Integration of Stable Isotopes across the Sciences (ISIS): Instrumentation for BioGeochemistry and Environmental Studies

Results from Prior NSF Support:

Keene State College has recently received support from the NSF for reforms in science education and curriculum development, including "Project INSPIRE: Investigations in Science for Pre-service teachers: promoting and producing Inquiry-based Relevant Exercises" (Sally Jean, PI, NSF Award #0088646), and "Geochemical Analysis across the Geology Curriculum and in related courses in Chemistry and Environmental Studies" (Timothy Allen, PI, NSF Award #0087860). These projects have only just started (May 1, 2001 and June 15, 2001, respectively), so we do not have any results to report yet.

Overview and Goals:

In the past two decades stable isotope analysis has facilitated new levels of understanding in diverse fields such as geology, hydrology, ecology, plant physiology, environmental science, and forensic science. Variations in the natural abundance of stable isotopes have been used to reconstruct paleoclimate (e.g. Koch 1998), determine the temperatures of metamorphism in rocks (Matthews 1994), trace water movement through the hydrologic cycle (e.g. Clark & Fritz 1997), determine the migration patterns of birds and monarch butterflies (Chamberlain et al. 1997, Wassenaar & Hobson 1998), evaluate resource partitioning and competitive interactions in plants (e.g. Gebauer & Ehleringer 2000), determine the sources of groundwater contamination from fertilizer or animal waste (e.g. Macko & Ostrom 1994), and to trace the origins of cocain (Ehleringer et al. 2000).

Although stable isotope analysis provides a powerful tool to explore sophisticated and exciting questions, few undergraduates have the opportunity to learn about and have hands-on experience with this modern technique. At present, faculty in Biology, Geology, and Environmental Studies at Keene State College use lectures and discussions of research papers to introduce students to the concept of stable isotopes and how they have been used to increase our understanding of patterns and processes from sub-cellular to global scales. However, content-oriented coverage in a lecture can be improved upon as a means of engaging students (Enns 1993, Allen et al. 1996). It is the combination of content, process, and application of concepts that attracts many students to and retains them in scientific careers (Felder 1993). We plan to expand and strengthen our science curriculum by developing inquiry-based laboratory and field exercises integrating modern stable isotope analytical techniques as a means to motivate student learning and to increase their understanding of basic concepts and principles (Lawson et al. 1990, Sundberg & Monaca 1994). This is consistent with national trends in science education towards student-centered and active participatory learning (e.g. Markovics 1990, Culotta 1994,). Students will collect, analyze, interpret, and apply stable isotope data through field and laboratory exercises in several courses in Biology, Geology, and Environmental Studies. These exercises are being designed by adapting a combination of exemplary models from other institutions (see below), examples from the published literature (e.g. Lajtha & Michener 1994, Clark and Fritz 1997), and from models for student-centered, inquiry based laboratories as exemplified in courses at the Natural Science Department at Hampshire College, Massachusetts (McNeal & D'Avanzo 1997).

The goals of this project are to (1) strengthen the science curriculum by providing new motivation for student learning and development of research projects; (2) increase the involvement of our students in modern scientific investigation and develop their scientific and technical skills (e.g. the ability to think independently and ask meaningful questions, to apply the scientific method to new situations, to understand the limitations of methodology, to comprehend and integrate information from the primary literature, and to communicate findings); and (3) enhance students' integrated understanding of biological and earth systems and their processes on different temporal and spatial scales. The specific content goals will vary with the course students are taking (see below). Our ultimate long-term goal is to train students to be broad thinking, critical, and experienced scientists who are well prepared for entering the professional work force or graduate school.

An Isotope Ratio Mass Spectrometer (IRMS) along with associated vacuum sample preparation lines will provide students and faculty from Biology, Geology, and Environmental Studies with an analytical tool to quantitatively address relevant and scientifically interesting problems. The new generation of IRMS is relatively easy to operate and maintain and therefore opens new educational research opportunities for undergraduates. The IRMS will facilitate active involvement of students in scientific investigations in several classes across the science curriculum.

Our specific objectives include: (1) strengthening introductory and intermediate-level laboratory courses such as *Ecology and Evolution*, *Environmental Geology, Glacial Geology, Stratigraphy, Hydrogeology,* and *Paleontology* by adding inquiry-based laboratory exercises involving stable isotopes; (2) re-designing upper-level courses in *Geochemistry, Physiological Ecology,* and *Community and Ecosytems Ecology* to include a new analytical laboratory component integrating research projects; and (3) supporting student-faculty cooperative research projects in *Independent Study*, and student projects in the Environmental Studies *Junior/Senior Seminar*.

Project Setting:

Keene State College (KSC) is a public four-year liberal arts college located in rural southwestern New Hampshire with a tradition and strength in teacher preparation. We have approximately 4500 full and part-time matriculated undergraduates from across New England, as well as about 90 graduate students (in Education). Approximately 40 to

50 % of each entering class are first-generation college students and 10 % are older than 25 years.

As a liberal art institution KSC is committed to rigorous student engagement in the Sciences, Mathematics, and Technology. We recognize the fundamental importance of science literacy for all students specifically for quality teacher training and student preparation for the rapidly expanding technological market. KSC is committed to this goal at every level of the institution. This is indicated by (1) construction of new laboratory space that facilitates collaborative group work (summer 2001) (see letter of support from the Dean of Sciences, Appendix); (2) recent restructuring of curriculum to include a small-group, active learning, case study approach (Environmental Studies), or to involve students into inquiry based laboratory and field research projects (Biology, Geology); and (3) a college-wide Science Education Initiative composed of science and education faculty to incorporate new pedagogy in training prospective K-12 teachers.

Biology, Geology, and Environmental Studies provide critical contributions to the science education at KSC (together the three departments have 181 majors), and to female students in particular (who comprise in average 54 % of these majors). Biology and Geology offer major and minor programs, attract a large proportion of the Science-Education majors (prospective K-12 teachers), contribute significantly to the interdisciplinary major and minor program in Environmental Studies (ENST), and serve a large number of non-science majors through the college's General Education program. The ENST program is an interdisciplinary program, which draws on contributions from Biology, Chemistry, Geology, Geography, and Economics as well as from English, Mathematics, and Social Studies. The PI (Renate Gebauer) shares an appointment between Biology and ENST (50:50), the first Co-PI (Tim Allen) shares an appointment between Geology and ENST (75:25), while the second Co-PI (Steve Bill) has a full time appointment in Geology.

Detailed Project Plan:

Exploring the variation in stable isotope distribution provides an excellent way to (1) involve students into the process of scientific inquiry, (2) study biological and geological processes and how they are affected by human activities, and (3) highlight the interconnection between different disciplines. We will adapt exemplary models for the use of stable isotopes in the classroom from Saint Louis University (http://www.eas.slu.edu/isotope/isotope.html), the University of Utah (http://ehleringer.net/bio_6473), Dartmouth College (see attached letter of support by Ross Virginia, Appendix), and the published literature (Ehleringer et al. 1993, Lajtha & Michener 1994, Clark & Fritz 1997 and references therein) to develop and design the laboratory and field exercises. Approaches practiced in courses at the Natural Science Department at Hampshire College, MA (NSF Awards # 9971486 and # 9980519, McNeal & D'Avanzo 1997) will serve as models for the student-centered, inquiry based approach.

Saint Louis University previously received NSF- support for incorporating stable isotope exercises into the undergraduate curriculum in geochemistry (DUE-ILI # 9751139, CCLI-A&I Award # 9952256). The project successfully showed that undergraduate students are capable of collecting, preparing, and analyzing stable isotope data and interpreting the data in a meaningful way. The University of Utah course in stable isotope ecology provides an excellent model of how to connect inquiry based field exercises with the wide range of topics covered in lecture class, as well as how to teach the methods and approaches used in stable isotope analyses (e.g. sample preparation and the use of the IRMS). It also provides a comprehensive reading list for a wide range of applications of stable isotopes. We plan to modify the field exercises of the University of Utah to New England ecosystems. We will further modify the University of Utah model (aimed at graduate students and post-docs) for undergraduates. Specifically we will (1) reduce the amount of theory and content covered and (2) emphasize the process of scientific investigation and the development of scientific and technical skills.

The faculty at Dartmouth College have developed a course on “Isotope Tracers in the Environment” that covers a wide range of applications in biology and geology (see letter from Ross Virginia, Appendix). We do not envision developing a similar dedicated isotope course, but we will integrate ideas from Dartmouth’s course. Additionally, we will modify exercises from the published literature (see below).

The incorporation of hands-on stable isotope techniques into our curriculum complements our pedagogical approach of creating a cooperative and engaging learning environment across the sciences (Beiersdorf & Beiersdorf, 1995).

In **introductory courses** (*Ecology and Evolution, Environmental Geology*) students will develop local field projects that explore the spatial or temporal *patterns* of stable isotope distribution. Students will work in groups of 3-4 to generate hypotheses based on a combination of background reading, concepts covered in the lecture portion of the class, observations in the field, and students' own interest. The entire class will reach a consensus on the hypothesis to be tested and collectively design an appropriate sampling scheme. Individual groups will, however, collect and be responsible for their own sample(s). During the next week's lab session we will acquaint students with the basic operation of the IRMS and students will operate the IRMS to analyze the stable isotopes of their already prepared group sample(s) (we will use experienced student workers hired by the Science Division to prepare the samples). The class data set will be made available on the course website and students will be asked to describe, interpret, and discuss the class dataset in their lab reports.

In **intermediate-level** courses (*Hydrogeology, Paleontology, Glacial Geology, Stratigraphy*), students will address similar questions about the patterns of stable isotope distribution and lab exercises will be structured similarly to the introductory courses. In addition, students will also learn techniques of safe extraction and preparation of samples at the vacuum lines and prepare their own samples.

Course-specific examples for both introductory and intermediate levels follow:

Bio 252/256 Ecology and Evolution (Gebauer) There are numerous lab and field exercises where stable isotopes are useful to increase students' understanding of concepts such as material flow in food webs and trophic level interactions, variation in photosynthetic pathways and changes in plant water use efficiency along environmental gradients, the contribution of nitrogen fixation to the total nitrogen economy of plants, and patterns of nitrogen cycling in ecosystems, etc. Students would collect organisms in the KSC campus pond to determine from nitrogen and/or carbon isotopes whether the organisms are herbivores or feed at higher trophic levels (adapted from University of Utah).

Geol 315 Environmental Geology (Allen) and *GEOL 460 Hydrogeology* (Allen) Stable isotopes are critical tools "to address problems related to ground water as a sustainable resource, and in particular to recharge, delineation of flow systems and quantification of mass-balance relationships" (Coplen et al. 2000), as well as to determine the sources and transport patterns of contaminants. For example, students could collect samples of precipitation (as at Saint Louis University), river water, and ground water to determine from oxygen isotopes the relative contributions of each source to the City of Keene's well field (e.g. Dysart 1988).

Geol 310 Glacial Geology (Bill) The oxygen isotopic composition of ice from cores of continental ice sheets is used as a proxy for paleo-temperature reconstruction (Stuvier & Grootes 2000). In field/lab exercises that demonstrate this approach, students will collect snow samples from Keene, New Hampshire (at low elevation) and from Tuckerman Ravine (on Mount Washington) to determine temperature differences between the two locations using oxygen isotopes.

GEOL 305 Paleontology and GEOL 306 Stratigraphy (Bill). Carbon and oxygen isotopes in carbonate rocks and fossils are useful for stratigraphic correlations and paleo-environmental analysis including paleo-temperature determinations. Similar to exercises at the University of Utah, students could analyze foraminifera from Ocean Drilling Project "flow in" material to determine whether it is from glacial or interglacial periods, or could analyze the shells of modern marine and freshwater bivalves to assess stable isotopes as an environmental discriminator.

In **advanced courses** (*Physiological Ecology, Ecosystems and Community Ecology, Geochemistry*) the use of stable isotopes will be introduced in multiple-week units and students will not only pose and ask questions about the *pattern* of stable isotope distribution, but also explore the *mechanism* of stable isotope distribution. As in the intermediate-level courses students will be responsible for and learn about the sampling, extraction and preparation of samples, and stable isotope analysis on the IRMS. During the entire course, emphasis will be placed on skills such as creative and analytical thinking, problem solving, quantitative reasoning, and communication skills. Exercises will involve group collaborations applying stable isotope techniques to problem-based questions with, at times, unforeseen results. The course will culminate in a multiple-week group research project, where students have the opportunity to use stable isotope analysis to address more complex questions. The group research project will entail problem definition, field work, sample preparation and analysis, data processing, data interpretation in light of published literature, and presentation. Since the students will be collecting and analyzing their own samples, unexpected results may occur, providing opportunities for discussion of uncertainties and the nature of scientific research (McConnaughay et al. 1999, McGinn & Roth 1999).

Course-specific examples for the advanced level follow:

BIO 385/87 Physiological ecology (Gebauer) Because stable isotope analysis has diverse applications in this area there are numerous lab exercises which can be designed (as at the University of Utah). For example students could be encouraged to investigate questions such as why carbon isotopic composition varies in plant material, what the effect is of different stresses on the carbon isotope composition (as an indicator of plant water-use-efficiency) or what the water and nitrogen sources are for different plant species. A possible example for a multiple week research project would be for students to use stable isotopes to investigate the phenomenon of hydraulic lift in red and sugar maples growing in the surrounding areas (Dawson 1993).

BIO 452/453 Ecosystems and Community Ecology (Gebauer) Lab exercises could expand on topics covered in *BIO 252/256* and address for example, the variation of carbon and nitrogen isotopes in soil organic matter, carbon and nitrogen transfers in food chains, sources of CO_2 in soils, etc (as in the University of Utah course). An excellent and fun student project could be modeled after the lab exercise developed at Dartmouth College, "Isotopic Lessons in a Beer Bottle" (Stern et al. 1997), which investigates microbial preferences for sugars from C3 and C4 plants.

GEOL 412 Geochemistry (Allen) Oxygen and carbon stable isotopes have a tremendous range of application to geological problems, such as for determining the sources of origin for rocks and geological fluids (including water), the extent of fluid interaction with rocks (Chamberlain & Rumble 1988), or the temperatures of formation or metamorphism of rocks. Students could use oxygen isotopes to explore the development of soil water isotopic profiles and their relationship to precipitation events (as at the University of Utah), or use carbon and oxygen isotopes to investigate the nature and origin of carbonate fracture fillings in local rocks or to determine the conditions of metamorphism for graphitic marbles. Multiple-week research projects could study temperature and pressure effects on the exchange reactions of oxygen and carbon among carbonate minerals, CO_2, water, and water vapor (as at Saint Louis University), or develop new methods to extend our existing analytical capabilities, such as extracting and analyzing waters from hydrous silicate minerals.

At the **most advanced level**, students will incorporate stable isotopes into independent research projects. Involvement of undergraduate students in real scientific research through student-faculty cooperative research projects is held out as an ideal approach to teaching science (e.g. Goodwin & Hoagland 1999). Student involvement in research outside of course-based projects is currently not a requirement in our science program. Students can, however, apply credits earned in *Independent Study* towards elective requirements in the major. Independent research projects are also supported by college funds and students can apply for grants of up to $ 750. Tim Allen has worked with a number of students on a variety of field projects exploring the dynamics of the sub-surface hydrologic cycle (O'Rourke et al. 1998), an area in which stable isotopes are important tracers (e.g. Clark & Fritz 1997). We are particularly interested in developing techniques for directly determining the rate (and mechanisms) of ground water recharge from precipitation, as this rate is usually estimated by difference in a watershed water budget. Renate Gebauer worked with students on a project on spring ephemerals in the understory of deciduous forests. The study could have been significantly improved if students would have had access to a stable isotope facility in order to study the nitrogen sources and water use efficiency of different plant species. In addition labeling studies with nitrogen stable isotopes can help us to better understand nitrogen pulse utilization of the spring ephemerals and the nitrogen turnover rates, particularly in the storage organs.

In the ENST *Junior/Senior Seminar* teams of students are expected during the junior seminar to develop a proposal for an independent research project, and to then conduct and accomplish the research project in the senior seminar. Most ENST students will have previously taken at least one of the Geology or Biology courses described above, and therefore have gained some experience with the stable isotope analysis. Examples of recent projects in this course that could also have benefited from the IRMS include the determination of the pumping-induced recharge from the Ashuelot river to the city of Keene's well fields, or the tracing of fertilizer-derived nitrate or sewage in surface- and ground water.

Equipment Request:

We seek to acquire a Finnigan DELTAplus IRMS with a dual-inlet sample introduction system. This configuration allow us to complete the stable isotope analysis within the timeframe of a course (commercial analysis can take 4-12 months) and enables students to participate in the entire process of the investigation. We also plan to construct two high-vacuum sample preparation lines for student use, each supporting two student groups.

We considered both continuous flow interfaces, as well as the more traditional dual-inlet sample introduction system. Continuous flow interfaces facilitate connection of automated "on-line" sample preparation systems, elemental analyzers and/or gas chromotographs to the IRMS. Given the costs and complexity of these attachments, we do not anticipate adding these in the foreseeable future. Dual-inlet systems provide for higher-precision analyses, and are appropriate when samples are prepared "off-line" as we intend to do with our students. Such “off-line” sample preparation will help students better understand some of the principles of stable isotope distribution. There are five manufacturers of stable isotope ratio mass spectrometers. All but Finnigan have an unstable or relatively short manufacturing history. Finnigan is the most well-established (part of the Thermo Electron Corporation), with a large user population and a strong support network.

Existing facilities to be used in this project include our current Stable Isotope BioGeochemistry Laboratory including a modular multi-purpose vacuum line for preparing water and carbonate samples for stable isotope analysis, with an Edwards RV3 vacuum pump and Edwards Active Pirani vacuum gauges (in courses the vacuum line can be used by one student group). The laboratory is also equipped with a NESLAB model RTE-211D refrigerated constant temperature water bath, a Thermolyne F48025-80 8-segment programmable muffle furnace, a Sartorius model 2462 Analytical Balance, a drying oven, a glass-blowing torch, and numerous dewars. Additionally, this project will make use of our existing Physiological Ecology Laboratory, Greenhouse, Well Fields and Hydrologic Field Equipment,

Weather Station, Rock Sample Preparation Laboratory, our forthcoming X-Ray Fluorescence Spectrometer (NSF Award #0087860), as well as our Laboratory for Environmental Chemical Analysis. For more details, see the Facilities and Equipment attachment.

Implementation, Responsibilities, Timetable:

The IRMS and student vacuum preparation lines will be housed in the Biology Department. KSC will make renovations as necessary (see letter of support from the Dean of Sciences, Appendix). KSC is also committed to maintaining the instrument (see letter of support from the Dean of Sciences, Appendix). Maintenance items would include vacuum pump oil and ion source filaments. We can anticipate that eventually repair or replacement of major components of the instrument (e.g. turbomolecular vacuum pump) may become necessary, although we will certainly take all steps to protect these components as much as possible. Consumables such as liquid nitrogen, dry ice, reagents, and sample tubes, will be accommodated within the Environmental Studies, Biology, and Geology departmental budgets.

Renate Gebauer (PI) will have the overall responsibility for the operation and the maintenance of the IRMS, and the installation of the student vacuum lines. KSC will grant her a one-course release (3 credit-hours) in fall 2002 to facilitate the project (see letter of support from the Dean of Sciences, Appendix). Tim Allen (Co-PI) will also provide support for all these tasks. They both will attend a week-long training workshop on the operation and maintenance of the instrument which is provided for by the manufacturer (see quote, Appendix). Renate Gebauer will visit the model institutions to observe the stable isotope facilities and their integration into the curriculum. The PI and both Co-PI's are responsible for the design and development of lab exercises in their own courses (as proposed above).

The instrument will be ordered in winter 2002 and installed in summer 2002 (expected delivery time 3 months). Over the summer of 2002 we will work on the calibration of the instrument, setting up the student vacuum lines and the development of class exercises. In fall 2002 stable isotope analysis will be included in *Ecology and Evolution* and *Environmental Geology*, in spring 2003 into *Ecosystems and Community Ecology*, *Hydrogeology*, and *Glacial Geology*, in fall 2003 into *Physiological Ecology*, *Stratigraphy*, and *Environmental Geology*, and in spring 2004 into *Geochemistry*, *Paleontology*, and *Ecosystems and Community Ecology*. The project is expected to be completed in May 2004.

Experience and Capability of the Principal Investigators:

Renate Gebauer held a post-doctoral fellowship at the Stable Isotope Ratio Facility for Ecological Research (SIRFER) at the University of Utah, where she used stable isotopes in her research on the desert plant community on the Colorado Plateau (Gebauer and Ehleringer 2000, Gebauer et al. 2001). She has trained several undergraduate students in methods of stable isotope analysis and was part of the annual short course on stable isotope applications in ecology. Recently, she has participated in several workshops on using the case-studies method in undergraduate science teaching. She provides leadership in the Writing Task Force at KSC and is on the curriculum assessment team that evaluates the writing component of the KSC general education program.

Tim Allen, for his doctoral dissertation at Dartmouth College, studied the petrology and stable isotope systematics of migmatites and other rocks in the White Mountains of New Hampshire (Allen, 1993, 1994, 1996). Together with Renate

Gebauer, he designed and built the new vacuum preparation line at KSC—drawing on his strong technical skills and experience and high degree of mechanical aptitude—and has worked with students on the isotopic analysis of water samples. He has been involved in the development of field-based laboratories in *Environmental Geology* and *Hydrogeology* (Allen, 1997, 1998), and the integration of geochemical analysis across the geology program (NSF Award #0087860). Tim Allen also serves as the coordinator of the ENST program.

Steve Bill's background is in paleo-environmental analysis and stratigraphy including micro-paleontology and stable isotope geochemistry. As part of his dissertation at Case Western Reserve University, he measured oxygen isotope ratios from biogenic silica to evaluate its use in correlating oceanic sediments lacking carbonate microfossils; he also has experience with isotopic studies of carbonates rocks and fossils. Steve Bill and has co-led several week-long workshops for in-service New Hampshire teachers focussing on hands-on science activities (funded by Eisenhower Grants from the NH Department of Education).

Project assessment:

The outcomes of our project to be assessed are whether the addition of inquiry based exercises using stable isotope analysis are effective at (1) motivating student learning and their involvement into scientific investigation, (2) improving students scientific research skills at all levels from introductory laboratories to the Senior Seminar and Independent Study, and (3) enhancing students conceptual (and integrated) understanding of biological and earth systems according to specific content goals for each of the individual courses. The evaluation plan will include qualitative and quantitative tools (Frechtling and Sharp 1997) and has the following two basic components: (1) pre- and post course assessment and (2) assessment by a team of external evaluators. We will adapt pre- and post-course surveys from the University of Oregon's assessment program (www.mth.pdx.edu/OCEPT/evaluation.htm) and other sources (Nuhfer 1995, Cross and Angelo 1988) to develop evaluation questions for individual courses. In addition we plan to have two on-site visits (Spring 2003 and Fall 2003) by the team of two external evaluators to (1) review relevant course materials to assess how well our course activities promote active involvement into scientific investigation and scientific thinking (2) evaluate the quality of research proposals and papers with respect to questions asked, hypotheses proposed, and effectiveness of the experiments and (3) assess during class visitation the involvement of students. We have asked Dr. Ross Virginia from Dartmouth College to serve as an independent evaluator of our project and he has graciously agreed to do so (see letter of support in the Appendix). Professor Ross Virginia has much experience with involving undergraduates in stable isotope research in independent studies, honors research, and courses. As Chair of Environmental Studies at Dartmouth College he has also been involved in curriculum development and assessment. A second evaluator will be selected through the consultancy service provided by the Council for Undergraduate Research (CUR; see http://www.cur.org/consulting.html).

Dissemination of Results:

We will expand our existing Stable Isotope Laboratory website (http://kilburn.keene.edu/ENST/isotopes/). The site is designed to inform current prospective students as well as interested faculty about the capabilities of the facility. Students will be encouraged to present results from their research projects at the annual KSC Academic Excellence Conference and regional scientific meetings (e.g. Geological Society of America Northeastern Section, National Council for Undergraduate Research, Eastern Colleges Science Conference) as appropriate. The PIs will share their experience with the curricular reforms in presentations at meetings of the Ecological Society of America or the Geological Society of and/or by publications in appropriate journals (e.g. Journal of Geoscience Education, Journal of College Science Teaching, American Biology Teacher, CUR Quarterly).

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