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Response of water column microbial communities to sudden exposure to deltamethrin in aquatic mesocosms

Charles W. Knapp [a], Thierry Caquet [b], Mark L. Hanson [b,c], Laurent Lagadic [b], David W. Graham [a,*]

[a] *Department of Civil, Environmental and Architectural Engineering, 4112 Learned Hall, 1530 W, 15th Street, University of Kansas, Lawrence, KS 66045, USA*

[b] *UMR 985 INRA-Agrocampus Ecobiologie et Qualité des Hydrosystèmes Continentaux, Equipe Ecotoxicologie et Qualité des Milieux Aquatiques, F-35042 Rennes, France*

[c] *Department of Environment and Geography, 213 Isbister Building, University of Manitoba, Winnipeg, Man., Canada R3T 2N2*

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Abstract

Sudden exposure of an aquatic system to an insecticide can have significant effects on populations other than susceptible organisms. Although this is intuitively obvious, little is actually known about how such exposure might affect bacterial communities and their relative metabolic activity in ecosystems. Here, we assessed small sub-unit (ssu)-RNA levels in open and shaded 9 m^3 aquatic mesocosms (16 units – 2×2 factorial design in quadruplicate) to examine the effects of sudden addition of deltamethrin to the units. When deltamethrin was added, a cascade of bacterial then phytoplankton "blooms" occurred over time. The bacterial bloom, which most likely included organisms from the plastid/cyanobacterial phylogenetic guild, was almost immediate (within hours), whereas the phytoplankton (algal) bloom lagged by about 4 days. This sequential response can be explained by an apparent sudden release of nutrients consequent to arthropod death that triggered a series of responses in the microbial loop. Interestingly, bacterial blooms were noted in both open and shaded mesocosms, whereas the algal bloom was only seen in open units, suggesting that both deltamethrin addition (and presumptive nutrient release) and an adequate light supply was required for the phytoplankton response. Overall, this work shows that microbial activities as reflected by ssu-rRNA levels can respond dramatically via apparently indirect effects following insecticide application.

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1. Introduction

Deltamethrin (3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylic acid cyano(3-phenoxyphenyl) methyl ester) is a pyrethroid ester insecticide that is often used for the control of mosquitoes and other nuisance arthropods in or close to aquatic systems. Typically, it is applied to an infested area over a short timeframe effectively killing the majority of the target insect(s) and many other co-existing invertebrates at the site [1]. However, Caquet et al. [2] showed that, although insecticide applications directly impact arthropods, they can also indirectly impact other organisms in the food web through both bottom-up and top-down mechanisms. For example, the elimination of herbivorous arthropods reduces grazing pressure that might result in increased levels of primary producers, such as periphyton [3] and phytoplankton [4,5]. Alternately, die-off of the target

* Corresponding author. Tel.: +1 785 864 2945; fax: +1 785 864 5379.
E-mail address: dwgraham@ku.edu (D.W. Graham).

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organisms might release secondary nutrients that become available for other microbial activity. As a result, ancillary ecological and chemical responses may cause microbial blooms or other ecosystem modifications not directly intended by the insecticide application [6,7].

Although such effects might be intuitive, little documentation exists on the exact nature of microbial community responses, especially at the bacterial level. Here we specifically assess microbial responses within the context of a much larger study assessing whole ecosystem recovery after exposure to deltamethrin [8]. In the larger study, the insecticide was added to a set of sixteen 9-m^3 aquatic field mesocosms, with and without screen lids (and appropriate controls), as a single dose targeted at 2 $\mu g\ l^{-1}$ nominal concentration to assess the short-term impact of insecticide addition and the effect of mesocosm covers on re-colonization after exposure. Although detectable deltamethrin was retained in the water column for less than 4 days, it had its desired effect on invertebrates with a loss of over 90% of emergent insects and a major reduction in zooplankton and benthic arthropods (e.g., insect larvae) over the week following insecticide addition [8]. Thus, conditions in the units (in conjunction with controls) were created to allow the study of "secondary" microbial community responses in the same systems, which was the primary goal of this work.

The key to successfully assessing microbial responses in the systems was to choose a technique that would detect both culturable and non-culturable species and also have the potential to target individual bacterial groups at higher taxonomic resolution. Therefore, ssu-rRNA (small sub-unit ribosomal RNA) hybridization techniques were adopted, which have advantages over other microbiological techniques in that they allow the detection of in situ microbial activities and not just abundance [9]. For example, ssu-rRNA methods provide a generally good representation of metabolically active organisms [10–12] because cell ribosome content tends to be proportional to growth rate [13–15], although some deviation is sometimes noted during non-steady-state growth experiments [16]. Further, sss-rRNA methods do not require microbial culturing that skews communities towards species that can be grown on plates. Finally, Pace and Cole [17] showed that increased bacterial activity (as suggested by ssu-rRNA levels) often precedes population growth and can be important when assessing a possibly rapid microbial response, which may be the case here. Although ssu-rRNA methods were primarily employed, direct microscopic counts were also performed to compare our molecular data with whole-organism data generated by others in this project.

The primary goal in this work, therefore, was to assess the effect of deltamethrin addition on microorganisms in aquatic systems. An ancillary goal of the work was to assess the influence of shading on the nature and extent of the observed bacterial and phytoplankton responses. It was hypothesized that the pulse addition of deltamethrin would result in increased bacterial and phytoplankton activities either by the release of nutrients from decaying arthropods or due to reduced grazing pressures. Our results demonstrate a rarely seen "top-down effect" in natural microbial communities resulting from insecticide exposure only previously seen in soil studies [18].

2. Materials and methods

2.1. Mesocosm experimental design

The experiment assessing the response among microbial communities to deltamethrin addition used aquatic mesocosms and was performed during Spring 2003 at the Rennes site of the Experimental Unit of Aquatic Ecology and Ecotoxicology of the Institut National de la Recherche Agronomique (INRA), France. It was a sub-component of a large two-year study that assessed aquatic arthropod recovery after sudden deltamethrin exposure as influenced by aerial re-colonization (or not) as controlled by screened lids on selected units. The experiment used a 2×2 factorial design with deltamethrin addition being one factor and the inclusion of white fiberglass 1-mm mesh screen lids (~37% reduction of Photosynthetically Active Radiation – PAR) being the other. The four treatments were (1) open control, (2) open deltamethrin, (3) covered control, and (4) covered deltamethrin. All treatments were maintained in quadruplicate. Further details of the experimental design are provided elsewhere [8].

The large experiment commenced in Spring 2002, although detailed molecular and microbial monitoring was only performed between mid March 2003 (~1 month prior to deltamethrin addition) and August 2003. Deltamethrin was added on 22 April 2003 by subsurface spraying, and monitoring was performed weekly with the exception of a two-week window of more intensive sampling immediately before and after deltamethrin addition.

2.2. Monitoring program

The physical, chemical and biological conditions in the 16 mesocosms were monitored by the collection of water samples for laboratory analysis and the real-time measurement of various parameters using field probes. Samples for water chemistry and molecular microbiological analysis were collected using rinsed, pre-sterilized PVC tube samplers (one sampler per mesocosm) that had a screen-covered one-way valve at the bottom for easy withdrawal to sample storage bottles (similar to Graham et al. [19]). Typically, individual volumes were

collected from four locations around each tank and then combined into single 1-L pre-sterilized amber bottles for analysis. The bottles were stored in a cooler on ice, returned to the laboratory, and processed immediately according to the requirements of each analytical procedure. Water analyses included total nitrogen (TN), total phosphorus (TP), dissolved organic carbon (DOC), and chlorophyll *a*. The PVC samplers were always rinsed with 95% ethanol after each sampling event, allowed to dry, and then wrapped in PVC film to minimize cross-contamination.

Real-time monitoring included dissolved oxygen (DO_{sat}, expressed as % dissolved oxygen saturation), pH and conductivity (Cond), which were measured weekly at the same location approximately 20 cm below the water surface using WTW portable apparatus (Wissenschaftlich-Technische-Werkstätten – WTW France, Champagne au Mont d'Or, France) equipped with OXI-320, pH-340 and LF-318 probes, respectively. Measurements were always made between 10:00 and 11:00 A.M. Water temperature was determined using each of the monitoring probes with individual readings being averaged and reported as a mean temperature value. Water temperature was also recorded continuously in four of the ponds using Stowaway Tidbit (Prosensor, Amanvillers, France) sensors. Further, PAR was continuously recorded in selected open and covered ponds using JYP 1000 sensors (SDEC France, Reignac-sur-Indre, France) connected to EasyLog EL-2-12Bit dataloggers (Lascar Electronics Ltd, Salisbury, UK) to monitor actual light conditions in the units.

2.3. Water chemistry

TN was determined spectrophotometrically after peroxydisulfate oxidation (Standard 11905-1, [20]). TP was also determined spectrophotometrically (Merck KgaA, 64271 Darmstadt, Germany) following a reaction with molybdate and ascorbic acid (European Standard EN 1189, [21]). DOC was determined by high-temperature catalytic oxidation and infrared detection with a Shimadzu TOC-505A instrument (Shimadzu France, Champs-sur-Marne, France). Samples for chlorophyll *a* analysis were initially separated using GF/C, 1.2-μm pore size filters (Whatman), which were then extracted overnight using a 10% (v:v) acetone:water mixture. Chlorophyll *a* was quantified spectrophotometrically using a double-beam UV–visible Uvikon 943 spectrophotometer (UVK-Lab Technologies, Trappes, France) according to Lorenzen [22].

2.4. Molecular microbiological analyses

Samples for ssu-rRNA analysis were collected similar to samples for water chemistry; however, the samples for molecular analysis were collected more frequently around the time of deltamethrin addition. Specifically, samples were collected on days −7, −4, −1, 0, +1, +2 and +4 (relative to the day of deltamethrin addition) to determine immediate effects of each treatment. Subsequent sampling was performed weekly in conjunction with the regular water-sampling program. Once the samples for ssu-rRNA analysis were returned to the laboratory, duplicate 100 ml aliquots were rapidly separated and sterile-filtered using 0.45 μm pre-sterilized Nalgene filter funnels. The filters were transferred to sterile centrifuge tubes and frozen at −80 °C for storage prior to final analysis.

Details on the ssu rRNA extraction and hybridization procedures can be found elsewhere [23]. In summary, total RNA was extracted in a low-pH buffer and phenol solution with a cell-disruptor (FastPrep™, Qbiogene, Irvine, CA, USA), and purified using sequential phenol, phenol–chloroform, and chloroform liquid extractions. The product RNA was precipitated with isopropanol and acetate solution, re-suspended in DEPC-treated water, and stored again at −80 °C. The extracted RNA was slot-blotted onto positively charged, nylon membranes (GE Osmonics, Inc., Trevose, PA, USA), and then pre-hybridized and hybridized using a suite of radio-labeled domain-level rDNA oligonucleotide probes (^{32}P-ATP; Perkin–Elmer Life Science, Wellesley, MA, USA). The specific probes included S-D-Bact-338-a-A-19 (eubacteria and cyanobacteria/plastids; 5′ GCTGCCTCCCGTAGGAGT), S-D-Bact-785-a-A-19 (eubacteria; 5′ CTACCAGGGTATCTAATCC), S-D-Euca-1379-a-A-16 (eukaryotes; 5′ TACAAAGGGCAGGGAC), and S-*-Univ-1390-a-A-18 (most organisms; 5′ GACGGGCGGTGTGTACAA).

After binding, blots were washed at appropriate dissociation temperatures (1 × SSC buffer) for each probe [23], and quantified using standard curves (2–200 ng RNA per slot) developed using extracted rRNA from pure cultures of *Escherichia coli* for the universal and eubacterial probes and *Saccharomyces cerevisiae* for the eukaryotic probe. Sample quantification was performed using a storage-phosphor K-screen (Eastman Kodak) with cassette (Molecular Dynamics) and scanned with a Molecular FX imager (Bio Rad). Resulting blot intensities were modeled using 1D Image Analysis Software v. 2.03 (Eastman Kodak) and compared to the standard curve to estimate mass quantities. The difference between the eubacterial-338 and -785 gene probe signals was used to provide an approximate estimate of cyanobacterial/plastid activity [9]. Corroborating bacterial and phytoplankton biomasses were determined microscopically using methods summarized in Ensz et al. [24].

2.5. Data analysis

Statistical analyses were performed using SPSS v.11 (Chicago, Illinois, USA). Non-parametric tests were

used for population comparisons among quadruplicate tanks under each treatment (due to small sample sizes and data distributions), including the Mann–Whitney test for two populations and the Kruskal–Wallis test for multiple populations. For assessments of ssu-rRNA levels over time, treatment averages were calculated and normalized to levels measured in the control open mesocosms. This normalization step was required to account for seasonal effects that influence all microbial communities and to also account for slight differences in rRNA extraction efficiencies (i.e., the samples were processed in two batches). Relationships between the results of molecular analyses and counts were assessed using Pearson's correlation coefficients.

3. Results

3.1. Water chemistry of the four treatments

Table 1 summarizes the mean water conditions from one-week prior to deltamethrin addition to 3 months after addition. Water conditions in all four treatments were mesotrophic ($0.025 < TP < 0.040$ mg-P l^{-1} and TN > 1.5 mg-N l^{-1}; [25]) with a moderate to high level of net photosynthesis as evidenced by comparatively elevated pH and DO_{sat} levels. With the exception of chlorophyll *a*, and conditionally for pH and DOC, there were minimal differences in mean water chemistry conditions among the four treatments (Kruskal Wallis test; $\alpha = 0.10$). Chlorophyll *a* levels were significantly higher in both treatments that were provided deltamethrin (compared with the non-treated units), whereas TP and DOC levels were significantly lower and higher, respectively, in the open control units compared with the covered deltamethrin units.

3.2. Comparison of between ssu-rRNA levels and microbial direct counts

In order to confirm that the ssu-rRNA hybridization data provides broadly similar data to conventional enumeration methods, ssu-rRNA values were compared to biomass estimates based on direct counts in a randomly selected sub-set of samples. Fig. 1 shows significant correlations between ssu-rRNA gene probe and bacterial biomass estimates (Bact-338, $r = 0.57$, $P = 0.02$; Bact-785, $r = 0.65$, $P = 0.01$, $n = 16$), suggesting that the gene probes and counts provide a roughly similar measure of the bacterial community size. Furthermore, Euca-1379 also correlates well with the phytoplankton biomass as estimated by direct count data ($r = 0.76$, $P < 0.01$, $n = 16$), suggesting that Euca-1379 approximates planktonic algae populations as noted in previous work [23].

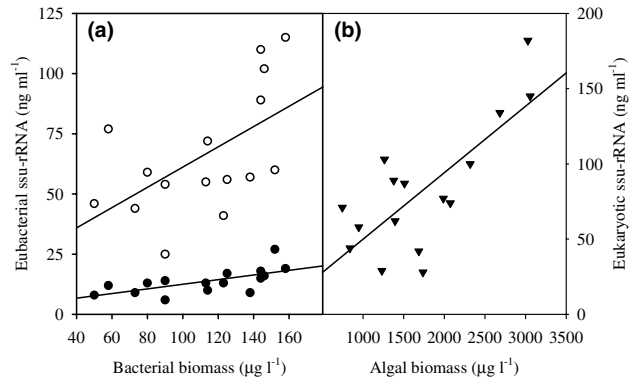

Fig. 1. Relationship between ssu-rRNA gene probe values and biomass: (a) Bact-338 (○) and Bact-785 (●) vs. bacteria (Bact-338: $r = 0.57$, $P = 0.02$; Bact-785: $r = 0.65$, $P = 0.01$) and (b) eukaryotic-1379 (▼) vs. algae ($r = 0.76$, $P < 0.01$).

3.3. Effects of covers in mesocosms without deltamethrin addition

In general, covering the mesocosms with the screen lids (reducing by 37% PAR light supply to the water column) resulted in reduced levels of photosynthetic activity and smaller microbial communities as evidenced by differences in mean pH (Table 1; Mann–Whitney test, $P < 0.01$), and universal (Fig. 2(a); $P = 0.05$, $n \sim 20$ each) and eukaryotic ssu-rRNA activities (Fig. 2(b); $P = 0.01$, $n \sim 20$ each). Euca-1379 signals, presumptively algae, were approximately 50% lower in the covered versus the open controls, whereas Univ-1390 signals were about 20% lower in the same units.

3.4. Effects of deltamethrin addition on microbial communities

Deltamethrin addition significantly increased the mean levels of chlorophyll *a* (Table 1; Mann–Whitney test, $P < 0.01$), Bact-338 ssu-rRNA (Fig. 2(d); all

Table 1
Average physico-chemical properties within each treatment condition (standard errors of mean in parentheses)

Treatment	pH	DO_{sat} (%)	Conductivity (µS)	TP (mg l^{-1})	TN (mg l^{-1})	DOC (mg l^{-1})	Chl *a* (µg l^{-1})
Open control	10.4 (0.1)	152 (11)	285 (5)	0.029 (0.009)	1.55 (0.51)	13.0 (3.2)	4.5 (3.8)
Open deltamethrin	10.2 (0.1)	146 (9)	284 (6)	0.035 (0.008)	1.75 (0.53)	10.8 (2.9)	7.6 (3.1)
Covered control	10.1 (0.1)	143 (14)	284 (13)	0.025 (0.008)	1.99 (0.52)	11.0 (2.7)	4.6 (6.0)
Covered deltamethrin	10.2 (0.1)	147 (12)	296 (5)	0.039 (0.017)	1.89 (0.34)	8.2 (1.4)	7.4 (3.2)

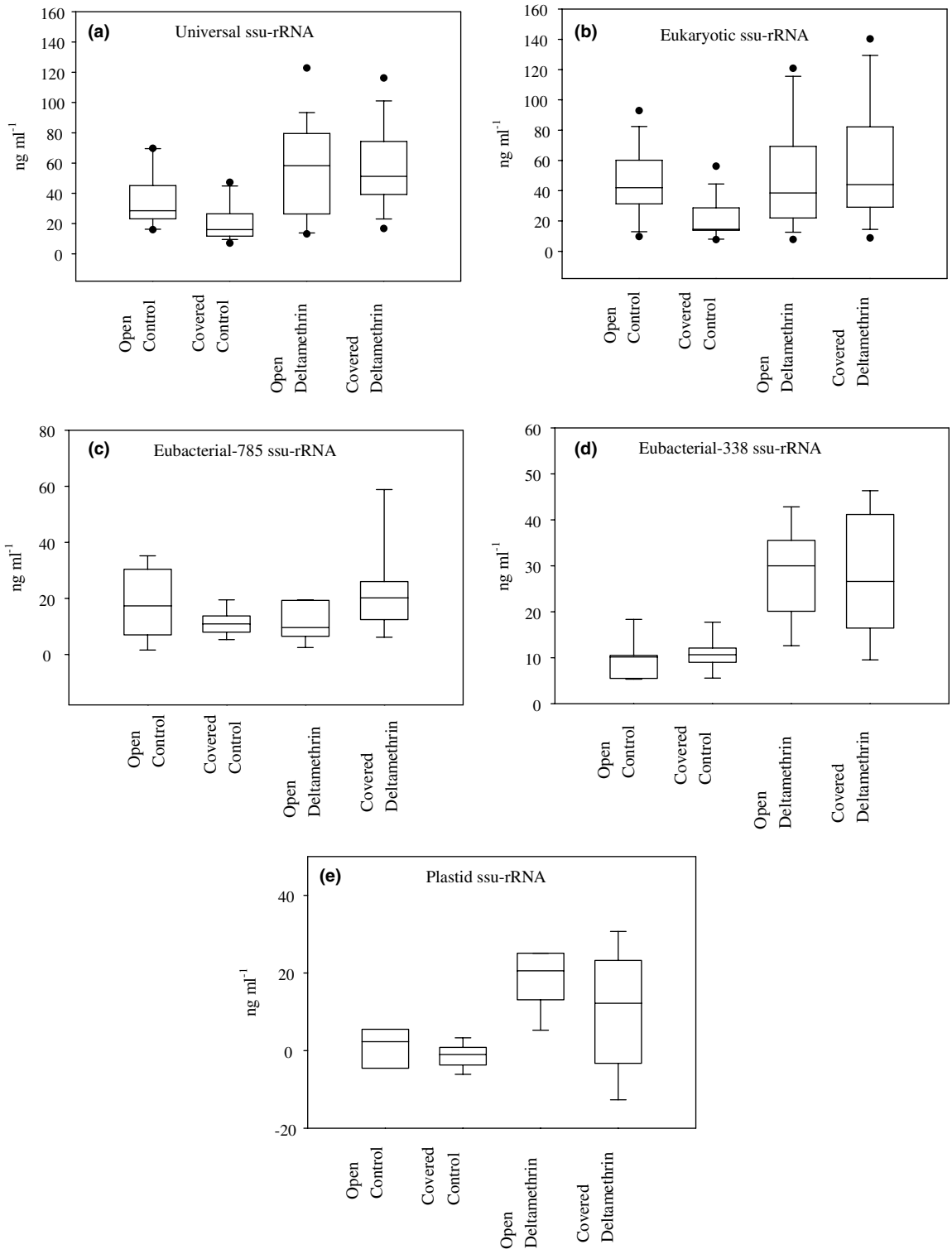

Fig. 2. Box-plot representations of the ssu-rRNA within each treatment: (a) universal, (b) eukaryotic, (c) eubacterial-785, (d) eubacterial-338, and (e) plastid/cyanobacterial values. Horizontal lines represents the median, inter-quartile ranges, and 5%/95% percentiles; solid circles denote outliers.

$P < 0.02$, $n \sim 16$ each), and plastid ssu-rRNA (Fig. 2(e); $P < 0.01$, $n \sim 12$ each) in both the open and covered mesocosms. Significant differences in chlorophyll *a* concentration ($P < 0.05$) were particularly noteworthy in the open deltamethrin-amended units between days +8 to +21 compared with the other treatments (data not shown). There were also small, but less significant increases in Univ-1390 (Fig. 2(a)) and Euca-1379 ssu-rRNA (Fig. 2(b)) levels after deltamethrin addition as compared to the controls, with the sole statistically

significant exception being the comparison between the covered deltamethrin units and the covered controls that indicated relatively increased Euca-1379 ($P = 0.05$) and Univ-1390 ($P = 0.01$) ssu-rRNA activities in the deltamethrin-amended units.

3.5. Temporal effects of deltamethrin addition

Figs. 3 and 4 summarize the temporal effect of deltamethrin exposure for Bact-338 and Euca-1379 ssu-rRNA activities before and after insecticide addition. The data are presented as normalized values (as a ratio of measured ssu-rRNA levels for each probe over time) for each treatment relative to observed ambient conditions in the open control mesocosms. This reporting strategy presents responses in a manner that minimizes the influence of seasonal effects, which naturally impact microbial communities in the spring, and also accommodates differences in RNA extraction efficiencies among sample runs.

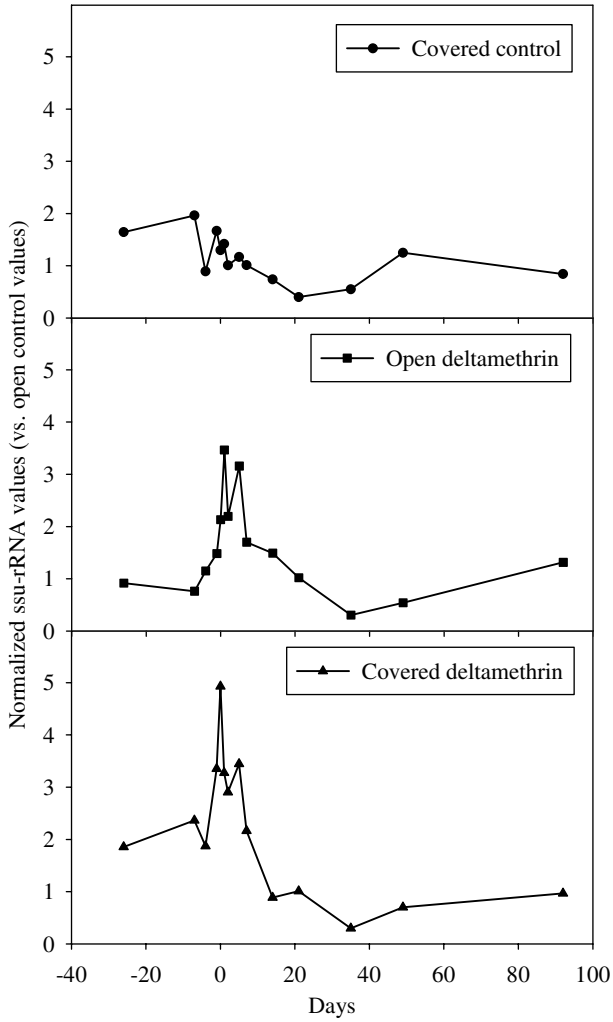

Fig. 3. Normalized eubacterial-338 ssu-rRNA values over time. Reported averages from the four units under each treatment proportional to ssu-rRNA levels measured in the open control mesocosms. The range of values for individual units under each treatment was ±25%; error bars not shown for visual clarity.

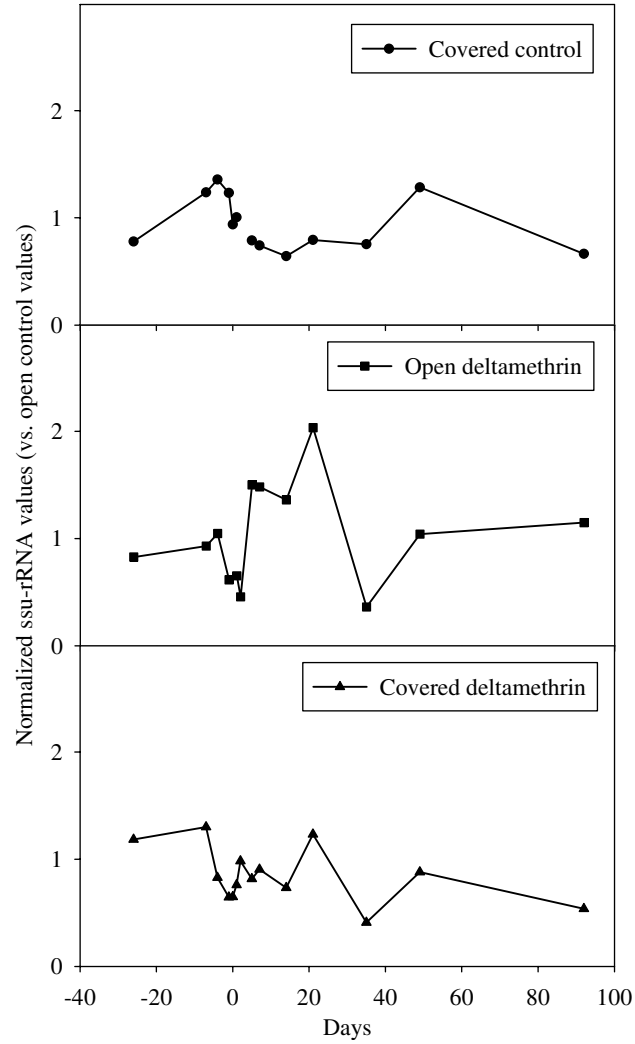

Fig. 4. Normalized eukaryotic ssu-rRNA values over time. Reported averages from the four units under each treatment proportional to ssu-rRNA levels measured in the open control mesocosms. The range of values for individual units under each treatment was ±22%; error bars not shown for visual clarity.

Fig. 3 shows that both the open and covered deltamethrin mesocosms had almost instantaneous Bact-338 peaks following insecticide addition. The maximum Bact-338 peak height was slightly higher in the covered versus the open units; however, both treatments retained elevated activities for about one week after deltamethrin addition. Alternately, no Bact-338 activity peak was noted in the covered control units and, in fact, the Bact-338 signal progressively declined over time presumably due to reduced "fresh" carbon supply levels in this treatment because of reduced photosynthesis by shading with the covers.

In contrast to Bact-338 activities, Fig. 4 shows a major Euca-1379 peak only in the open

deltamethrin-amended units. This peak appeared 4 days after insecticide addition, crested after about two weeks, and lasted for about three weeks. The timing of this peak closely paralleled observed increases in chlorophyll *a* [8], suggesting that it was likely related to phytoplankton rather than zooplankton. Importantly, no major Euca-1379 activity peaks were observed in either of the covered treatments (even deltamethrin-amended covered units), suggesting that both deltamethrin addition and higher light supplies were required for the formation of a Euca-1379 peak after insecticide exposure.

4. Discussion

Numerous studies exist on the indirect effects of the exposure to insecticides on non-target organisms, especially related to organisms higher up the food chain. For example, the indirect influence of deltamethrin additions on micro- and macro-invertebrate communities have been long since established (summarized by Solomon et al. [1]); however, much fewer studies exist on the indirect impact of such contaminants on organisms lower on the food chain, especially at the bacterial level. One might argue that such impacts are not ecotoxicologically important because microbial biodiversity is great, niche substitution readily occurs in microbial systems, and microbial communities appear to self-organize and tolerate transient disturbances. Regardless, little is known about the timing and extent of such effects down the food chain, and the goal of this study was to determine such information.

In the larger study, deltamethrin was added to mesocosms with and without covers to assess the impact of insecticide addition on macroinvertibrates (e.g., emerging insects) and zooplankton in the units, including rates of recovery after exposure [8]. In summary, deltamethrin addition had the intended effects; i.e., the benthic arthropods (including larvae of aerial insects) and zooplankton (except rotifers) were almost completely exterminated after exposure. Further, the recovery rate of affected populations was heavily dependent upon the presence of the covers, suggesting that aerial re-colonization was more important than internal re-growth for insects in the mesocosms.

The results presented in our work are highly complementary with observations from the larger study. Table 1, and Figs. 2–4 show that the sudden die-off of insects and other arthropods after deltamethrin addition impacted microbial communities. Table 1 and Fig. 2 show that all microbial domains were affected by deltamethrin addition with chlorophyll *a*, and Bact-338, plastid (Bact-338 − Bact-785), Euca-1379, and Univ-1390 ssu-rRNA activities all increasing with differing levels of statistical significance. Fig. 3 further shows that the bacterial "bloom" was almost immediate, including a significant cyanobacterial/plastid component (since no parallel increase in Bact-785 ssu-rRNA was noted), possibly as a rapid consequence of the sudden release of "fresh" nutrients after higher organism death [26]. Further, Fig. 4 shows that a eukaryotic bloom, almost certainly planktonic algae (confirmed by chlorophyll *a* data), closely followed the bacterial bloom, which is consistent with the assumption that bacteria are innately more efficient at nutrient uptake than algae and the fact that bacteria tend to have much shorter generation times [27].

Unfortunately, although DOC and other nutrients were measured in the experiment to detect such nutrient releases, the frequency of sampling after deltamethrin addition was not sufficiently high to quantify immediate nutrient changes. The extremely rapid responses after deltamethrin addition were unexpected, which is partially defendable because no precedent data existed on this response. However, it is possible that such nutrient releases might not have been detected by routine DOC analysis anyway (or other measures) because strong evidence suggests that the most readily available nutrients are consumed very rapidly in aquatic systems (nearly instantaneous) and tend not accumulate as detectable DOC after production [24,28,29].

Although the two blooms were consistent with an assumed release of nutrients from decaying benthic arthropods, it is also possible that responses result from reduced grazing pressure due to the simultaneous die-off of zooplanktonic arthropods (e.g., daphnids and copepods). Grazing by invertebrates can account for 50% and 80% of total losses of bacteria and phytoplankton in any system, respectively [30–32]. However, although the sudden reduction in zooplankton might allow bacterial and phytoplankton populations to temporarily flourish, such blooms would still require a new source of nutrients for growth that would ultimately come from arthropod die-off.

An interesting observation related to recovery is the effect of the mesocosm covers on the phytoplankton bloom (see Fig. 4). A major phytoplankton bloom only occurred when covers were not present, which implies that both the release of nutrients due to die-off and higher light supplies were required to promote elevated phytoplankton numbers after deltamethrin addition. This effect was seen most clearly in the ssu-rRNA data and was not so clear in the chlorophyll *a* data [8], which may seem somewhat contradictory. However, phytoplankton often regulate their chlorophyll *a* levels according to light conditions, therefore this apparent inconsistency may reflect differences in internal chlorophyll *a* levels rather than differences in community abundance [33]. Regardless, the relationship between light intensity and the nature of the ssu-rRNA bloom response is novel, and should be a point of new investigation, especially coupled with more intensive nutrient sampling immediately after contaminant addition.

In summary, a significant cascade microbial response was observed after deltamethrin addition among treatments, with "bacterial" response being rapid and phytoplankton response being somewhat delayed. The results suggest that the addition of an insecticide can have rippling impacts both up the food chain and also down the food chain. New work, therefore, is justified in examining this down-food-chain effect, especially as it might relate to issues of contaminant fate, which is primarily driven by bacteria, and recovery rates of higher organisms in the system. With further work, it may be possible to provide alternate mechanistic explanations for both zooplankton and maybe insect recovery as a function of bloom responses among organisms at the microscopic scale.

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References

[1] Solomon, KR., Giddings, J.M. and Maund, S.J. (2001) Probabilistic risk assessment of cotton pyrethroids: I. Distributional analyses of laboratory aquatic toxicity data. Environ. Toxicol. Chem. 20, 652–659.

[2] Caquet, T., Thybaud, E., Le Bras, S., Jonot, O. and Ramade, F. (1992) Fate and biological effects of lindane and deltamethrin in freshwater mesocosms. Aquat. Toxicol. 23, 261–278.

[3] Brock, T.C.M., Roijackers, R.M.M., Rollon, R., Bransen, F. and Van der Heyden, L. (1995) Effects of nutrient loading and insecticide application on the ecology of *Elodea*-dominated freshwater microcosms. II. Responses of macrophytes, periphyton and macroinvertebrate grazers. Arch. Hydrobiol. 134, 53–74.

[4] Hurlbert, S.H. (1975) Secondary effects of pesticides on aquatic ecosystems. Res. Rev. 57, 81–148.

[5] Van Donk, E., Prins, H., Voogd, H.M., Crum, S.J.H. and Brock, T.C.M. (1995) Effects of nutrient loading and insecticide application on the ecology of *Elodea*-dominated freshwater microcosms. I. Responses of plankton and zooplanktivorous insects. Arch. Hydrobiol. 133, 317–439.

[6] Ruber, E. and Ferrigno, F. (1964) Some effects of DDT, Baytex, and endrin on salt marsh productivities, copepods, and *Aedes* mosquito larvae. Proc. N. J. Mosq. Exterm. Assoc., 84–94.

[7] Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A. and Thingstad, F. (1983) The ecological role of water column microbes in the sea. Mar. Ecol. Prog. Ser. 10, 257–263.

[8] Hanson, M., Caquet, T., Graham, D.W., Azam, D., Coutellec, M.-A., Knapp, C.W., and Lagadic, L. Recovery of isolated experimental aquatic ecosystems to the application of an insecticide. I. Rationale, study design and general assessment of environmental conditions. Environ. Toxicol. Chem., submitted for publication.

[9] Knapp, C.W. and Graham, D.W. (2004) Assessment of different ssu-rRNA probing protocols for the characterization of aquatic microbial communities. J. Microbiol. Meth. 56, 323–330.

[10] Muttray, A.F. and Mohn, W.W. (1999) Quantitation of the population size and metabolic activity of a resin acid degrading bacterium in activated sludge using slot-blot hybridization to measure the rRNA:rDNA ratio. Microb. Ecol. 38, 348–357.

[11] Ka, J.O., Yu, Z. and Mohn, W.W. (2001) Monitoring the size and metabolic activity of the bacterial community during biostimulation of fuel-contaminated soil using competitive PCR and RT-PCR. Microb. Ecol. 42, 267–273.

[12] Leser, T.D., Boye, M. and Hendriksen, N. (1995) Survival and activity of *Pseudomonas* sp. Strain B13(FR1) in a marine microcosm determined by quantitative PCR and a rRNA-targeting probe and its effect on indigenous bacterioplankton. Appl. Environ. Microbiol. 61, 1201–1207.

[13] Srivastava, A.K. and Schlessinger, D. (1990) Mechanism and regulation of bacterial ribosomal RNA processing. Ann. Rev. Microbiol. 44, 105–129.

[14] Kemp, P.F., Lee, S. and LaRoche, J. (1993) Estimating the growth rate of slowly growing marine bacteria from RNA content. Appl. Environ. Microbiol. 59, 2594–2601.

[15] Kerkhof, L. and Ward, B.B. (1993) Comparison of nucleic acid hybridization and fluorometry for measurement of the relationship between RNA/DNA ratio and growth rate in a marine bacterium. Appl. Environ. Microbiol. 59, 1303–1309.

[16] Kerkhof, L. and Kemp, P. (1999) Small ribosomal RNA content in marine Proteobacteria during non-steady-state growth. FEMS Microb. Ecol. 30, 253–260.

[17] Pace, M.L. and Cole, J.J. (1996) Regulation of bacteria by resources and predation tested in whole-lake experiments. Limnol. Oceanogr. 41, 1160–1448.

[18] Tu, C.M. (1980) Influence of five pyrethroid insecticides on microbial population activities in soil. Microb. Ecol. 5, 321–327.

[19] Graham, W.H., Graham, D.W., deNoyelles Jr., F., Smith, V.H., Larive, C.K. and Thurman, E.M. (1999) Metolachlor and alachlor breakdown product formation patterns in aquatic field mesocosms. Environ. Sci. Technol. 33, 4471–4476.

[20] International Organization for Standardization (ISO), 1997. Water quality –Determination of nitrogen – Part I: Method using oxidative digestion with peroxydisulfate. International Organization for Standardization, Geneva, Switzerland.

[21] European Committee for Standardisation (ECS), 1996. Water Quality – Determination of phosphorus. Ammonium molybdate spectrometric method. European Standard EN 1189. European Committee for Standardisation, Brussels, Belgium.

[22] Lorenzen, C.J. (1967) Determination of chlorophyll and pheopigments: spectrophotometric equations. Limnol. Oceanogr. 12, 343–346.

[23] Knapp, C.W., Graham, D.W., Berardesco, G., deNoyelles, F., Cutak, B.J. and Larive, C.K. (2003) Nutrient level, microbial community conditions, and alachlor transformation in aerobic aquatic mesocosms. Water Res. 37, 4761–4769.

[24] Ensz, A.P., Knapp, C.W. and Graham, D.W. (2003) Influence of autochthonous dissolved organic carbon and nutrient limitation on alachlor biotransformation in aerobic aquatic systems. Environ. Sci. Technol. 37, 4157–4162.

[25] OECD (1982) Eutrophication of Waters, Monitoring, Assessment and Control. Organisation for Economic Cooperation and Development, Paris.

[26] Fasham, M.J.R., Platt, T., Irwin, B. and Jones, K. (1985) Factors affecting the spatial distribution of the deep chlorophyll maximum in the region of the Azores Front. Prog. Oceanogr. 14, 129–165.
[27] Joint, I., Henriksen, P., Fonnes, G.A., Bourne, D., Thingstad, T.F. and Riemann, B. (2002) Competition for inorganic nutrients between phytoplankton and bacterioplankton in nutrient manipulated mesocosms. Aquat. Microb. Ecol. 29, 144–159.
[28] Amon, R.M.W. and Benner, R. (1993) Rapid cycling of high molecular weight dissolved organic matter in the ocean. Nature 369, 549–551.
[29] Amon, R.M.W. and Benner, R. (1996) Bacterial utilization of different size classes of dissolved organic matter. Limnol. Oceanogr. 41, 41–51.
[30] Riemann, B. (1985) Potential importance of fish predation and zooplankton grazing on natural populations of freshwater bacteria. Appl. Environ. Microbiol. 47, 632–638.
[31] Vadstein, O., Harkjerr, B.O. and Jensen, A. (1989) Cycling of organic carbon in the photic zone of a eutrophic lake with special reference to the heterotrophic bacteria. Limnol. Oceanogr. 34, 840–855.
[32] Pace, M.L., McManus, G.B. and Findlay, S.E.G. (1990) Plankton community structure determines the fate of bacterial production in temperate lake. Limnol. Oceanogr. 35, 795–808.
[33] Kopf, W. (1983) Investigations of the dynamic of chlorophyll concentration in natural populations of phytoplankton. Arch. Hydrobiol. 98, 173–180.