Field assessment of oxytetracycline exposure to the freshwater macrophytes *Egeria densa* Planch. and *Ceratophyllum demersum* L.

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**Abstract**

In a microcosm study, two aquatic macrophytes, *Egeria densa* and *Ceratophyllum demersum* were exposed to nominal concentrations of 0, 5, 20, 50, and 250 μg/L oxytetracycline (n = 3), plus 20 μg/L oxytetracycline amended with additional nitrogen (N) and phosphorus (P). Responses were monitored bi-weekly over a six-week exposure period. Both plant species exhibited a significant decline in growth in the 250 μg/L oxytetracycline and the N- and P-amended units. Decreased light penetration resulting from accumulating oxytetracycline by-products appears to be the primary modifier in the growth of these plants. Increased susceptibility to oxytetracycline exposure was noted in some paired plantings (e.g., *E. densa* root development), relative to individual plants in these treatments, however, no clear explanation for this response is available. Based on the toxicity data generated in this study, we estimate that current concentrations of oxytetracycline in freshwater environments do not pose a direct risk to *E. densa* and *C. demersum*.

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1. Introduction

Knowledge of the ecological effects of antibiotics, their metabolites, and degradation products is generally lacking (Boxall et al., 2003). Tetracyclines are of particular significance because they are used frequently in concentrated animal feeding operations (CAFO; Mellon et al., 2001) and have been shown to reach the aquatic environment via several transport routes (Halling-Sørensen et al., 1998; Kolpin et al., 2002). Although various tetracyclines are used in CAFOs, oxytetracycline is of particular interest because it is used in both Europe and North America; it has broad-spectrum bacteriostatic activity (Chopra and Roberts, 2001); it has been detected in many surface water locations in the U.S. at concentrations up to 0.34 μg/L (Kolpin et al., 2002) and Colorado rivers in the range 0.08—0.15 μg/L (Yang et al., 2004); and it can be persistent in the environment, especially in interstitial waters and sediments where its epimers and degradation products can persist for extended durations and retain similar toxicological potency towards bacteria as the parent oxytetracycline (Halling-Sørensen, 2001; Halling-Sørensen et al., 2002, 2003; Hektoen et al., 1995), although it can also be quite photosensitive in well-lit systems (US Food and Drug Administration, 1989; Lam et al., 2004; Sanderson et al., 2005). Therefore, oxytetracycline in the environment might conditionally pose an ecotoxicological threat, especially where the drug is used heavily, has continuous release, and/or resides in light-restricted settings; and deserves evaluation among potentially exposed organisms, including macrophytes (Daughton and Ternes, 1999).
Some toxicity data already exists for tetracyclines and selected macrophytes (Pomati et al., 2004), but there is limited information on the aquatic toxicity of oxytetracycline. For example, the floating macrophyte *Lemma gibba* was shown to have a wet mass EC_{50} of 1.01 mg/L oxytetracycline after 7 days of exposure (Brain et al., 2004), and affected frond production in *Lemma minor* in 7-day exposures (EC_{50} = 4.92 mg/L) and the growth of *Chlorella vulgaris* in 48-h exposures (EC_{50} = 6.4 mg/L) (Pro et al., 2003). Additionally, oxytetracycline was a component in a four antibiotic mixture study (along with chlorotetracycline, tetracycline and doxycycline) that found significant growth inhibition in macrophytes with increasing concentrations of the mixture. However, it was unclear whether this effect was due to direct toxicity on the plants or resulted from reduced light availability due to water colouration (Brain et al., 2005). Further, this study used very high net tetracycline loadings (~1.2 mg/L), which did not necessarily reflect the majority of likely environmental exposure scenarios.

This current study was initiated, therefore, to assess more environmentally relevant concentrations and to examine different plants that might be suitable for similar future environmental assessments. The plants chosen were *Ceratophyllum demersum* L. (commonly called Coontail) and *Egeria densa* Planch. (commonly called Brazilian waterweed and also known as *Elodea densa*). *C. demersum* is found throughout North America as a native species and is an important food source for waterfowl and shelter for fish and arthropods, while *E. densa* (Planch.) is an introduced invasive species, mainly via the aquarium trade, from Brazil and is also found throughout North America as a nuisance species (Washington State Department of Ecology, 2005). Neither plant species has been used broadly in ecotoxicological field-scale studies (except *C. demersum*, Detenbeck et al., 1996), although *C. demersum* has been used extensively in the laboratory (Bunluesin et al., 2004; Kumar and Prasad, 2004). *E. densa* has been studied from the context of eradicating the species from certain waterways (e.g., Hofstra and Clayton, 2001), although not within the context of ecotoxicological effects. Therefore, these two plants were chosen for assessment because of their ubiquity in North American ecosystems, their availability, and the limited information at the field-scale for environmental impacts on them caused by chemical contaminants.

An ancillary goal in this study was to assess the influence of plant population density on the response to a toxicant, which is not in the current framework for laboratory and field assessment of rooted macrophytes (ASTM, 1999; Davy et al., 2001; Forbes et al., 2003). Submersed aquatic plant stands often create zones of increased pH and dissolved oxygen, while simultaneously depleting dissolved carbon dioxide concentrations, suppressing the photosynthetic efficiency and decreasing the growth rate of adjacent species (Jones et al., 1996). Therefore, the implications of community density on an individual plant’s response to a toxicant might be profound. For example, competition between invasive and non-invasive species of *Elodea* spp. in laboratory systems indicated that growth rates were affected by the presence or absence of different competing plant species (James et al., 1999; Barrat-Segretain and Arnaud, 2004). Additionally, macrophytes, including *C. demersum* and *E. densa*, are known to release allelopathic compounds (Gross et al., 2003; Nakai et al., 1996) that are capable of inhibiting epiphytic and algal growth. These allelopathic compounds could reduce the ability of neighbouring plant species to respond successfully to a stressor.

This study was a component of a larger investigation to ascertain the ability of antibiotics, in this case oxytetracycline, to influence and modify freshwater microbial communities (Knapp et al., submitted for publication). The specific objectives of the work reported here are associated with effects on the aquatic plants in the microcosms (1) assess the toxicity, if any, of oxytetracycline to common aquatic macrophytes under semi-natural field conditions; (2) investigate the influence of intra-plant interactions on their response to oxytetracycline; (3) evaluate the utility of these plants in assessing toxicity; and (4) use the generated toxicity data for a preliminary risk assessment for macrophytes and oxytetracycline.

2. Materials and methods

2.1. Study design

The microcosm systems were located at the Nelson Environmental Study Area (NESA) of the University of Kansas near Lawrence, Kansas, USA and have been used previously to characterize fate and toxicity of chemical contaminants (Graham et al., 1999; Knapp et al., 2003; Gordon et al., in press). Each microcosm is a flat-bottomed fiberglass tank, 3.2 m in diameter and 1.4 m deep, filled with 11.3 m³ of uncontaminated water from a protected area-pond. Three sets of six microcosms (18 total) were placed into shallow "host" ponds to minimize water temperature fluctuations in the microcosms. Approximately 12 L of sediment from an uncontaminated pond at NESA was divided amongst three 37 × 53 cm plastic trays and added to each microcosm to act as a source of microbial communities.

Target oxytetracycline concentrations for the five non-nutrient amended treatments (n = 3) were 0, 5, 20, 50 and 250 µg/L oxytetracycline. Concentrations were chosen based on their anticipated influence on microbial communities. Their background nutrients were nitrogen (N) at ~0.48 mg-N/L and phosphorus (P) at ~0.012 mg-P/L. Three additional units, treated with 20 µg/L oxytetracycline, were provided potassium nitrate (KNO₃) and dilute phosphoric acid (H₃PO₄) to maintain higher targeted N and P levels of 1.44 mg-N/L and 0.036 mg-P/L, respectively, to assess the impact of nutrient conditions on oxytetracycline fate and biological community responses. Addition of N and P commenced on August 23rd, 2004 and continued weekly until the end of the experiment. Addition of oxytetracycline commenced on September 15th, 2004 (the first day of the study) with supplementary treatments performed every 2–4 days as dictated by routine oxytetracycline analyses in order to maintain target concentrations.

2.2. Oxytetracycline analysis and physico-chemical analysis

Details of the sampling routine and measurements of oxytetracycline can be found elsewhere (Knapp et al., submitted for publication). In brief, weekly measurements of DO, pH, conductivity, turbidity and water temperature were made at three depths (0.3, 0.7, and 1.2 m) using a Water Checker Field Monitor (Horiba Instruments) to generate water-column averages. Water-column light readings (PAR, photosynthetically active radiation) were determined at the surface and a depth of 0.5 m for each unit at midday using an LI-COR spherical quantum sensor #LI185A (Lincoln, NE, USA) in order to calculate light transmittance.

Water chemistry samples (1 L) were collected with depth-integrated samplers made with PVC tubing with a valve at one end (Graham et al., 1999).
Total phosphorus (TP) was analyzed spectrophotometrically (Shimadzu UV-160) following wet digestion with potassium persulfate (APHA et al., 1998), whereas total nitrogen (TN) was measured spectrophotometrically following alkaline persulfate digestion (APHA et al., 1998). Water-column phytoplankton chlorophyll-a was analyzed spectrophotometrically following hot ethanol extraction (Nusch, 1980). Water samples for oxytetracycline analysis were collected and analyzed using ELISA (RIDASCREEN Tetracycline kit, R-Biopharm, Darmstadt, Germany). Details of these methods and their results can be found in Knapp et al. (submitted for publication).

2.3. Macrophyte assessment

_E. densa_ Planch. (Hydrocharitaceae) was purchased from Carolina Biological Supply (Burlington, North Carolina, USA) and _C. demersum_ L. (Ceratophylaceae) was obtained from a natural population in the west campus reservoir at the University of Kansas. Plants were acclimatized for at least 4 days at the NESA field station in microcosm water prior to their introduction to specific microcosms. The plants were transferred to 150 ml plastic “cone-tainers” in a planting tray (Stuewe and Sons, Corvallis, OR, USA). The cone-tainers were 21 cm long with a 3.8 cm internal diameter. Prior to the plant transfer, each tube was filled to the top with a clay–sila granule mix (Oil Dri Corporation of America, Alpharetta, GA, USA) that had been soaked and rinsed several times in microcosm water.

Plants of both species were cut to 5-cm apical shoot lengths, removing all evident side shoots. Each shoot was planted approximately 2–3 cm into the artificial rooting media to secure the plants. Every microcosm was supplied with a total of six cone-tainers of single _E. densa_, six cone-tainers of _C. demersum_, four cone-tainers with two _E. densa_, and six cone-tainers with two _C. demersum_. Planting trays were randomly assigned to each microcosm 2 days prior to the first addition of oxytetracycline.

Plants were sampled 1 day prior to treatment with oxytetracycline, and 14, 28, and 42-day post-treatment. The final day of the macrophyte study (42 days) was October 27, 2004. At the sampling points, two cone-tainers of each type were removed and evaluated, except for day – 1. On day – 1, 10 plants of each species were evaluated as 5 cm apical shoots for the endpoints measured: shoot dry mass and chlorophyll-a content. Relative growth rates were calculated according to Hunt (1990). Chlorophyll-a levels in the plants were determined on a fresh-weight basis according to Nusch (1980).

2.4. Individual plant toxicity data analysis

Plant response data were analyzed using General Linear Models of SAS 8.0 (SAS Institute, Cary, NC, USA). The effect of oxytetracycline on each endpoint at specific time-points was evaluated in a one-way analysis of variance (ANOVA). Nominal concentrations of oxytetracycline were used to conduct the statistical evaluations because the compound rapidly degraded, although nominal values proved to be good approximations of actual oxytetracycline levels (see below). If significance (\( p > 0.05 \)) was found, the means were compared to the control using Dunnett’s test, from which a no observed effect concentration (NOEC) and a lowest observed effect concentration (LOEC) were determined.

Concentration–response analyses were performed in SAS Version 8.0 according to the procedure for plant toxicity outlined in Stephenson et al. (2000) using a logistic or linear equation. The EC_{0%}, EC_{25}, and EC_{50} plus confidence intervals, were calculated for those endpoints showing a distinct concentration–response. Prior to use in the regression analyses, –1 day values were subtracted from later sampling dates for data on shoot growth, wet mass, dry mass and node number so that only new growth was utilized for assessment of effects. This permits a more conservative estimate of toxicity, especially at the initial stages of the study when growth is starting to commence (Hanson et al., 2003).

2.5. Analysis of interactions

The response of _E. densa_ and _C. demersum_ when planted as pairs of the same species in a cone-tainer was assessed in an attempt to characterize possible plant–plant interactions that might modify their response to oxytetracycline. Data were converted to percent of their respective control values and endpoints at each sampling date were compared between the individual response and the paired response using a Student’s t-test (\( \alpha = 0.05 \)). Endpoints evaluated were the same as for the assessment of individual plant responses to oxytetracycline (except chlorophyll-a, which was not measured).

2.6. Risk assessment

A hazard quotient approach was utilized where an Expected Environmental Concentration (EEC) is divided by a Toxicological Benchmark Concentration (TBC) (Suter, 1995). The lowest calculated EC_{10} from the individual plant response test was used as the TBC to assess the risk of these plants to oxytetracycline. The point estimate of 0.34 \( \mu \)g/L, the highest environmental concentration reported to date (Kolpin et al., 2002), was used as the estimate of exposure for the EEC. Values greater than 1.0 indicate a potential for toxic effects to occur and values of less than 1.0 indicate toxicity is not likely to occur (Suter, 1995). No uncertainty factors were applied due to the study being conducted at the field-level and the lack of persistence of oxytetracycline in the water column.

2.7. Statistical sensitivity of macrophyte data

Coefficients of variation (CVs) were calculated from the raw control data for each time-point to assess the statistical sensitivity of the two plant species used in the study (Hanson et al., 2003). The minimum detectable differences (MDD) for an ANOVA with _E. densa_ and _C. demersum_ were calculated using the average CV as described previously (Hanson and Solomon, 2004) with \( n \) set to 3 (the replication), and \( k \) set to 5 (the number of data groups).

3. Results

3.1. Oxytetracycline analysis and physico-chemical analysis

General physico-chemical water conditions are provided in Table 1 with pre-treatment and post-treatment means reported. Actual mean oxytetracycline levels were 7.8, 21.2, 55.2, and 301 \( \mu \)g/L, for the 5, 20, 50, and 250 \( \mu \)g/L oxytetracycline treatments, respectively (Knapp et al., submitted for publication). The N- and P-amended microcosms had a measured mean oxytetracycline level of 20.7 \( \mu \)g/L (nominal of 20 \( \mu \)g/L). Briefly, measured nutrient levels in the unfertilized tanks were basic and above saturation levels, respectively. Nutrient addition resulted in more eutrophic water conditions (mean Z = 0.041 mg-P/L, for the 5, 20, 50, and 250 \( \mu \)g/L oxytetracycline treatments, respectively (Knapp et al., submitted for publication). The point estimate of 0.34 \( \mu \)g/L, the highest environmental concentration reported to date (Kolpin et al., 2002), was used as the estimate of exposure for the EEC. Values greater than 1.0 indicate a potential for toxic effects to occur and values of less than 1.0 indicate toxicity is not likely to occur (Suter, 1995). No uncertainty factors were applied due to the study being conducted at the field-level and the lack of persistence of oxytetracycline in the water column.
the transition from summer (August) to fall (September—November).

Light transmittance (as PAR) in the tanks was affected by two factors: (1) amount of oxytetracycline added and its putative degradation products, and (2) the amount of secondary nutrients added to the water column. Tanks receiving higher doses of oxytetracycline resulted in progressively greater coloration of waters, thus reduced light transmittance (Table 1). The nutrient amended microcosms (at 20 µg/L oxytetracycline) had ~30% less light transmittance than their low-nutrient counterparts (also receiving 20 µg/L oxytetracycline). The change in light transmittance was essentially immediate, occurring within hours of the addition of oxytetracycline to these systems.

3.2. Individual plant toxicity responses

A decline in most endpoints was observed for *E. densa* at the highest oxytetracycline level (250 µg/L) and in the plants exposed to 20 µg/L oxytetracycline plus nutrients, although many of the responses were not statistically significant and could not be modeled in a concentration—response manner (Tables 2 and 3). The response of *E. densa* root number, as seen in Fig. 1, demonstrates the typical response for this plant at the tested treatments and time-points. With continued exposure the endpoints deviated further from control. The strongest responses in *E. densa* were observed in endpoints related to root development and plant length. Responses were closely linked to the level of light transmission at each time-point, with *r*² values of up to 0.93 for linear relationships between endpoint response and light transmittance (Table 4). As light attenuation decreased, plant length increased, resulting in a negative slope, while all other endpoints decreased with decreased light attenuation, resulting in positive slopes. *C. demersum* did not generally respond to either oxytetracycline or nutrient treatments with the exception of node number on day 28 at 250 µg/L oxytetracycline (*p* < 0.05).

3.3. Influence of planting density on toxicity response

Statistically significant differences were observed between plants as individuals and those planted as intra-species pairs (Table 5). The majority of differences were noted in paired *E. densa* in the 20 µg/L oxytetracycline plus nutrient treatment. Root measures tended to be the most responsive, with reduced root growth in the paired *E. densa* noted, as seen in Fig. 2. Few differences were noted in *C. demersum*, likely due to a lack of overall growth in any of the planting scenarios, though it should be noted that the differences observed were significant increases from control.

3.4. Risk assessment

Oxytetracycline appears to pose minimal hazard to the plants tested at typical environmental concentrations as indicated by the hazard quotient (HQ) approach. The HQ calculated from the day 14 root length EC₁₀ (47.9 µg/L
Table 2
The average response ($n = 3 \pm$ standard deviation) and the calculated no observed effect concentrations (NOECs) for the suite of endpoints monitored over the course of the 42-day study on the effects of oxytetracycline on Egeria densa in outdoor microcosms

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Day</th>
<th>Concentrations (µg/L)</th>
<th>0</th>
<th>5</th>
<th>20</th>
<th>20 NP</th>
<th>50</th>
<th>250</th>
<th>NOEC (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant length (cm)</td>
<td>14</td>
<td>11.2 ± 0.1</td>
<td>10.5 ± 0.8</td>
<td>11.2 ± 0.5</td>
<td>12.0 ± 1.7</td>
<td>11.5 ± 2.6</td>
<td>14.2 ± 0.1</td>
<td>nc</td>
<td></td>
</tr>
<tr>
<td>Root number</td>
<td>14</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
<td>4 ± 0</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
<td>2 ± 1</td>
<td>nc</td>
<td></td>
</tr>
<tr>
<td>Total root length (cm)</td>
<td>14</td>
<td>19.7 ± 5.4</td>
<td>22.3 ± 2.7</td>
<td>22.1 ± 2.1</td>
<td>15.6 ± 2.6</td>
<td>19.5 ± 3.4</td>
<td>10.7 ± 0.3</td>
<td>250n</td>
<td></td>
</tr>
<tr>
<td>Node number</td>
<td>14</td>
<td>30 ± 6</td>
<td>28 ± 3</td>
<td>28 ± 6</td>
<td>30 ± 3</td>
<td>33 ± 10</td>
<td>34 ± 9</td>
<td>nc</td>
<td></td>
</tr>
<tr>
<td>Wet mass (mg)</td>
<td>14</td>
<td>1669.0 ± 289.6</td>
<td>1460.6 ± 35.2</td>
<td>1506.9 ± 106.8</td>
<td>1305.6 ± 232.4</td>
<td>1477.4 ± 129.1</td>
<td>1345.3 ± 245.6</td>
<td>nc</td>
<td></td>
</tr>
<tr>
<td>Dry mass (mg)</td>
<td>14</td>
<td>2161.3 ± 39.6</td>
<td>2098.9 ± 30.1</td>
<td>2005.0 ± 8.5</td>
<td>110.3 ± 9.6</td>
<td>182.7 ± 31.0</td>
<td>139.2 ± 12.8</td>
<td>250c</td>
<td></td>
</tr>
<tr>
<td>Chlorophyll-a (µg/mg)</td>
<td>14</td>
<td>0.78 ± 0.06</td>
<td>0.77 ± 0.04</td>
<td>0.81 ± 0.09</td>
<td>0.99 ± 0.05</td>
<td>0.77 ± 0.01</td>
<td>0.92 ± 0.19</td>
<td>nc</td>
<td></td>
</tr>
</tbody>
</table>
| All responses were modeled using a linear regression with the parameters in the form of the following equation $y = mx + b$ where $x$ is the actual concentration (i.e., µg/L), $y$ is the response or change from control of the endpoint modeled, and $b$ is a constant.

nc-Not calculated.

a 20 NP represents those microcosms treated with 20 µg/L oxytetracycline with nutrient amendment of nitrogen and phosphorus.

b The plant length in the 250 µg/L microcosms was significantly greater than controls.

c The 20 µg/L oxytetracycline with nutrient amendment also declined significantly from control.

d Relative growth rate.


oxytetracycline) was determined to be 0.007, which is well below the threshold of 1 normally required to indicate a risk.

3.5. Statistical sensitivity of macrophyte data

E. densa was found to have lower CVs and, therefore, lower MDDs than C. demersum (Table 6). E. densa had CVs ($n = 3$) as low as 1% for plant length and as high as 32% for root length. C. demersum had CVs as low as 5% for plant length and as high as 58% for dry mass. Plant length was the least variable endpoint for both plant species, while the most variable endpoint was root length in E. densa and dry mass in C. demersum.

4. Discussion

Oxytetracycline was observed to dissipate rapidly from the water column of the microcosms (Knapp et al., submitted for publication) with observed dissipation rates greater than those seen in a previous microcosm-scale study (Brain et al., 2005).

Table 3
Effective concentrations (µg/L) required to cause a decrease in the endpoint of interest by 10%, 25%, and 50% (EC10-values, EC25-values, and EC50-values) as calculated using linear regression, with associated 95% confidence intervals, for endpoints with significant responses in Egeria densa and Ceratophyllum demersum exposed to oxytetracycline in aquatic microcosms

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Endpoint</th>
<th>Day</th>
<th>EC10 (95% CI)</th>
<th>EC25 (95% CI)</th>
<th>EC50 (95% CI)</th>
<th>Parameters</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. densa</td>
<td>Root number</td>
<td>14</td>
<td>62.1 (17.0, 98.8)</td>
<td>155.2 (63.3, 247.0)</td>
<td>310.4 (126.6, 494.1)</td>
<td>$b = 3.95, m = -0.0064$</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Root length</td>
<td>14</td>
<td>47.9 (28.6, 67.2)</td>
<td>119.8 (71.5, 168.1)</td>
<td>239.6 (143.1, 336.2)</td>
<td>$b = -22.42, m = -0.2138$</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>Dry mass</td>
<td>14</td>
<td>56.5 (24.1, 88.9)</td>
<td>141.2 (60.2, 222.1)</td>
<td>282.3 (120.3, 444.3)</td>
<td>$b = 43.96, m = -0.1284$</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>Node number</td>
<td>28</td>
<td>59.5 (24.4, 94.6)</td>
<td>148.8 (61.1, 236.5)</td>
<td>297.6 (122.2, 473.0)</td>
<td>$b = 8.98 m = -0.6628$</td>
<td>0.46</td>
</tr>
</tbody>
</table>

All responses were modeled using a linear regression with the parameters in the form of the following equation $y = mx + b$ where $x$ is the actual concentration (i.e., µg/L), $y$ is the response or change from control of the endpoint modeled, and $b$ is a constant.
Oxytetracycline half-lives ranged from 2.6 to 5.0 hours in the current study, whereas Brain et al. (2005) observed half-lives ranging from 24 to 66 hours. This difference is likely due to much lower soluble organic matter (SOM) levels here compared with the earlier study that had a different water source and a more extensive sediment zone. Although oxytetracycline dissipation rates were higher here, oxytetracycline addition rates were adjusted accordingly and consistent levels were maintained in all the units. As previously noted, oxytetracycline had a distinct impact on PAR transmittance in our units, reducing it by ~50% at the highest oxytetracycline concentration tested versus the controls, which is suspected to be due to degradation products of oxytetracycline in the system (US Food and Drug Administration, 1989; Brain et al., 2005; Sanderson et al., 2005).

When we consider the toxicity data, both macrophytes exhibited distinct responses in the 250-μg/L oxytetracycline and 20-μg/L oxytetracycline with nutrient amendment treatments, most noticeably declines in overall growth, especially root development, relative to controls. Still, much of the observed effect can appear to be attributed to light attenuation and not to the toxicity of oxytetracycline itself. For example, plant growth endpoints in *E. densa* were, on average, 100%, 93%, and 88% that of the controls at 20 μg/L oxytetracycline where light was reduced approximately ~5% at days 14, 28, and 42, respectively. Those plants in microcosms treated with additional nutrients at the same oxytetracycline concentrations were 87%, 72%, and 61% that of controls at days 14, 28, and 42, respectively, where light transmittance was reduced by ~30%. Plant length typically increased with decreased light penetration, whereas other growth endpoints decreased

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Table 4

The coefficients of determination ($r^2$) and slope for *Egeria densa* as a function of light (photosynthetically active radiation or PAR) transmittance and the percent of control for the evaluated endpoints at three time-points upon exposure to oxytetracycline in microcosms

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Time-point</th>
<th>$r^2$</th>
<th>Slope</th>
<th>$r^2$</th>
<th>Slope</th>
<th>$r^2$</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant length</td>
<td>14</td>
<td>0.88</td>
<td>-100</td>
<td>0.37</td>
<td>-61</td>
<td>0.91</td>
<td>-239</td>
</tr>
<tr>
<td>Root number</td>
<td>28</td>
<td>0.82</td>
<td>155</td>
<td>0.93</td>
<td>133</td>
<td>0.43</td>
<td>72</td>
</tr>
<tr>
<td>Root length</td>
<td>42</td>
<td>0.84</td>
<td>185</td>
<td>0.88</td>
<td>158</td>
<td>0.69</td>
<td>136</td>
</tr>
<tr>
<td>Wet mass</td>
<td>5</td>
<td>0.61</td>
<td>48</td>
<td>0.50</td>
<td>60</td>
<td>0.10</td>
<td>43</td>
</tr>
<tr>
<td>Dry mass</td>
<td>250</td>
<td>0.80</td>
<td>141</td>
<td>0.68</td>
<td>164</td>
<td>0.55</td>
<td>124</td>
</tr>
</tbody>
</table>

* The 20 μg/L nutrient amended microcosms were excluded from this analysis.

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Table 5

Statistically significant differences, as determined by a Student’s $t$-tests ($p < 0.05$), between cone-tainers with one plant versus those with paired plants of the same species at the same oxytetracycline concentration

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Endpoint</th>
<th>Concentration (μg/L)</th>
<th>Day</th>
<th>$p$-Value</th>
<th>Percent difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 <em>E. densa</em></td>
<td>Plant length</td>
<td>20 NP</td>
<td>28</td>
<td>0.03</td>
<td>-27</td>
</tr>
<tr>
<td></td>
<td>Root number</td>
<td>20 NP</td>
<td>28</td>
<td>0.02</td>
<td>+42</td>
</tr>
<tr>
<td></td>
<td>Root length</td>
<td>5</td>
<td>28</td>
<td>0.002</td>
<td>-33</td>
</tr>
<tr>
<td></td>
<td>Longest root</td>
<td>5</td>
<td>28</td>
<td>0.04</td>
<td>-28</td>
</tr>
<tr>
<td></td>
<td>Dry mass</td>
<td>5</td>
<td>28</td>
<td>0.04</td>
<td>-29</td>
</tr>
<tr>
<td>2 <em>C. demersum</em></td>
<td>Node number</td>
<td>250</td>
<td>14</td>
<td>0.04</td>
<td>+28</td>
</tr>
<tr>
<td></td>
<td>Wet mass</td>
<td>250</td>
<td>14</td>
<td>0.02</td>
<td>+36</td>
</tr>
<tr>
<td></td>
<td>Dry mass</td>
<td>250</td>
<td>14</td>
<td>0.01</td>
<td>+42</td>
</tr>
</tbody>
</table>

* Some values, while, not statistically significant, were deemed to be sufficiently close as to warrant reporting.

* This percent represents the absolute difference between the controlled response and the paired-interaction response. A negative value indicates the response of the paired plants was less and a positive value indicates that they were greater as compared to controls by the percentage reported.

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Fig. 1. Root number of *Egeria densa* expressed as a percent of control following oxytetracycline treatment in outdoor microcosms. NP refers to microcosms treated with additional nitrogen and phosphorus.

Fig. 2. Root length of *Egeria densa* planted as individuals and as pairs expressed as a percent of control following oxytetracycline in outdoor microcosms. NP refers to microcosms treated with additional nitrogen and phosphorus. An asterisk indicates a significant difference between the plants at a specific treatment level (two-tailed Student’s $t$-test, $p < 0.05$).
as light penetration diminished, with light penetration being a function of oxytetracycline level or nutrient amendment. This response is typical of aquatic plants, where higher white light or UV-B can inhibit shoot elongation (Li et al., 2005), which is a similar response observed by Brain et al. (2005) to a tetracycline mixture in field microcosms.

The addition of nutrients had a distinct impact on the plant responses in the microcosms, especially on *E. densa*. In fact, the most dramatic reduction in growth was seen in plants exposed to 20 μg/L oxytetracycline plus nutrients, even compared with higher oxytetracycline exposures (i.e., 250 μg/L). This is different than other field studies on the macrophytes *Elodea nuttallii* or *Aponogeton elongates* where growth enhancement was noted after additional nutrient (Best et al., 1996; Crossley et al., 2002). These convergent observations are not necessarily inconsistent because many higher nutrient lakes can exist in two general equilibrium states; a clear state dominated by macrophytes and a turbid state dominated by algal biomass (Scheffer et al., 1993) with both states tending to be equally self-reinforcing. There was an increase in chlorophyll-α concentrations in the nutrient amended systems, implying a shift to an algal dominated system; which tends to confirm the minimal impacts of oxytetracycline itself at the concentrations assessed in this study.

The examination of plant—plant interactions that might affect response to oxytetracycline exposure found increased toxicity in *E. densa* at 20 μg/L with nutrients relative to the same treatments of individual plants. It is unclear what is responsible for this increase in susceptibility to oxytetracycline or what role direct versus indirect oxytetracycline toxicity plays. Still, planting density does modify the response of these plants to the toxicant, in this example resulting in an accentuated decrease in overall growth. Root endpoints exhibited the highest number of significant interactions. Root development, in the form of root mass or root diameter, can be reduced when a species is planted at increasing densities (Spencer and Ksander, 2005). This could have implications at the population and community levels that would not normally be anticipated based on individual level testing for a toxicant, but not likely in the case of oxytetracycline, considering the orders of magnitude between environmental exposure and effect concentrations.

When one considers the statistical variation and sensitivity, both important in determining effects and estimating risk, of *E. densa* and *C. demersum*, plant length was the least variable and hence most statistically sensitive endpoint for both plants. The most variable endpoint for *E. densa* was root length, which was seen similarly in *Myriophyllum* spp. in previous studies (Hanson et al., 2003; Hanson and Solomon, 2004). Due to the lack of a strong concentration—response in the plants versus oxytetracycline observed here, it is not possible to rank the toxicological sensitivity of the endpoints or to meaningfully compare the difference in sensitivity between plant species, but *E. densa* would appear to be the more sensitive indicator plant of toxicity than *C. demersum*. The generally low rate of growth of *C. demersum* in this study made it difficult to assess, therefore *E. densa* would appear to be the better test species for similar microcosm studies.

Overall, oxytetracycline dissipates rapidly from the water column of outdoor microcosms, likely due to photolytic degradation, which implies exposure effects to aquatic plants will be attenuated or minimal. *E. densa*, which shows promise as a test species in the assessment of toxicity, exhibited a distinct attenuation or minimal.

### References


