster for microorganisms to process (Petersen and Cummins, 1974). This may be attributable to the nutritional dependence of detritivores on the microorganisms present on the litter more than on the substrate itself (Cummins, 1974). In addition to biological influences, leaf litter breakdown rates have also been shown to affect the physical and chemical properties of stream ecosystems (Clapcott and Barmuta, 2010). Further work should investigate whether the microbial communities that develop vary in species composition and abundance at different concentrations of TCS.

Although in this experiment the only test animals used were *L. unicolor*, the findings of this work could be applicable for many of the 1340 caddisfly species known in North America, as well as other freshwater detritivores (Thorp and Covich, 1991). Additionally, because the macroinvertebrate structure of areas is related to factors such as water chemistry (Maltchick *et al.*, 2009), researching the effect of TCS on *L. unicolor* larvae may have implications for the ecology of their riparian habitats. Benthic invertebrates have been

suggested to be particularly sensitive to pollution stress, and for some pollutants, the sensitivity of benthic organisms may be due to the greater concentrations of those compounds in sediments than in the water column (Schindler, 1987). This may be the case for TCS, which is highly sorptive and has been shown to persist in sediments for over 40 years (Miller *et al.*, 2008).

Additional Concerns for TCS

There are many additional concerns associated with the presence of TCS in the environment. As a result, the findings of this study may actually be quite conservative when other negative effects of TCS are also taken into consideration.

The formation of compounds from the chlorination of TCS is thought to expand the potential for TCS to cause environmental and human harm. It has been found that TCS and its chlorinated derivatives are readily converted into various chlorinated dibenzo-p-dioxins by heat and ultraviolet irradiation (Kanetoshi *et al.*, 1987). Chlorinated dioxins accumulate in the fatty tissues of humans and animals and are considered persistent organic pollutants (Kanetoshi *et al.*, 1987). TCS also reacts with free chlorine under drinking water treatment conditions to form chloroform, which is an anticipated human carcinogen that has been banned from consumer products since 1976 (Rule *et al.*, 2005; National Institute of Environmental Health Sciences, 2009). TCS undergoes both biodegradation and photodegradation; one degradation product, methyl-triclosan is more persistent in the environment than TCS and is more lipophilic and has a higher potential to bioaccumulate (DeLorenzo *et al.*, 2008). Detectable concentrations of PPCPs and their metabolites have been reported in surface water, sewage effluent, soils, sediments, groundwater, and drinking water (Daughton and Ternes, 1999; Kolpin *et al.*, 2002), but the exposure routes of such micropollutants remain poorly understood (Halling-Sørensen *et al.*, 1998).

Controversy currently exists over the widespread use of TCS, as the potential hazards of long-term exposure remain largely unknown. Aiello *et al.* (2007) argued for closer evaluation of antibacterial product claims by governmental regulators, due to the lack of additional health benefit to using TCS containing consumer soaps over regular soaps and laboratory data demonstrating a risk of selection for antibiotic resistant bacteria. There are also concerns about the potential human health impacts of TCS exposure. TCS, due to its

similar chemical structure to polychlorinated biphenyls and polybrominated diphenylethers, is thought to have similar thyroid hormone receptor effects (Pearce and Braverman, 2009). TCS was found to disrupt thyroid hormone-regulated gene expression in bullfrogs at low concentrations (Veldhoen *et al.*, 2006). In the United States National Health and Nutritional Examination Survey in 2003 to 2004, TCS was detectable in 75% of urine samples (Calafat *et al.*, 2008). Detectable levels of TCS have also been reported in human milk (Adolfsson-Erici *et al.*, 2002), which is of concern as infants are thought to be particularly susceptible to endocrine disruptors (Pearce and Braverman, 2009).

There are also concerns about the potential for the development of microbial resistance to TCS. This research is tied closely to work investigating the mechanism by which TCS affects bacteria, which remains inconclusive. Work by McMurry *et al.* (1998) found that TCS acts on a specific target, the enoyl reductase enzyme involved in lipid synthesis, in *Escherichia coli*. This would reduce the potential of TCS resistance to develop, as only those organisms with intrinsically TCS-insensitive enoyl reductases would be capable of doing so (McMurray *et al.*, 1998). However, TCS has also been considered a non-specific biocide, with numerous intracellular and cytoplasmic target sites (Russell, 1997). It has been argued that the effects of TCS on multiple factors, including fatty acid synthesis, culminate in bacterial death (McDonnell and Pretzer, 1998). This theory has been supported by work demonstrating the ability of other species of bacteria to develop resistance to TCS, such as *Staphylococcus aureus* (Suller and Russell, 2000). Although there is debate about how TCS works, there is undeniable evidence that some bacteria species are capable of developing resistance to TCS.

Continued human population growth has resulted in an increased demand for the Earth's limited supply of water, making the protection of water resources one of the most pressing global environmental issues (Kolpin *et al.*, 2002). Although with regard to preserving water resources most attention has been paid to pesticides, herbicides, and other chemicals used in large-scale operations, the documented widespread presence and persistence of organic wastewater contaminants, which are typically released in smaller amounts by consumers into the environment, make them worthy of concern.

The United States Environmental Protection Agency (2010) has accelerated the registration review process for TCS, intending to begin in 2013, ten years earlier than

originally planned, due to the rapid expansion of scientific research on the environmental and human effects of the compound. However, TCS remains widely used, with approximately 1500 tons used worldwide each year (Oliveira *et al.*, 2009); there is little effort currently to better regulate its use.

Future Research

Further work on the long-term effects of TCS on organisms and ecosystems is necessary in order to understand the implications of its sustained release and presence in the environment. As noted earlier, shifts in detritus-based food webs may take several generations of consumers to be noticed; even one-generation long studies may be insufficient to detect effects. Long-term monitoring is necessary to distinguish natural sources of stress from anthropogenic stress on aquatic ecosystems (Schindler, 1987). Future research should also be wider in scale to allow for better understanding of the effects of TCS on ecosystems.

Although focus has remained mainly on the effects of TCS on aquatic organisms, TCS has been detected in terrestrial ecosystems. Reiss *et al.* (2009) noted that the use of sewage sludge containing TCS on agricultural soil could expose the compound to earthworms, terrestrial plants, and soil microorganisms; TCS could then be transmitted to higher trophic level animals, such as birds and mammals, which feed on organisms exposed to TCS. As such, future research should investigate further the risk of TCS exposure in the terrestrial environment. Acute toxicity studies have been conducted for TCS on terrestrial organisms including birds, mammals, soil dwellers, and plants (Reiss *et al.* 2009).

Due to the widespread occurrence of many environmental contaminants in aquatic and terrestrial ecosystems, organisms are expected to be exposed to mixtures of compounds, of which PPCPs only compose a portion (Dussault *et al.*, 2008). Much is unknown about the potential interactive effects of mixtures of organic wastewater contaminants in the environment (Kolpin *et al.*, 2002). The combined concentration of drugs that share the same mechanism of action in surface waters would most likely have an additive effect and increase the effective concentration of pharmaceuticals (Richards & Cole, 2006). In fact, even compounds with different modes of action may have an increased physiological threat when combined (Pickrell, 2002). DeLorenzo and Fleming (2008) found that the presence of PPCP mixtures could decrease the toxicity threshold for phytoplankton populations, which could

have impacts on nutrient cycling and food availability to higher trophic levels. Parrott and Bennie (2009) conducted a study on the effects of life-cycle exposure of fathead minnows to a mixture of six common pharmaceuticals and TCS. Additionally, synergistic anti-biofilm activity has been observed when biofilms are treated with triclosan and then an antibiotic and additive activity has been observed with planktonic cells (Tabak *et al.*, 2009). As such, future studies should investigate the ecological effects of mixtures of environmental contaminants.

Summary

With human populations continuing to grow, it is expected that the use of PPCPs such as TCS will increase in the future. The results of this 8 week long mesocosm experiment suggest that TCS has the potential to impact base trophic levels of aquatic food webs through the reduction of autochthonous and allochthonous food sources, such as algal growth and leaf litter decomposition respectively. As such, if TCS exposure continues to spread and/or worsens in the environment, it is possible that detrital-based food webs will be negatively affected. However, the work conducted here should not be considered in isolation; there are many other environmental and human problems associated with TCS usage and exposure. Instead, usefulness of this work would be maximized if taken into consideration in the context of other research, such as chemistry and medicine, to better understand the whole spectrum of effects that TCS may have, both ecological and societal.

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