Focussing Review

Collaborative Research in Invertebrate Biology and Analytical Chemistry at Lafayette College

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Abstract

This paper describes a unique collaborative undergraduate research program in analytical chemistry and invertebrate biology that was begun at Lafayette College in the 1980s by Sherma (an analytical chemist) and Fried (a parasitologist). The program has resulted in 64 publications co-authored with 76 undergraduates in various peer-reviewed chemical and biological journals. Most of our collaborators have been junior and senior chemistry, biology, and biochemistry majors. The research has involved mainly studies on the chemical content, i.e., lipids, phospholipids, pigments, sugars, amino acids, and metals, of parasitic flatworms and medically important snails. We have also analyzed pheromones released by parasites and snails and the chemical constituents of various food items. Numerous analytical techniques have been used, including thin layer chromatography, gas chromatography, GC/mass spectrometry and atomic emission and absorption spectrometry, along with microscopic techniques such as cryostat microtomy and transmission electron microscopy. The students have assumed a major role in all aspects of the collaborative work, including modifying research protocols, most of the hands-on work, data analysis, literature searching, preparation of figures, and writing of initial drafts of papers for publication. The operation of our program and its numerous benefits to both the students and their mentors are discussed in the article.

Keywords: invertebrate biology, analytical chemistry, parasitology, malacology, Lafayette college, undergraduate research, TLC

1. The Importance of Research at an Undergraduate College

Undergraduate research is the most powerful tool for engaging students in active learning and is the culminating, capstone experience in an undergraduate liberal arts education. Through research, students learn the process and spirit of science as they can in no other way, and they have opportunity for an integration and depth of learning unmatched by formal courses. Students become colleagues of faculty in the teaching and learning of science and collaborators in the development of new knowledge through the research project, and they experience first-hand the excitement of discovery. No longer are they just working for a grade in a course, but they are challenged to think for themselves while working on a project that they believe is interesting and important. Students get to see how scientists make false starts and mistakes before finding the right course in a research project, and they gain the spirit of inquiry, initiative, integrity, independence, sound judgement, patience, persistence, alertness, imagination, and intellectual flexibility.

Research transforms students from mostly passive learners sitting in a classroom into young scientists who translate the theoretical material they learn in courses into practical application in a research project and thereby assume a new status on campus and in the larger scientific establishment. Students often think of postgraduate research for the first time after becoming excited and motivated by their research, and they are much better prepared for their future careers as a result of the research experience. Teaching and research are mutually reinforcing activities for faculty, who become more dynamic and up-to-date classroom teachers as a result of the mentoring process and can experience the satisfaction and excitement of watching the students grow to their full potential. The intellectual challenges and discoveries of research help faculty to bring a special energy and enthusiasm into the classroom that reaches students at all levels.

Although programs involving student research are common today in completely and predominantly undergraduate colleges, the active
research programs being carried out in the Chemistry and Biology departments in the late 1950s and early 1960s when we arrived at Lafayette College were quite unusual. Most undergraduate research programs involve projects in one discipline, such as analytical chemistry or in one course (e.g., molecular biology, parasitology, organic chemistry, and physical chemistry), supervised by a single faculty member with expertise in that area. The research projects may lead to an oral presentation by the student or faculty member at a local, regional, or national scientific or education conference, but seldom to a research paper published in a peer-reviewed journal and less often to multiple papers co-authored by the research student. Described below is the collaborative analytical chemistry-invertebrate biology undergraduate research program that we began in the early 1980s, in addition to our thriving individual programs in analytical method development (Sherma) and parasitology (Fried), that is unique in its interdisciplinary nature and the numbers of students that have been involved and student co-authored publications in peer-reviewed journals that have resulted.

2. The Collaborative, Interdisciplinary Program

In an earlier paper [1], we reviewed our collaborative studies in analytical chemistry-parasitology during the 1980s. In brief, these studies included analyses of lipids in parasitic flatworms and snails, of presumptive pheromones in parasitic flatworms, and the effects of dietary changes on the lipid composition of medically important snails. The studies all used qualitative and quantitative thin layer chromatography (TLC) or high performance thin layer chromatography (HPTLC). Our first collaborative study with a Lafayette College undergraduate used TLC with scanning densitometric measurement of the separated zones to examine the effects of starvation on the neutral lipid composition of the medically important planorbid snail *Biomphalaria glabrata* [2]. Since that work was done, we have published 64 collaborative research papers with 76 Lafayette

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<th>Techniques</th>
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TLC  Neutral lipids in two strains of H. trivolvis snails were compared Park, Y.Y.; Fried, B.; Sherma, J. Comp. Biochem. Physiol. 1991, 100B, 127-130.


AAS  Calcium analysis of freshwater snails Boston, C.J.; Layman, L.R.; Fried, B.; Sherma, J. The Veliger, 1994, 37, 121-123.


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<td>TLC</td>
<td>Analysis of lipids in the intestinal mucosa of mice infected with echinostomes</td>
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TLC  Analysis of neutral lipids in larval echinostomes  Marsit, C.J.; Fried, B.; Sherma, J. *J. Helminthol.* 2000, 74, 365-367


Note: TLC = classical thin layer chromatography and/or high performance thin layer chromatography. Abbreviations: AAS = atomic absorption spectrometry; GC = gas chromatography; GC/MS = gas chromatography/mass spectrometry; HPLC = high performance liquid chromatography; ICP-AES = inductively coupled plasma-atomic emission spectrometry; LM = light microscopy; TEM = transmission electron microscopy.
College undergraduate chemistry, biology, and biochemistry majors named as co-authors. A chronological listing of our joint publications with students is presented in Table 1.

We have analyzed numerous types of samples in our research projects, but most have involved parasitic flatworm material from experimentally infected mice and from the tissues of medically important snails. For example, we have examined neutral lipids in the medically important trematode *Echinostoma caproni* maintained in mice on a high fat diet [3] and also studied neutral lipids in the tissues of *B. glabrata* maintained on various diets [4]. We have also examined neutral lipids in the blood (hemolymph) of *B. glabrata* snails [5] and in the serum of mice infected with *E. caproni* [6]. Because of our interest in chemotactants (pheromones) produced by either flatworm parasites or their snail intermediate hosts, we have examined water conditioned by snails (known as snail conditioned water or SCW) for the presence of various presumptive lipophilic and hydrophilic pheromones (e.g., neutral lipids [7] and amino acids [8], respectively). We have also analyzed the chemical contents of various diets used to feed our snails [4] and determined the lipid composition of tissues and organs of domestic chicks infected with trematode parasites [9] and of hen’s eggs labeled in markets as being "low fat" compared with normal hen’s eggs [10].

In all of these studies and those mentioned later, methods for sample handling and preparation and for determination of the analytes had to be developed and validated.

A variety of analytical techniques have been used by our students in their research, including TLC and HPTLC with scanning densitometry, gas chromatography, and GC/mass spectrometry for qualitative and quantitative determination of organic compounds, and inductively coupled plasma-atomic emission spectrometry and flame and graphite furnace atomic absorption spectrometry for determination of metals. These techniques have been useful in providing data related to our work on the chemical constituents of medically important trematodes, pheromones released from trematodes and snails, and the chemical constituents in the diets that we have used to maintain the snails. We have used cryostat microtomy for the histochemical analysis of lipids, and transmission electron microscopy for the ultrastructural analysis of tissues of snails maintained on high-fat diets. We have also used various in vitro bioassays to determine the effects of pheromones on the behavior of parasitic worms and medically important snails.

We have examined various analytes in our projects, including neutral lipids (sterols, triacylglycerols, cholesteryl esters, free fatty acids, and mono- and diacylglycerols) and polar lipids (phospholipids and glycolipids) in trematodes, snails, and host tissues parasitized by either larval or adult trematodes [11,12]. Various metals have been determined in both invertebrate and vertebrate snail hosts affected by trematodes [13,14]. Recent work has examined sugars in a variety of uninfected medically important snails and those infected with larval trematodes. We have reexamined reports in the literature stating that the hemolymph and tissues of certain marine and freshwater snails contain trehalose as a major non-reducing sugar, and our research proved that, at least for the species of these snails that we have studied, trehalose is not detectable at the sensitivity limits of our analytical methods [15].

These projects were valuable in showing students that published papers do not necessarily report accurate data and that confirmation of certain questionable results can be an important area of research. We have identified and quantified lipophilic plant pigments (carotenoids, xanthophylls, and chlorophylls) in snails with and without larval trematode parasites, and in conjunction with these studies we analyzed the pigment content of spinach leaves [16,17].

Research papers co-authored by our students have been published in a wide assortment of peer-reviewed journals. These have included separation-science journals (e.g., *Journal of Planar Chromatography - Modern TLC, Acta Chromatographica, Journal of Liquid Chromatography and Related Technologies, and Biomedical Chromatography*), an analytical chemistry journal (*Microchemical Journal*), biological journals (*Comparative Biochemistry and Physiology, The Veliger, Journal of Parasitology, Transactions of the American Microscopical Society* (now titled *Invertebrate Biology*, *Parasitology Research, Journal of the Helminthological Society of Washington* (now titled *Comparative Parasitology*), and *Parasite*) and ecology journals (*Comparative Parasitology* and *Parasite*). Almost every student involved in our research program has been named as a co-author with us on a published research paper; many papers include two or more student co-authors involved in the same project, and students are often cited as co-authors on more than one paper. Although not a typical case, one especially talented and highly motivated student who has worked with us for 2.5 years has six papers already published or in-press, with the prospect of two more papers based on her Honors thesis research and an additional project she has not yet completed.

New students are selected to join our program by coming to us to express interest after speaking with our current or former research students or through our invitation based based on meeting them in our courses. Most of our students are AB or BS Chemistry, Biology, or Biochemistry majors, but we have involved some students with non-science majors, such as Philosophy, Anthropology, or Language, who have taken science courses and have interest in...
Scientific research. Some of the students have worked for academic credit during the school year in our Honors or Independent Study courses, while others have been supported by the college EXCEL Scholar program for part-time research during the school year or full-time research during the three-week interim session between semesters or for 10 weeks during the summer. Some students have done both credit and EXCEL research with us at different times. The EXCEL Scholar program provides a stipend (plus free room during the interim and summer) from college funds, and we have also supported some students from intramural or extramural grants made to one or both of us. Some of our students have worked with us as postgraduates for various periods of time (usually from two to eight weeks). Their work has been supported by special college funds available to us as recipients of endowed chairs at Lafayette College.

Our students receive important training in many different areas. We personally help train new students but rely heavily on our experienced students to provide training for the new students. Training of students by other research students is an extension of the group or team learning approach that is currently in formal courses.

Most students who enter our program have had courses in invertebrate biology, analytical chemistry, biochemistry, and molecular biology, in addition to the usual first level courses in biology, chemistry, physics, and mathematics. These courses provide valuable pretraining for our research students. The types of additional training needed are in [1] care and maintenance of animals (both invertebrates and vertebrates), [2] biological sample preparation, [3] analytical determinations, [4] data interpretation and report writing, and [5] writing drafts for publication (students generally initiate the first draft of papers describing their completed research, including preparation of publication-quality line drawings, half-tones, and computer-generated scans of chromatograms and densitograms). Our students are also taught to do initial literature searches using computer-based and traditional methods. The extent of training is in accord with their backgrounds when they initiate work with us. We have had students begin their work with us as sophomores, but more typically we involve juniors and seniors in our program. The types of formal courses that students have will determine the training they need in specific areas. For example, students who have taken a course in analytical chemistry will need less specialized training in the use of volumetric glassware for solution preparation and in instrument operation. Conversely, students with a course in invertebrate biology will need minimal training in the care and handling of the various invertebrates we use. Students who use vertebrate animals are trained in their care and maintenance by one of us (BF) following institutional guidelines for experiments involving the handling of warm-blooded animals.

After they graduate, our students pursue various fields of endeavor. Most enter medical, dental, or veterinary schools, while others choose a graduate school to pursue an advanced degree in chemistry, biochemistry, or biomedical sciences. Some of our students enter the job market directly upon graduation and become involved in research in industrial, hospital, or governmental laboratories or non-research positions such as pharmaceutical marketing.

Some of our students who graduated from Lafayette College in 1997-2000 are currently in graduate or professional schools. Our students who graduated prior to 1996 are employed in a variety of professions including physicians, dentists, veterinarians, lawyers, chemists, biomedical scientists, and pharmaceutical sales representatives. Many of our students have had high grade point averages (>3.5-4.0/4.0) and some were among the academically elite of their class. Others had more modest grades (B-C range), but all have had considerable success in our program and have benefited from it greatly. Our more experienced students help us instruct new students who enter the program. Biology and chemistry majors who ordinarily would not meet in the classroom setting are able to interact and share diverse knowledge and experiences. A symbiotic, mutually enriching relationship usually forms between the biology and chemistry majors working together in our laboratories. Chemistry students, much to their surprise, learn about the diverse nature of biological data with very high standard deviations around the mean because of the nonreproducibility of the replicate biological samples. Biology students are exposed to analytical training they would not ordinarily receive as undergraduates and have a better knowledge of statistical analysis and handling sophisticated analytical instrumentation they would not encounter in their courses.

We recommend projects to our students based on our experience rather than letting them choose their own research topics. This approach is the most productive and rewarding for students because the work is likely to be completed and lead to publishable results in the time available. We have seen much effort expended in programs based on student-initiated research at undergraduate colleges that has not led to discovery of any new knowledge worthy of publication because of lack of expertise on the part of the supervising faculty member and absence of required equipment and supplies.

It is an important aspect of our program that students are not laboratory assistants who just do menial "slave labor" related to our own research projects (e.g., literature searching, cleaning glassware, animal maintenance, or operating an instrument for routine analysis) as in some other programs, but they do essentially all of the research
work. When the students complete their projects, they feel that they have accomplished something really meaningful and important, and the importance is confirmed through publication. Students are usually named as the first or senior author on the resultant paper to recognize the overriding extent of their contributions.

Our students sometimes present their research orally at meetings, but the focus of our program is on publication in peer-reviewed journals. Publications are critically important for students when they apply for medical or graduate schools or for employment upon graduation. Our students are taught that research performed but not published is incomplete and the results are of no use to other scientists, and they learn the long-standing tradition of scientists exposing their research results to the scrutiny of expert referees during the peer-review process. In short, our students learn that publication is the necessary culmination of successful research. A co-authored publication helps to launch the career of the student scientist and enhances the reputation of the college.

The purpose of this article is to illustrate one possible program for joint research involving chemistry and biology faculty and students. Although our particular specialities have blended together particularly well, we are confident that similar collaborations are possible in other research areas and could enhance the research opportunities available to students at undergraduate colleges with limited resources and number of faculty compared to universities, as well as undergraduates in universities worldwide. For example, we have collaborated with a biologist specializing in herpetology and several biology majors to publish a paper involving the analysis of scent gland lipids from snakes [18]. In addition, joint research in materials science has been published involving physical chemistry and chemical engineering professors working together with chemistry and chemical engineering majors at Lafayette.

We intend to continue this program into the future and will try and involve one or two chemistry majors and/or one or two biology majors per year in this program. New instrumentation will be introduced for analyses as it becomes available. We will continue to explore new areas on topics related to the chemical analysis of invertebrates, particularly gastropod molluscs and parasitic flatworms. We will also continue to explore areas of research related to the pathobiochemical effects of larval trematodes on gastropod molluscs.

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References