Pesticides and Nutrients in Surface Waters Associated with Golf Courses and Their Effects on Benthic Macroinvertebrates

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Goals:

- Measure the concentration of pesticides and nutrients residing in the water column of streams associated with golf courses.
- Measure the concentration of pesticides residing in the sediments and sediment porewater of streams associated with golf courses.
- Assess the impact of golf courses on stream macroinvertebrate communities.
- Determine the sublethal impacts of selected pesticides on benthic macroinvertebrates.

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Golf courses provide citizens with a convenient recreational opportunity while preserving green space and natural settings. Yet, their intensive management necessitates the use of pesticides and fertilizers, thus provoking concerns of environmental damage. One of the overall goals of this project is to determine if surface waters, and their sediments, associated with golf courses are contaminated by pesticides and/or fertilizers. Contamination is especially expected to occur especially in association with high runoff events such as storms. However, because contamination varies with time, a second overall goal is to develop the use of stream macroinvertebrates and their communities as long-term indicators of water quality; this will allow us to determine if pesticides and/or fertilizers are impacting stream macroinvertebrate communities.

Water samples for nutrient level measurement have been collected and analyzed once or twice every month since March, 1998. In addition, we have collected water from five run-off events and have analyzed this water for nutrients. Water and sediment samples for pesticide analysis have also been collected five times following run-off events. The water samples have been filtered and processed using solid phase extraction. On the basis of this data, it does appear as though pesticides and fertilizers used on golf courses are moving in streams associated with golf courses, especially in association with run-off events of a large magnitude such as those on 31-July-1998 and 8-October-1998, and are in higher concentrations at the downstream locations. However, routine sampling for nutrient levels indicated that golf courses do not cause nutrient enrichment of streams.

Macroinvertebrates associated with natural leaf packs are collected using artificial leaf pack samplers. Five leaf packs, each consisting of dried leaves (standardized by leaf taxa and dry weight) connected to a brick with a strap, are placed in the stream 21 days prior to the sampling date to allow for colonization by benthic macroinvertebrates. On the sampling date, the leafpacks are collected and water quality parameters measured. In the laboratory, invertebrates in each sample are sorted, preserved, and identified to family level. Community comparisons, using taxonomic diversity and invertebrate density, have been performed by calculating various community statistics for each golf course and site.

During 1997 and 1998, invertebrates were collected five times. These samples yielded 42,557 individuals representing 79 families of invertebrates. The most abundant types of invertebrates collected were members of the families Chironomidae (midge flies), Simuliidae (black flies), Hydropsychidae (net-spinning caddisflies), Elmidae (riffle beetles), and Capniidae (winter stoneflies). The overall analysis for taxa richness showed a significant difference (increase) in the number of taxa at sites downstream of the course. However, the analysis did not indicate a significant difference in taxa richness at upstream and downstream locations for any of the courses when the analysis by course was completed. The overall analysis for total invertebrate abundance showed a significant difference (increase) in abundance at sites downstream of the course. In addition, analysis of the data showed a significant difference (increase) in abundance of invertebrates at the downstream location for two of the courses. Total abundance of invertebrates can either increase or decrease in the presence of pollution, depending on the type of pollution. However, pesticide presence (at least at toxic levels) would be expected to be correlated with a decrease in total abundance.

While the communities at upstream and downstream sites do appear to differ slightly, at least in terms of taxa richness and total abundance, the pesticide and nutrient data don't provide any explanation for these differences. The pesticides concentrations, even at their highest, at sites downstream of the courses are not close to toxic levels for fish and aquatic invertebrates. In addition, when the pesticide levels are high in the water columns from sites downstream of the course, the EPT richness and taxa richness of benthic macroinvertebrates from the same sites are higher than they are at the upstream sites. Also, the nitrate and phosphorus concentrations at sites downstream of the course are less than (in the case of nitrate) or equal to (in the case of phosphate) the concentrations at sites upstream of the course.

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Title of Project:
Pesticides and Nutrients in Surface Waters Associated with Golf Courses and Their Effects on Benthic Macroinvertebrates

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Project Timetable:
Initiated February 1, 1998; to terminate January 31, 2000.

Project Summary:

Golf courses provide citizens with a convenient recreational opportunity while preserving green space and natural settings. Yet, their intensive management necessitates the use of pesticides and fertilizers, thus provoking concerns of environmental damage. This project focuses on the potential run-off of chemicals from golf courses, into surface running waters, and its impact on stream macroinvertebrate communities. To accomplish these objectives, we have worked with five golf courses to study run-off of pesticides and nutrients, the effects of golf course management practices on benthic macroinvertebrate communities, and the sublethal impacts of pesticides on benthic macroinvertebrates. Information gained from this project will aid development of management practices that are ecologically sound without causing undue economic hardships on golf courses.

The specific research objectives are:

1) To measure the concentration of pesticides and nutrients residing in the water column of streams associated with golf courses,
2) To measure the concentration of pesticides residing in the sediments and sediment porewater of streams associated with golf courses,
3) To assess the impact of golf courses on stream macroinvertebrate communities, and
4) To determine the sublethal impacts of selected pesticides on benthic macroinvertebrates.

Research Initiated and Progress to Date:

Objective 1. To measure the concentration of pesticides and nutrients residing in the water column of streams associated with golf courses.

The goal of this objective is to determine if pesticides and/or nutrients applied to golf courses are moving into streams associated with the golf courses, especially through run-off events such as storms.

Progress and Results to Date:

Water samples, from streams associated with golf courses, were collected five times during 1998 and once in 1999 within 24, and usually 12, hours of a run-off event (e.g. thunderstorms). Samples were collected from Course 1 five times (24-July-1998, 31-July-1998, 11-August-1998, 8-October-1998, and 24-August-1999) and from Course 3 one time (18-September-1998). These water samples, which were collected from two sites upstream (collectively referred to as the upstream location) from the golf courses and two sites downstream (collectively referred to as the downstream location) of the golf courses, were analyzed for pesticides and nutrients. In addition, water samples for nutrient level analysis have been collected and analyzed once or twice every month since March, 1998.

Nutrients (nitrogen and total phosphorus) were analyzed using the HACH DR 890 colorimeter. Nitrate concentrations were measured using E.P.A. cadmium-reduction methods. Total phosphorus was measured by treating the water via acid persulfate digestion (to convert all of the phosphorus into a soluble form- orthophosphate) and then measuring the orthophosphate concentrations using E.P.A. approved ascorbic acid methods. In addition, fluoride concentrations were measured using SPADNS method in order to determine if sewage contamination of the streams was a possible source of nutrient input.

Water samples were analyzed for pesticides using solid-phase extraction and gas chromatography/mass spectrometry (G.C./Mass Spec.). Water samples were analyzed for approximately 40 different pesticides, including five commonly used on Maryland golf courses. These five pesticides were the fungicides chlorothalonil and metalaxyl, the insecticides chlorpyrifos and malathion, and the herbicide pendimethalin. Two different G.C./Mass Spec. methods were used for analysis of the water samples. Negative chemical ionization (N.C.I.) was used to analyze for water samples for several pesticides including chlorothalonil, chlorpyrifos, and malathion. Electron impact (E.I.) analysis was used to detect other pesticides including metalaxyl and pendimethalin.

Water samples collected from Course 1 showed concentrations of several pesticides- pendimethalin, chlorothalonil, malathion, and chlorpyrifos- that were greater at sites downstream of the golf course than at sites upstream of the course. The largest increases in pesticide concentrations at sites downstream of the course were observed especially in association with run-off events of the largest magnitudes (measured in terms of precipitation). However, the concentration of these pesticides were lower at sites downstream of the course, compared to those upstream from the course, in water samples collected from Course 3.

On the basis of this data, it does appear as though pesticides used on golf courses are moving in streams associated with golf courses (at least Course 1)- especially in association with run-off events of a large magnitude such as those on 31-July-1998 and 8-October-1998- and are in higher concentrations at the downstream locations. However, in the case of Course 3, pesticide levels are consistently lower at sites downstream of the course. We believe that these differences are probably because Course 1 management includes mowing the turf up to the stream, therefore the stream is not protected by a buffer zone of any type, while a well developed buffer zone is maintained along most the stream's length at Course 3.

Water samples for nutrient analysis were collected during run-off events and from each course once to twice a month. The concentrations of nitrate and total phosphorus were measured using the methods discussed above. Analysis of the data shows a significant difference (decrease) in nitrate concentration when one compares the nitrate concentrations at upstream locations with downstream locations for each of the courses except Course 2. In addition, an overall analysis using data collected from all of the courses also showed a significant difference (decrease) in nitrate concentrations at the locations downstream of the course. Finally, nitrate concentrations were analyzed by season to determine if nitrate concentrations were higher at sites downstream of the course during those times of the year (spring/fall) when nitrogen is applied to the courses. Once again, there was a significant decrease in nitrate concentrations at sites downstream of the course during every season. The by course analysis was completed in order to determine if any differences in nutrient concentration trends (i.e. higher upstream at one course and higher downstream at a different course) were related to different management practices used at each of the courses.

Analysis of the data did not show any significant differences in phosphorus concentrations between upstream and downstream locations, in any analysis including an overall analysis, by course analysis, or by season analysis. In addition, fluoride concentration analysis did not show any differences between upstream and downstream locations.

Water samples collected during the same run-off events were also analyzed for nitrate and total phosphorus. During run-off events of a large magnitude (31-July-1998 and 8-October-1998), an increase in nitrate concentrations were seen at sites downstream of the golf course. However, for Course 3, a decrease in nitrate concentrations was seen at sites downstream of the course. These same trends were seen with total phosphorus. Once again, it appears as though movement of these chemicals (nitrate and phosphorus) into streams associated with golf courses is occurring during run-off events, especially those of large magnitude- at least in the case of Course 1. However, the different trends seen with Course 1 (increases at downstream sites) and Course 3 (decreases at downstream sites) may be due to the absence of a buffer zone at Course 1 and the presence of one at Course 3.

The increase in taxa richness and total invertebrate abundance (see Objective 3) at the sites downstream of a golf course may be indicative of increased productivity due to nutrient enrichment. Nutrient enrichment may be the result of run-off of fertilizers applied to golf courses. However, if stream productivity is nitrogen or phosphorus limited, nutrient enrichment of the streams may not be detected when nitrate and phosphorus levels in the water column are analyzed. Because aquatic ecosystems are often nutrient limited, an nutrients that move into the system are quickly used by aquatic organisms resulting in higher productivity at enriched sites. Therefore, it might be possible to show nutrient enrichment not through elevated levels of nutrients in the water column, but through increased stream productivity.

To determine if biological productivity at sites upstream and downstream from golf courses differed, periphyton growth was measured. Periphyton growth is often an indicator of primary productivity in a stream and is related to nutrient levels. Frosted acrylic plates were used as a artificial substrate on which periphyton could colonize and grow. PVC frames were constructed from which four acrylic plates of a standard area were suspended. The frames were constructed such that they could float on the water's surface while the plates were submerged a couple of inches below the surface. One PVC frame was placed at each of the upstream and downstream sites in areas that are similar in terms of light penetration through the canopy.

The plates were left in the stream for a period of four weeks. At the end that time period, they were collected and the periphyton growing on one side of the plates was scraped of the plates, dried, and weighed. Ash-free dry mass of the periphyton was then determined. The ash-free dry mass of the periphyton at upstream and downstream sites was then analyzed in order to determine if there were any differences.

The ash-free dry mass of the periphyton from the upstream location did not differ significantly from that of the downstream location. Therefore, it does not appear as though primary productivity differs between these sites.

On the basis of the nutrient data, it does not appear as though nutrient enrichment of streams associated with golf courses is occurring. The nitrate concentrations of the upstream sites are consistently greater than those of the downstream sites. Furthermore, the total phosphorus concentrations are not different when comparing the upstream and downstream sites. Finally, periphyton growth at the upstream and downstream sites does not differ. The only increases in nutrient concentration at sites downstream of the golf courses were detected in water samples collected from course 1 in association with run-off events of larger magnitude. Therefore, it appears as though, during these larger run-off events, that golf courses are a source of nutrient input into the streams, however, it does not result in nutrient enrichment (i.e. an over increase in nutrient levels or primary productivity).

Objective 2. <u>To measure the concentration of pesticides residing in the sediments and sediment porewater of streams associated with golf courses</u>.

The proposal called for the collection of sediment samples and their analysis for pesticides for five run-off events. However, since many pesticides bind to soil materials, it was been decided that analyzing the sediments suspended in water samples collected for pesticide analysis might be a better method of determining whether or not pesticides are moving into streams via run-off events.

Progress and Results to Date:

Water samples were collected during five run-off events during 1998. Suspended sediments were trapped on filter paper during filtration of the samples using a Millipore pressure filter. Sediments trapped from water samples collected on 31-July-1998 and 8-October-1998 were analyzed for pesticides; because the concentration of pesticides in the water samples were greatest on these dates, we decided to analyze the sediments collected from these water samples for pesticides.

Pesticides were removed from the sediments using Soxhlet extraction. The solvent/pesticide mixture obtained via Soxhlet extraction was then analyzed for pesticides using both N.C.I and E. I. G.C./Mass Spec. Of the pesticides commonly used on Maryland golf courses, chlorothalonil and chlorpyrifos were detected bound to the sediments. Chlorothalonil was detected from sediments collected on 31-July-1998 from the upstream sites; this compound was also detected in high concentrations in the water samples collected from the upstream sites on this same date. Chlorpyrifos was detected from sediments collected on 8-October-1998 from the downstream sites and was also detected in high concentrations in the water samples from the downstream sites on this sample date. However, these were the only two instances in which pesticides were detected bound to the sediments. Sediments were also analyzed for malathion, metalaxyl, and pendimethalin and, other than chlorothalonil and chlorpyrifos being detected at one location on one date each, pesticides were below quantification limits for all sites on all sample dates. Therefore, it does not appear as though sediments moving into the streams during run-off events are a source of pesticide contamination of the streams.

Objective 3. To assess the impact of golf courses on stream macroinvertebrate communities.

Progress and Results to Date:

Benthic macroinvertebrates were sampled using quantitative sampling methods five times in 1997 and 1998 in order to statistically compare community structure. Sampling was timed to reflect key times of invertebrate life history: 1) mid-April before spring emergence, 2) early June, 3) mid-August, 4) mid-September after egg hatch, and 5) early November.

Macroinvertebrates associated with decomposing leaf packs were collected using artificial leafpack samplers. Five leaf packs, each consisting of dried leaves (five grams of oak leaves) connected to a brick with a strap, were placed in the stream 21 days prior to the sampling date to allow for colonization by benthic macroinvertebrates. On the sampling date, the leafpacks were collected several physical and chemical parameters of the streams were measured. These physical and chemical parameters were measured in order to determine if any changes in the benthic macroinvertebrate community are due to environmental variation or if they are due to influences from golf course management practices.

In the laboratory, invertebrates in each sample were sorted, preserved, and identified to the lowest taxonomic level possible or needed, usually genus. Community comparisons, using various biomonitoring indices and community structure analysis were completed using data obtained via the collection of benthic macroinvertebrates (specifically aquatic insects).

Table 1 provides a summary of the invertebrate samples and Table 2 indicates the most abundant types of invertebrates collected. Community structure was analyzed several ways, including the use of bioassessment indices, in order to determine if the structure at sites upstream of the courses differ from the structure downstream of the courses. One index is EPT richness; this index is a measurement of the community of sensitive organisms. EPT richness is calculated as the number of mayfly, stonefly, and caddisfly taxa present in a sample. In theory, the number of sensitive organisms decreases as water quality decreases. EPT richness values did not differ significantly between upstream and downstream sites in an overall analysis using data from all the courses from which invertebrates were collected in 1997 and 1998 (courses 1, 2, and 3). However, Course 3 did shown an significant increase in EPT richness at the downstream locations when this parameter was analyzed by course (i.e. the upstream and downstream values were compared for each course, separately) (Table 3).

A second index used to analyze community structure was taxa richness. The overall analysis showed a significant difference (increase) in taxa richness at sites downstream of the course (Table 3). However, the analysis did not indicate a significant difference in taxa richness at upstream and downstream locations for any of the courses when the analysis by course was completed. In theory, taxa richness decreases with decreasing water quality.

The third bioassessment index used to analyze community structure was total invertebrate abundance. The overall analysis showed a significant difference (increase) in abundance at sites downstream of the course (Table 3). In addition, analysis of the data showed a significant difference (increase) in abundance of invertebrates at the downstream location for courses 2 & 3. Total abundance of invertebrates can either increase or decrease in the presence of pollution, depending on the type of pollution. However, pesticide presence (at least at toxic levels) would be expected to be correlated with a decrease in total abundance.

Finally, community structure at upstream and downstream sites was also compared using discriminant analysis. On the basis of this analysis, which uses taxa composition to measure community composition, there were no significant differences in community composition at upstream and downstream sites at any of the courses (Table 4).

The by course analyses were completed in order to determine if any differences in community structure trends (i.e. increases in EPT richness, taxa richness, or total abundance at upstream sites at one course and decreases at downstream sites at a different course) were related to different management practices used at each of the courses.

Table 5 shows a summary of comparisons of physical and chemical parameters at upstream versus downstream sites. The values of these physical and chemical parameters from the upstream and downstream sites were compared in order to determine if any natural variation in the ecosystem could explain changes in the community structure. Only alkalinity differed at between upstream and downstream locations, and while the difference was significant, it was most likely not great enough to explain any shifts in community structure.

Table 1: Calculations concerning numbers of individuals and families of invertebrates collected using a total of 649 leafpacks.

Parameter Calculated	Individuals	Families
Total number collected using leafpacks	42,557	79
Average number collected per leafpack	65.6	2.8
Least number collected per leafpack	0	0
Greatest number collected per leafpack	2,437	13

Table 2: Number of individuals of the eleven families of aquatic insects most commonly found in the leafpacks.

Family	# Upstream	# Downstream
Chironomidae	16,934	17,911
Simuliidae	2,035	2,721
Hydropsychidae	215	637
Elmidae	224	248
Capniidae	95	173
Tipulidae	59	133
Empididae	62	127
Coenagrionidae	10	90
Nemouridae	18	61

Table 3: Results of repeated measures analysis using One-Way ANOVA comparing EPT Richness, Taxa Richness, and Total Abundance at locations upstream and downstream of the courses.

Index	Approximate F-ratio	p-Value
EPT Richness		
Overall Analysis	1.5045	0.3348
Course 1	0.9848	0.03342
Course 2	3.9760	0.0635
Course 3	4.4952	0.0481
Course 4	3.2916	0.1072
Course 5	0.1071	0.7518
Taxa Richness		
Overall Analysis	47.2500	0.0205
Course 1	0.1409	0.7118
Course 2	2.2728	0.1511
Course 3	2.8592	0.1081
Course 4	0.0000	1.000
Course 5	0.4414	0.5251
Total Abundance		
Overall Analysis	11.5283	0.0009
Course 1	0.5576	0.4591
Course 2	13.8510	0.0009
Course 3	7.7003	0.0077
Course 4	2.2921	0.1412
Course 5	0.1752	0.6786

Table 4: Discriminant analysis results.

Course	Approximate F-ratio	p-Value
Course 1	17.624	0.0550
Course 2	1.045	0.5411
Course 3	0.653	0.7569
Course 4	0.900	0.5693
Course 5	0.900	0.5673

Table 5: Physical and chemical parameters measured.

Physical Parameters	Chemical Parameters
Depth	pH
Current	Alkalinity
Discharge	Hardness
Photosynthetic Active Radiation	Conductivity
Temperature	
Turbidity	
Dissolved Oxygen	

Benthic macroinvertebrate communities at sites upstream of the courses do appear to differ from those at sites downstream of the courses. Taxa richness and total abundance of the invertebrates is greater at sites downstream of the courses. However, there do not appear to be any major shifts in community structure. Those taxa that are the most common at the upstream sites are the same taxa that are most common at the downstream sites. In addition, discriminant analysis of the invertebrate communities did not show any significant differences in their structure based on taxa composition.

While the communities at upstream and downstream sites do appear to differ slightly, at least in terms of taxa richness and total abundance, the pesticide and nutrient data don't provide any explanation for these differences. The pesticides concentrations, even at their highest, at sites downstream of the courses are not close to toxic levels for fish and aquatic invertebrates. In addition, when the pesticide levels are high in the water columns from sites downstream of the course, the EPT richness and taxa richness of benthic macroinvertebrates from the same sites are higher than they are at the upstream sites. Also, the nitrate and phosphorus concentrations at sites downstream of the course are less than (in the case of nitrate) or equal to (in the case of phosphate) the concentrations at sites upstream of the course.

Objective 4. To determine the sublethal impacts of selected pesticides on benthic macroinvertebrates.

Progress and Results to Date:

Area golf courses routinely use fungicides for disease control; these fungicides include Daconil 2787, Bayleton, Aliette, Banol, and Subdue. Furthermore, the application of nitrogen and phosphorus is commonplace on area golf courses. Therefore, laboratory and field studies are being used to determine if pesticides and/or fertilizers influence consumption and decomposition of coarse particulate organic matter (CPOM).

Field studies were completed to study the decomposition and consumption of organic matter in streams associated with golf courses. Mesh bags containing predetermined amounts of leaf material were left in the field for various amounts of time during which the leaves were allowed to decompose or were subjected to consumption by benthic macroinvertebrates. At the end of the study period, the bags were removed from the stream and reweighed. From this information, the percentage leaf mass lost will be determined for sites upstream and downstream of the courses. Using this information, it is possible to determine if pesticides and/or fertilizers applied to golf courses are influencing the processing of organic matter via alterations in decomposition of the leaf matter by periphyton or consumption of the matter by benthic macroinvertebrates.

Laboratory studies included analysis of the decomposition of maple leaf discs in the presence of the several fungicides in order to determine if the presence of these fungicides inhibits decomposition of organic matter by fungi and bacteria. In addition, laboratory studies were completed in order to study the effect of the presence of fungicides on the consumption of maple leaf discs by peltoperlid stoneflies. It has been shown that consumers of organic matter are really using the periphyton growing on the organic matter as an energy source. Therefore, we are trying to determine if the presence of these pesticides has a sublethal affect on invertebrates through altering their consumption of organic matter- possibly due to altering periphyton growth on organic matter.

Proposed Research Schedule:

Objective 1**:** To measure the concentration of pesticides and nutrients residing in the water column of streams associated with golf courses.

Water samples from each of the courses will continue to be collected one to two times a month through the spring of 2000. These samples will be analyzed for nitrate and phosphorus in order to obtain a larger database which can be analyzed.

Objective 2**:** To measure the concentration of pesticides residing in the sediments and sediment porewater of streams associated with golf courses.

Completed.

Objective 3: To assess the impact of golf courses on stream macroinvertebrate communities.

Additional analyses of the benthic macroinvertebrate community data will most likely be completed in order to determine if differences in the community structure from sites upstream and downstream of the course can be detected. These analyses may include multivariate analyses and cluster analyses, such as TWINSPAN. In addition, the physical and chemical parameters and the community structure (taxa composition) will most likely be studied via a correlation analyses in order to try and determine the causes behind the shifts in the benthic community that have been detected (i.e. increases in taxa richness and total abundance).

Objective 4: To determine the sublethal impacts of selected pesticides on benthic macroinvertebrates.

Laboratory and field experiments on the processing of organic matter in the presence of pesticides and fertilizers have been completed. Processing of the leaf matter in order to obtain percentage mass lost from leaf bags will be completed by December, 1999, as will analysis of the data.

Current personnel:

Amy Soli - graduate research assistant
Chris Long, Alicia Reges, and Brian Hoffman - undergraduate laboratory assistants

Publications:

Soli, A.M.S. and Lamp, W.O. 1999. Benthic macroinvertebrate response to golf course management practices. Abstract 176, 47th Annual Meeting, North American Benthological Society, Duluth, Minnesota.

Soli, A.M.S. and Lamp, W.O. 1998. The response of benthic macroinvertebrates communities to nutrient and pesticide loading associated with mid-Atlantic golf courses. Abstract 228, 46th Annual Meeting, North American Benthological Society, Prince Edward Island, Canada.

Soli, A.M. 1998. Do golf course management practices impact benthic macroinvertebrate communities? Poster presentation at the 1998 UMCES/MEES Colloquium, Solomons, Maryland.

Soli, A.M. and Lamp, W.O. 1998. Do golf course management practices impact benthic macroinvertebrate communities? Poster presentation at the 1998 Annual Meeting, Entomological Society of America, Las Vegas, Nevada.