

## **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.

### **Cooperators:**

Dr. Kevin Mathias  
Dr. Laura McConnell  
Dr. Steven J. Lehotay  
Ms. Jennifer Harman-Fetcho

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. The sediment samples are being stored using methods required to maintain the integrity of any pesticides. We are now in the process of analyzing the samples using gas chromatography and mass spectrometry. These samples are being analyzed using protocols developed at USDA.

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, are being performed by calculating various community statistics for each golf course and site.

During 1997 and 1998, invertebrates were collected five times. During 1997, these samples yielded 24,555 individuals representing 46 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). No significant differences were seen in either taxonomic richness ( $P=0.59$ ) or invertebrate density ( $P=0.65$ ) when comparing upstream with downstream sites, illustrated in Figures 1 and 2. Of the physical and chemical parameters measured, only turbidity showed a trend across all golf courses; water from sites upstream from the courses were more turbid than water from downstream the courses.

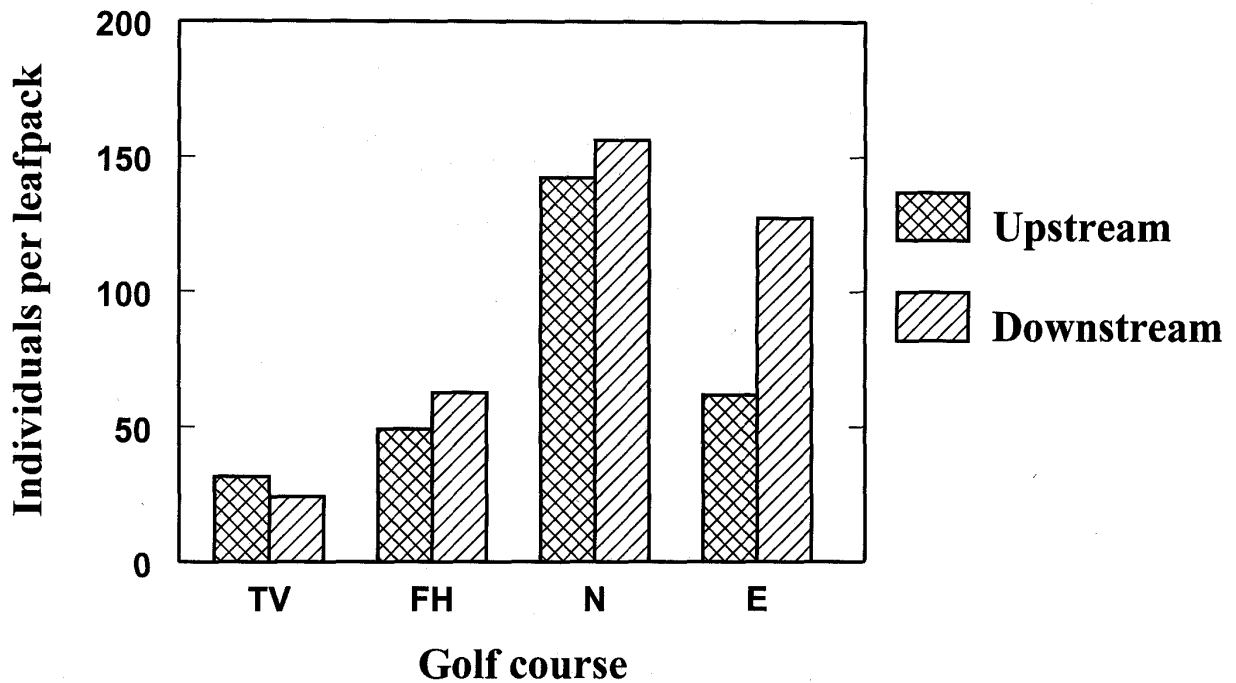
On the basis of this preliminary analysis, golf course management practices are not significantly impacting the invertebrate community. However, when one analyzes the trends seen in the invertebrate density and taxonomic diversity data, it is apparent that there is an increase in these two population indices at the downstream sites at three of the four courses, albeit, they are not significant increases. We will collect additional data and perform further analyses over the next year.

Area golf courses routinely use five fungicides: 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).

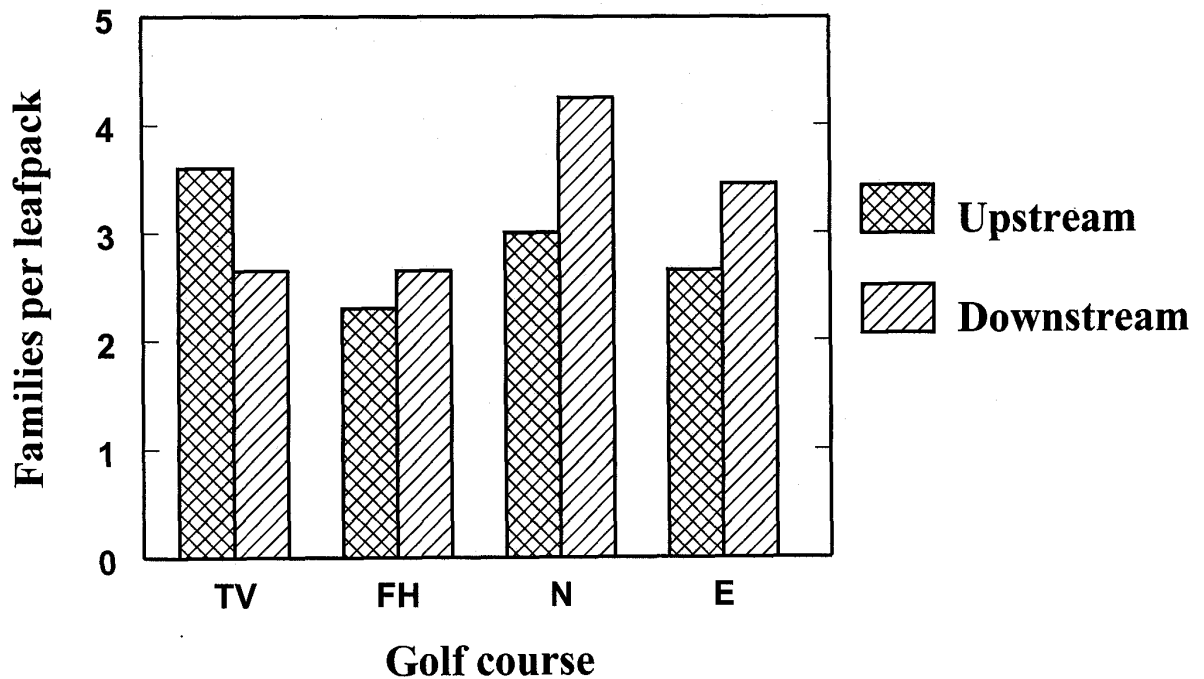
Specifically, field studies are being conducted to measure the decomposition and consumption of organic matter in our streams associated with golf courses. Mesh bags containing predetermined amounts of leaf material are left in the field for various amounts of time during which they leaves are allowed to decompose or are subjected to consumption by benthic macroinvertebrates. At the end of the study period, the bags are removed from the stream and mass is determined for the remaining leaf matter. Using this information, we can determine if 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 are being conducted to measure the decomposition of maple leaf discs in the presence of the five fungicides listed above. We will try to determine if the presence of these fungicides inhibits decomposition of organic matter by fungi and bacteria. In addition, laboratory studies will look at the effect of the presence of these fungicides on the consumption of maple leaf discs by 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.

**Figure 1: Average Invertebrate Density**  
All Sample Dates - 1997



**Figure 2: Average Taxonomic Diversity**  
All Sample Dates - 1997



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**Submitted by:**

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**Title of Project:**

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

**Principal Investigators:**

William O. Lamp, Department of Entomology  
Judd O. Nelson, Department of Entomology  
Amy M. Swope Soli, Ph. D. Candidate, M.E.E.S. Program

**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 runoff of chemicals from golf courses, a nonpoint pollution source, into surface running waters, and its impact on stream macroinvertebrate communities. Macroinvertebrates are excellent indicators of water quality, and play an important role in the trophic structure of streams. To accomplish these objectives, we have worked with four golf courses to study runoff of pesticides and nutrients, the effects of golf course management practices on benthic macroinvertebrates 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.

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 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.

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.**

##### **Methods:**

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. The proposal called for collecting water samples during five run-off events and analyzing these samples for several commonly used pesticides. In addition, the proposal included measuring nutrient levels once to twice every month and during the five run-off events.

Water samples are being analyzed for nutrients using the HACH DR890 Colorimeter. We are measuring nitrate, reactive (dissolved) phosphorus, total phosphorus, and fluoride concentrations. Except when prohibitive due to time or weather constraints, nitrate, reactive phosphorus, and fluoride levels are being measured in the field. Total phosphorus is measured using preserved water samples. Nitrate levels are being determined using a cadmium reduction method. Reactive phosphorus is being analyzed using ascorbic acid treatment and total phosphorus analysis uses an acid persulfate digestion followed by the ascorbic acid treatment. Measurement of fluoride levels utilizes SPADNS reagent. We are measuring the fluoride levels in order to determine if any elevated nutrient levels may be the result of sewage contamination instead of input from the golf courses. All analyses utilize protocols and reagents developed by HACH Company and are, except for the nitrate analysis, USEPA approved.

Water samples are being analyzed for pesticides using protocols developed by Dr. Laura McConnell, Dr. Steven J. Lehotay, and Jennifer Harman-Fetcho at the USDA in Beltsville, Maryland. Briefly, water samples are collected in properly cleaned steel canisters and are then filtered in order to separate the suspended materials from the water. The water samples are then analyzed for pesticides using solid phase extraction, gas chromatography, and mass spectrometry. The suspended materials that are trapped during filtration are saved, and it may be possible to process these materials such that they can also be analyzed for pesticides.

**Progress to Date:**

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 samples for pesticide analysis have also been collected five times following run-off events. The samples have been filtered and processed using solid phase extraction. We are now in the process of analyzing the samples using gas chromatography and mass spectrometry.

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

**Methods:**

The proposal called for the collection of sediment samples and their analysis for pesticides for five run-off events. The sediment samples are collected following ASTM guidelines and are being analyzed for pesticides following protocols developed by the Drs. McConnell and Lehotay and Jennifer Harman-Fetcho at the USDA. The sediments are collected from areas of soft sediment deposition in the stream bed using a corer. Briefly, analysis of the sediments includes the homogenization of the sediments followed by treatment with appropriate solvents in order to isolate the pesticides. The solvent-pesticide extract will then be analyzed for pesticides using solid phase extraction, gas chromatography, and mass spectrometry.

**Progress to Date:**

Sediments have been collected from the stream bed following five run-off events. These sediments are being stored using methods required to maintain the integrity of any pesticides that may be in the sediments. Within the next couple of months, the sediments will be processed and analyzed for pesticides.

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

**Methods:**

Quantitative sampling is being conducted to statistically compare community structure. Sampling is 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 are collected using 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 the following water quality parameters measured: water temperature, water depth,, above-water photosynthetic active radiation (using a ceptometer), current rate (using a pygmy current meter), dissolved oxygen, conductivity, and pH. These parameters are

measured in order to determine if any changes we may see in the benthic macroinvertebrate community are due to environmental variation or if they are due to influences from chemicals applied to the golf courses.

In the laboratory, invertebrates in each sample are sorted, preserved, and identified to family level. Community comparisons, using taxonomic diversity and invertebrate density, are being performed by calculating various community statistics for each golf course and site. These indices include of richness and evenness compared by analysis of variance with each stream as a replicate and upstream/downstream of the golf course as treatments.

#### Progress to Date:

The proposal called for the sampling of invertebrates for benthic community analysis five times a year. Invertebrates were collected five times during 1997. They have also been collected four times during 1998 and we are finishing our fifth collection during November. The insects collected during 1997 have been counted and identified; we are in the process of counting and identifying the insects collected during 1998. Preliminary results have been obtained from the 1997 data.

#### Results:

Table 1 provides a summary of the invertebrate samples and Table 2 indicates the most abundant types of invertebrates collected. No significant differences were seen in either taxonomic richness or invertebrate density when comparing upstream with downstream sites (Table 3, Fig.1, Fig. 2). Table 4 shows a summary of comparisons of physical and chemical parameters at upstream versus downstream sites.

Table 1: Calculations concerning numbers of individuals and families of invertebrates collected using a total of 287 leafpacks.

<u>Parameter Calculated</u>	<u>Individuals</u>	<u>Families</u>
Total number collected using leafpacks	24,555	46
Average number collected per leafpack	85.2	3.12
Least number collected per leafpack	0	0
Greatest number collected per leafpack	2,441	12

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

**Top 11 Aquatic Insect List**

<u>Family</u>	<u># Upstream</u>	<u># Downstream</u>
Chironomidae	8,357	8,705
Simuliidae	1,782	2,510
Hydropsychidae	137	160
Elmidae	142	153
Capniidae	135	134
Empididae	37	84
Tipulidae	23	75
Coenagrionidae	8	60
Baetidae	15	34
Taeniopterygidae	17	16
Heptageniidae	9	24

Table 3: Results of ANOVA tests using Proc Mixed, SAS comparing the number of individuals and families at upstream sites with downstream sites.

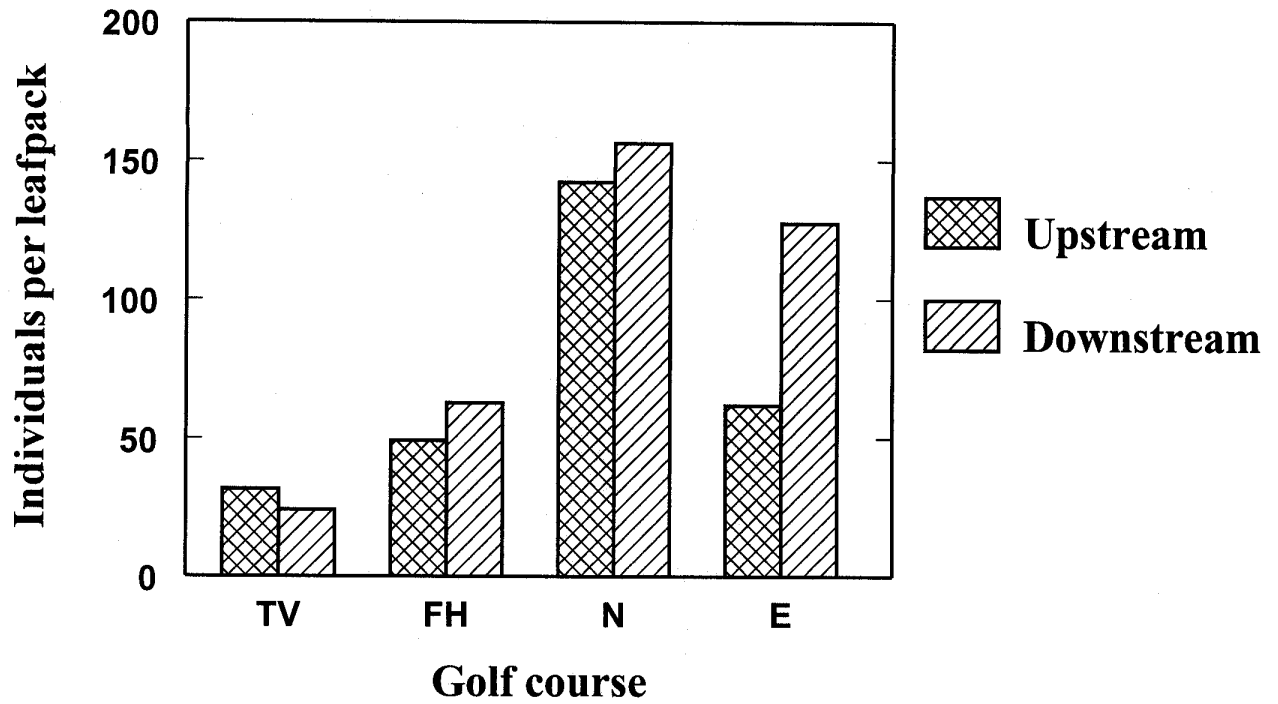
**Analysis of Variance**

<u>Source</u>	<u>NDF/DDF</u>	<u>F-Value</u>	<u>Prob.</u>
<u>Individuals per leafpack:</u>			
Location	1/6.31	0.23	0.65
Period	4/22.6	0.94	0.46
L*P	4/22.6	1.10	0.38
<u>Families per leafpack:</u>			
Location	1/5.93	0.33	0.59
Period	4/22.4	3.43	0.02
L*P	4/22.4	0.37	0.83



**Figure 1: Average Invertebrate Density**

All Sample Dates - 1997



**Figure 2: Average Taxonomic Diversity**

All Sample Dates - 1997

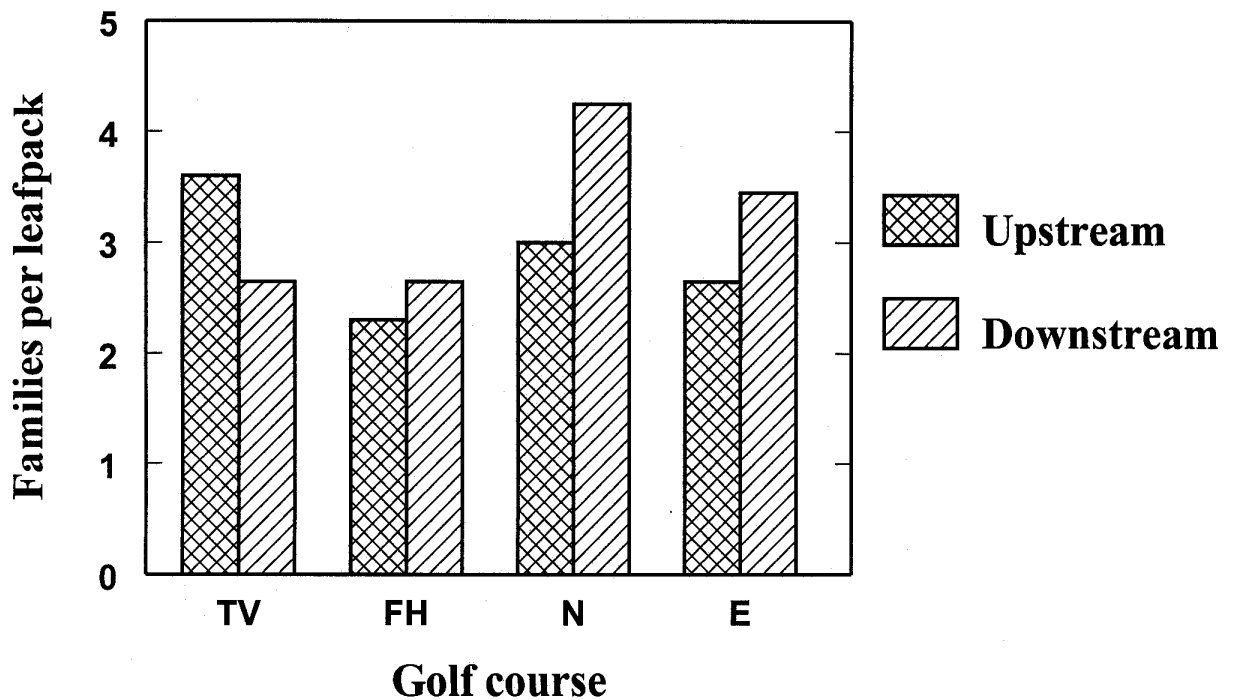


Table 4: Summary of trends identified when comparing data on various physical and chemical parameters of upstream sites with downstream sites.

<u>Physical Parameters</u>	<u>Trends Identified</u>
Water temperature	similar at upstream and downstream sites
Current	no trends identified
Depth	no trends identified
Turbidity	usually greater at upstream sites
PAR	no trends identified
<u>Chemical Parameters</u>	<u>Trends Identified</u>
Dissolved oxygen	similar at upstream and downstream sites
Alkalinity	usually similar at upstream/downstream sites
Hardness	no trends identified
pH	no trends identified

On the basis of this preliminary comparison, we conclude that golf course management practices are not significantly impacting the invertebrate community. However, when one analyzes the trends seen in the invertebrate density and taxonomic diversity data, it is apparent that there is an increase in these two population indices at the downstream sites at three of the four courses, albeit, they are not significant increases (Table 3, Fig. 1, Fig. 2). These trends leave us with several questions:

1) Are these minor differences the result of environmental influences, such as the chemical and physical parameters?

In order to answer this question, we looked at the physical and chemical parameters in order to see if any trends could be identified when comparing the data from upstream and downstream sites. In general, for most of the parameters, no trends were identified or the parameters were similar at both sites (Table 4). However, turbidity was usually greater at upstream sites. Therefore, analysis of the literature needs to be done in order to determine if the magnitude of the difference is great enough to explain the differences in diversity and density.

2) Could analysis of a different type better show the impact of management practices on invertebrate community?

In order to answer this question, the data will be studied using different types of analyses including TWINSPAN and biomonitoring indices such as the family biotic index and percent contribution of dominant family.

3) Could this general increase in diversity and density indicate increased productivity at downstream sites due to nutrient enrichment?

It has been suggested that the increase in taxonomic diversity and invertebrate density seen at the sites downstream of a golf course is indicative of increased productivity due to nutrient enrichment. This nutrient enrichment is probably the result of runoff 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. This is because these nutrients 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. Therefore, in response to this suggestion, we decided to undertake a study looking at periphyton productivity in our golf course streams.

Frosted acrylic plates are being used as a substrate on which periphyton can colonize and grow. PVC frames were constructed from which four acrylic plates on standard area are suspended. The frames were constructed such that they can float on the water's surface while the plates are submerged a couple of inches below the surface. One PVC frame is placed at each of the upstream and downstream sites in areas that are similar in terms of light penetration through the canopy.

The plates are being left in the stream for a period of four weeks. At the end that time period, they will be collected and the periphyton growing on one side of the plates will be scrapped of the plates, dried, and weighed. Ash-free dry mass will then be measured by ashing the periphyton. This data will then be analyzed to determine if there is increased productivity at the downstream sites.

The data from this study will be used in conjunction with the data collected from the field study on decomposition to determine if nutrient enrichment in streams associated with golf courses can be shown through increased periphyton productivity.

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

Methods:

Area golf courses routinely use five fungicides: 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 are looking at the decomposition and consumption of organic matter in our streams associated with golf courses. Mesh bags containing predetermined amounts of leaf material are left in the field for various amounts of time during which they leaves are allowed to decompose or are subjected to consumption by benthic macroinvertebrates. At the end of the study period, the bags are removed from the stream and ash-free dry mass is determined for the remaining leaf matter. Using this information, we can 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 that are being completed this fall will include analysis of the decomposition of maple leaf discs in the presence of the five fungicides listed above. We will try to determine if the presence of these fungicides inhibits decomposition of organic matter by fungi and bacteria. In addition, laboratory studies will look at the effect of the presence of these 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.

**Progress to Date:**

As of October, 1998, the field experiments have been run four times. The leaves used in the experiments have been dried and are in the process of being ashed. After the leaves have been ashed, the data can be analyzed in order to determine if there is any evidence of pesticides and/or fertilizes affecting the processing of organic material in stream ecosystems. Preliminary laboratory experiments have been conducted in order to refine the protocols being used in our consumption and decomposition studies.

**Proposed Research Schedule:**

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

We plan to continue our routine sampling of nutrient levels through 1999 at the four sites on each golf course stream. In addition, we will sample during key run-off events during 1999 for analysis of pesticides.

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

Following analysis of samples taken during 1998, we will assess the need to collect more of these samples during 1999.

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

We will continue to sort, identify, and count invertebrates from our 1998 samples, and perform additional statistics on data from 1997 and 1998. Analysis is expected to indicate where further data is needed. Periphyton experiments using plexiglass plates will be repeated during 1999, concurrent with more frequent nutrient analyses.

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

We will finish sample processing for the decomposition and consumption field studies, and perform analyses of the data. Additional field experiments will be conducted if warranted. In addition, we will complete the development of a laboratory bioassay for decomposition and consumption, and follow with specific experiments with five fungicides.

**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. 1998. The response of benthic macroinvertebrates communities to nutrient and pesticide loading associated with mid-Atlantic golf courses. Abstract 228, 46<sup>th</sup> 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.