Project Description

Goals and Objectives

We propose to introduce the nematode worm *Caenorhabditus elegans* as an experimental model organism into a spectrum of related Biology courses. This approach will provide an integrated laboratory experience for our Biology Majors. In lower division courses (Zoology, Introductory Cell Biology and Genetics) the students will be come familiar with the worm life cycle and behavior. In upper division courses (Advanced Cell Biology, Molecular Biology and Recombinant DNA Technology) the entire laboratory portion of the courses will be devoted to experiments involving *C. elegans* as the model organism.

We are proposing this approach in order to achieve a number of curricular and pedagogical goals. The curriculum in our department employs the traditional approach, which compartmentalizes the curriculum in separate courses and therefore often fails to encourage the students to apply knowledge gained in one course to another course in the curriculum. One potential problem pointed out in the department's last external review was that the department's curriculum was organized in such a way as to require the "least interaction and collaboration." The department recognizes this problem, and is engaged in a number of approaches to solving it. The program described in this proposal is one of them. The current curriculum fails to recognize appropriately the contribution of one sub discipline to another. Furthermore, students often do not realize that systematic laboratory studies of multiple aspects of the same organism at the behavioral, genetic, cellular and molecular levels can be integrated to gain an understanding of the organism as a whole. We intend to adapt and implement experiment-based laboratories utilizing a progressive set of exercises. We will begin with simple observational studies in lower level courses, and in upper level ones,

incorporate project-based investigations with *C. elegans* as the experimental organism. Precedents for this approach include integration based on a common experimental system has been utilized by following a given cloned gene through several courses (DUE 9950647), experiments with a common theme (DUE 9950879) or by using the same organisms in different courses e.g. *Worms in Class at NYU* (Fitch, Hubbard and Clark, 1999).

We believe the *C. elegans* system to be ideal for our purposes for a number of reasons. The organism is inexpensive to rear and can be frozen and revived. It haves well-understood genetics; the genome has been sequenced and there are a large number of mutant strains available. *C. elegans* larvae and adults are nearly transparent and almost all of their internal structures can be observed by differential interference microscopy. These organisms have been adopted as an experimental organism in a large number of research laboratories, and their potential as organisms for undergraduate laboratories has recently become apparent (W.O.R.M. Initiative: http://www.loci.wisc.edu/outreach/about/about.html).

Our expectation is that as a result of our adaptation and implementation plan, detailed below, that our students will:

A. Be able to demonstrate understanding of the integrated nature of biological science by application of knowledge gained in one sub discipline or course to questions a second discipline.

B. Be able to explain why *C. elegans* is an appropriate model for the study of behavior, genetics, molecular and cell biology.

C. Be able to effectively use the newly introduced technology to answer scientific questions about the behavior, cellular and molecular biology of *C. elegans.*

The Current Program:

Buffalo State College is the largest four-year college the SUNY system. Students at the College come from across the state, but the majority of students are from Erie and Niagara counties. Many of them live at home or off campus. The College's current enrolment is made up of about 50% minorities and includes students with diverse educational backgrounds. Consistent with its teacher training and liberal arts missions, the Biology Department offers two undergraduate (BA in Biology and BS in Biology Secondary Education) degrees and two Masters degrees (MA in Biology and MS in Biology Secondary Education). Our majors in the BA program are very diverse in their preparation for college and in their career goals. Some of them go on to graduate and professional school; others are hired as environmental biologists or biotechnologists by academic or commercial institutions; some pursue careers outside biology. Our BS in Biology Secondary Education graduates earn temporary certification and teach Biology (7-12) in NY state. The required core curriculum for both BA and BS students consists of; Introductory Botany (BIO 115) Introductory Zoology (BIO 116), Introductory Cell Biology (BIO 214), Genetics (BIO 303), Ecology (BIO 315) and Evolution (BIO 405). Students interested in cell and molecular biology can choose from the following set of elective courses: Molecular Biology (BIO 305), Advanced Cell Biology (BIO 314) or Recombinant DNA Technology (BIO 450). Many of these students choose to do either Honors Research or (BIO 498) or Independent Project (BIO 495). Laboratory sections in these courses range in size from 22 students in BIO 115, BIO 116, BIO 214, to 8-18 students in BIO 303 BIO305, BIO314 and BIO450.

Detailed Plan

Our project will place emphasis on experimentation-based exercises in all the affected courses. We will introduce students to current research technology and experimental designs in each affected laboratory course by adapting currently available laboratory exercises and published research using C. elegans, and using what we have learned from our current experiences using *C. elegans* in our Recombinant DNA Technology course. We are starting with the Zoology, Cell Biology, Genetics, Molecular Biology, Advanced Cell Biology and Recombinant DNA technology courses, but we hope to involve more of the curriculum in this integrative effort as we proceed.

BIO116 (Zoology): Two separate laboratory exercises will be introduced into this course. The first will investigate the anatomy of nematodes, the phases of the nematode life cycle, differences in the sexes, and simple behaviors such as locomotion, feeding and chemotaxis. This exercise will emphasize microscope skills, observation, data collection and basic nematode biology. In a second exercise near the end of the semester, students will design a simple experiment to investigate if worms display associative learning. Students will be asked to determine if worms starved of bacteria (unconditioned stimuli) in the presence of NaCl (conditioned stimuli) will loose their chemotaxic response to NaCl (Saeki et al, 2001). This exercise will emphasize experimental design and the utility of a model organism to address broad biological questions. Therefore students in BIO116 will acquire the basic worm biology skills and techniques (use of **dissecting microscopes, Nomarski optics** and worm isolation) that will be needed in more advanced courses.

BIO 214 (Introductory Cell Biology): The current laboratory exercises in this course emphasize cell structure, enzyme activity, organelle function, protein diversity and the use of

restriction enzymes. Because of the technical challenges involved, we have not previously been able to demonstrate the important concept of transcriptional control of gene expression in eukaryotes in the Cell Biology laboratory. The availability of transgenic *C. elegans* with green fluorescent protein (gfp) fusion reporters makes it possible to directly observe genes expressed in all tissues of the organism (housekeeping genes), cell specific expression of genes (luxury genes) and the expression of genes induced by external stress (e.g. heat shock). Transgenic worms expressing each type of reporter gene are available from the Caenorhabditis Genetics Center (CGC) University of Minnesota. Students will be able to easily observe each type of expression in worms at different stages in development by using **Nomarski and fluorescence optics**. Students will also be able to compare the promoter sequences for the genes they observe and identify common and divergent features, e.g. heat shock elements TATA box etc.

BIO303 (Genetics): To better integrate the laboratory portion of this course with other courses in the curriculum, two *C. elegans* exercises will be incorporated. In the first exercise students will observe the relationship between molecular genotype and phenotype by using PCR to investigate deletions in mutant alleles of a chemotaxis gene such as *odr-1* (CGC stock CX2065). PCR products will be analyzed by electrophoresis and visualized on the **Imaging Workstation.** The second exercise will introduce students to RNAi. Students will use RNAi to inhibit expression of a gfp transgene that they observed in BIO214 *i.e*. the *unc-22* gene for uncoordinated movement and the *odr-1* gene for chemotaxis (Timmons et al. 2001). These RNAi experiments will demonstrate the relationship between the molecular biology of a gene and its corresponding phenotype. Further, it will introduce students to the

utility of RNAi as a simple technique for manipulating gene expression in *C. elegans*; a technique that they will use in more advanced courses.

BIO305 (Molecular Biology): This course is a junior level elective. Approximately 90% of the students in the course are in the Biology BA program; the remaining 10% are in the Biology BS Ed. or Chemistry BA program. The first two thirds of BIO305 covers the molecular biology of eukaryotic gene expression with an emphasis on regulation; the final third of the course focuses on the structure and evolution of eukaryotic genomes. The laboratory portion of the course is structured around exercises that last several laboratory periods. For this project, we propose to introduce exercises investigating germ cell specification in *C. elegans*. We chose this aspect of *C. elegans* biology for four reasons. First, the end product of germ cell specification, oocyte and sperm, are easily observed by **Nomarski or fluorescence microscopy**. Second, specification involves regulation of translation, an aspect of gene regulation covered in lectures but not explored in current laboratory exercises. Third, components of the regulatory mechanism have recently been characterized at the genetic and molecular levels (Puoti et al, 2001; Eckmann et al 2002). Fourth, analysis of the regulation will introduce students to key experimental approaches to investigating regulation of gene expression. As an approach to experimental investigation of this system, students will be introduced to the most recent model for regulation of germ cell specification. Based on the model, students will work in groups to predict how RNAi knockdown of the expression of three genes involved in sperm and oocyte specification, *fem-3, fbf-1*, and *gld-3* would affect germ cell specification. [FEM-3 is necessary for spermocyte specification, FBF-1 represses translation of fem-3 mRNA allowing oocyte production and GLD-3 inhibits activity of FBF-1 allowing sperm production.] They will then test their

predictions by using RNAi to knockdown the expression of each of these genes. The effect of RNAi treatment on oocyte and sperm formation will be observed using **Nomarski optics** or with DAPI staining of nuclei and **fluorescence microscopy**. In a second exercise, students will use the yeast two-hybrid system (Odum and Grossel, 2002) to assay the interaction of FBF-1 with GLD-3. Specifically, students will isolate plasmids from the two-hybrid system that express FBF-1 and transform yeast cells with the plasmid. They will use a chemiluminescent western blot assay to analyze expression of the FBF-1 protein using the **Imaging Workstation**. Finally, students will assay for the interaction of FBF-1 and GLD-3 by detecting B-galactosidase activity in the two-hybrid system.

BIO 314 (Advanced Cell Biology): This is a junior level elective with similar student demographics to BIO 305. The emphasis in this course is on the structure, function, synthesis, processing, targeting and regulation of proteins. For this project we intend to concentrate on the role of the endoplasmic reticulum (ER) in the synthesis, folding, and targeting of secretory and membrane proteins in eukaryotes. The initial exercises will focus on the lysosomal enzyme acid phosphatase. The students will build a phylogenetic tree for lysosomal acid phosphatases from protein sequences in the Genbank database and thereby identify the *C. elegans* homologue of human acid phosphatase (which will emphasize the role that model organisms can play in investigations). This will be followed having the students localize acid phosphatase activity in the gut cells of *C. elegans* adults and embryos through histochemical staining (Beh et al, 1991). The remaining time will be devoted to systematic examination of the *C. elegans* unfolded protein response (UPR). The UPR counteracts stress caused by the accumulation of unfolded membrane and secretory proteins in the ER by up regulating the transcription of ER chaperone genes, hsp-*3, hsp-4, hsp-16* etc., (Shen et al,

2001; Urano et al 2002). When *C. elegans* are exposed to agents that interfere with protein folding in the ER, the UPR is induced. Students will monitor this induction visually by following the expression of a *gfp:hsp-4* fusion reporter in transgenic *C. elegans* (CGC 4005) using **fluorescence/Nomarski microscopy**. Students will also observe the response in worms with a mutant transcriptional activator of UPR genes, (*xbp-1* mutants carrying the *gfp:hsp-4* reporter; CGC SJ17). They will assay for hsp-4 expression using the gfp fusion reporter. As a control, students will verify the presence of the reporter gene in the worms by Southern blotting using **chemiluminescent imaging.** The transmembrane protein IRE-1 detects unfolded protein in the ER lumen and activates the translation of XBP-1 by splicing the xbp-1 transcript in the nucleus (Shen et al, 2001). The students will be able to demonstrate the splicing phenomenon by extracting total RNA from induced and uninduced mixed stage *C. elegans* cultures, amplifying the *xbp-1* mRNA by reverse transcriptase PCR and analyzing the amplified sequence for spliced product. As a summative experience the students will be asked to formulate a hypothesis to predict the localization and accumulation of PHO-1 acid phosphatase in ER stressed worms with the *xbp-1* mutation and to propose and execute simple experiments to test their hypothesis.

BIO450 (Recombinant DNA Technology): This course is a senior level laboratory-intensive elective and is intended to be the culminating academic experience for undergraduate students interested in cell or molecular biology. BIO450 involves a single semester-long research project using recombinant DNA technology. This semester (Fall 2002) students investigated the physiological role of the hsp110 in *C. elegans*. Students identified the *C. elegans* hsp110 homologue by using the mouse sequence as query sequence in a BLAST search of the *C. elegans* genomic database. They designed PCR primers based

on *the C. elegans* sequence and amplified an exon from the gene. They cloned the exon into a plasmid, they used the plasmid to express dsRNA within *E. coli*, fed these *E. coli* to *C. elegans* to induce RNAi knockdown of hsp110 expression and they scored the phenotypes. Based on the students' success with this project, we came to see the potential of *C. elegans* as a laboratory system for undergraduates.

We are proposing here to modify BIO450 to take advantage of the knowledge of *C. elegans* that students will have acquired in lower level courses. In the first weeks of the course we will have each student develop their own experimental question involving *C. elegans*. Their questions will be based on laboratories from lower division courses. For example, students may choose to investigate the molecular biology of associative learning or chemotaxis they observed (BIO 116), or other phenomena they observed in previous courses. Once students have selected an experimental question, they will prepare a two-page proposal describing an RNAi based experiment, which addresses their question. In the remaining 12 weeks of the semester, students will use PCR to amplify their gene of interest, clone the gene into a dsRNA-expressing vector, use RNAi to knockdown gene expression (Timmons et al, 2001) and measure phenotype associated with their knockdown. This laboratory will utilize all the instrumentation we have requested. Students will describe their results in a research paper and present their results at the College's Student Research and Creativity Celebration.

Experience and Capability of Principal Investigators

Dr. Douglas Easton (Professor of Biology):

Dr. Easton has been a member of the Buffalo State College Biology faculty for 25 years. He has developed and taught laboratory courses in Cell Biology, Developmental Biology,

Advanced Cell Biology and Recombinant DNA Technology. His research interests are in the structure function and evolution of molecular chaperones.

Dr. Gregory Wadsworth (Associate Professor of Biology):

Dr. Wadsworth has been a member of Buffalo State College Biology faculty for 10 years. He has developed and taught laboratory courses in Cell Biology, Genetics, Molecular Biology and Recombinant DNA Technology. His research interests involve the structure and evolution of plant gene families.

Evaluation Plan:

An external evaluator, Dr. Joseph Zawicki, in collaboration with the principal investigators, will evaluate this project. Dr. Zawicki is an Assistant Professor of Science Education at Buffalo State College. He has a PhD and MS Ed. in Science Education and a BA in Biology. His expertise is in assessment of student learning outcomes.

Formative Evaluation will be conducted in each course throughout the semester. Pre- and post-test will be administered in each course to assess the course's impact on student knowledge of *C. elegans* as an experimental system and student understanding of the experimental designs and technology. Pre-lab discussions will be used to elicit student comments to determine how well they are able to use their previous experience with *C. elegans* biology to approach the experimental question posed in that laboratory. Copies of student laboratory reports will also be collected. At the end of each semester, all of the participating faculty will meet with the evaluator to discuss implementation of the project. The evaluator will assess the following:

a) Were the planned laboratory experiments implemented in appropriate courses?

b) Could the students successfully complete to proposed experiments?

c) Were there sufficient resources available to implement the experiments?

d) Was there evidence that students were achieving project objectives?

e) Which activities were most effective in helping students reach the goals?

Summative Evaluation will be conducted in the final year of the project to assess whether the three objectives outlined earlier in the narrative were achieved. The evaluator, in collaboration with participating faculty, will develop an instrument to measure achievement of the three learning objectives associated with the project. This instrument will be administered at the end of two key courses in the curriculum, BIO303 Genetics (which is the last laboratory course in our plan that is required of all BS Ed. and BA students), and BIO450 Recombinant DNA Technology (a culminating laboratory experience for BA students interested in cellular biology and biotechnology). In the final year of the project, it is anticipated that both courses will have a mixed student population. For example, some of the students will have completed several courses that have incorporated the laboratories described in this project. However, there will also be students that have not had these laboratory experiences because they are transfer students (approximately 50% of our graduates are transfer students from other colleges). The external evaluator will use matched pair analysis to identify differences in achievement of the objectives. Matching will be based on age, gender and grade point average. Therefore the evaluation will assess the impact of the project on three categories of students, BS Ed. students, and BA students interested in cellular biology/biotechnology and BA student's interests in other sub disciplines of biology.

Dissemination Plan:

A web page will be maintained describing all phases of the project. The web page will include detailed descriptions of all the laboratory exercises developed for the project,

including examples of student work. As evaluation results become available these will also be reported. The principal investigators plan to attend a Council on Undergraduate Research National Meeting and an International *C. elegans* meeting. They will report the results of this project at these meetings. Once the evaluation is complete, a manuscript will be prepared for publication in *Cell Biology Education* or another appropriate journal describing the results of our project.

Instrumentation Budget Items & Justification:

a. Item	b. NSF Contribution	c. Matching	d. Total
Research Microscope BX51 DIC/GFP (Olympus)	**13,205 (14,672)**	**13,205 (14,672)**	**26,410 (29,344)***
CCD Camera DP-12 (Olympus)	**1,440 (1,600)**	**1,440 (1,600)**	**2,880 (3,300)**
8 Stereo Zoom Microscopes SZ40 (Olympus)	**9,304 (10,336)**	**9,304 (10,336)**	**18,608 (20,672)**
2 Refrigerated Incubators 0 C to +50 C (Fisher Scientific)	**2,495**	**2,495**	**4,991**
Foto/Analyst Luminary Imaging Workstation (Fotodyne) BO24-FD6730	**10,450**	**10,450**	**20,900**

***Numbers in parentheses are list prices for discounted items.**

The research microscope we have requested combines both Nomarski differential interference contrast (DIC) optics and fluorescence capabilities. Both of these capacities will be required observation of detailed worm anatomy (DIC) and for the observation green fluorescent protein (gfp) and DAPI stained nuclei. The **Stereo Zoom** microscopes are equipped with both transmitted and incident lighting and have optical qualities, which exceed those of ordinary student stereomicroscopes. The eight which we are ordering will

supplement the 10 **SZ 40 microscopes** we have on hand and allow us to have a full compliment of microscopes for a lab section. These microscopes will be used for worm observation, manipulation and to observe phenotypes and worm behavior. This level of quality is recommended in most protocols for this type of work. The **refrigerated incubators** are required for worm culture and two are required to allow culturing at two different temperatures**.** The **Foto/Analyst imaging station** is capable of imaging electrophoresis gels, bacterial colonies and micro-titer plates by visible light and UV trans illumination , epi-illumination and chemiluminescence. Software is provided to quantitatively analyze the images. This will allow us to do quantitative and qualitative analysis of images generated from electrophoresis of PCR products, electrophoresis of cloning intermediates, Southern blots, western blot and bacterial colonies generated by transformation. All of these operations are required to perform the Bio 303, 305, 314, and 450 labs.

Implementation and Instrumentation Maintenance:

Implementation: All of the instrumentation will be required at the beginning of the project. Therefore, the instrumentation is budgeted for the first year. We expect to order equipment during the summer and to familiarize ourselves with it at that time. The first use of the equipment will be in BIO116, BIO214 and BIO303 in the fall semester. At the end of the semester the faculty involved will meet with our evaluator for a formative evaluation of those courses. In the spring semester of the first year we will continue our implementation in the above courses as modified by our evaluation. We will then add either BIO314 or BIO305 laboratories. We will meet with our evaluator at the end of spring semester to evaluate progress in all the courses. In the fall semester of the second year we will continue with the

BIO116, BIO214 and BIO303 labs and add either BIO314 or BIO305, depending upon which of these was taught in the spring of the first year. Finally, in the Spring Semester of the second year, we will institute the program in the final course in the program, BIO450. A final formative evaluation will be done at semester end and plans for the Fall semester of year three will be made, which will include the same courses as in the fall of the first year. At the end of the semester, the summative evaluation will be performed. Publication of the results (including results of the summative evaluation) will follow.

Instrument maintenance: The major instrumentation in our plan is microscopes and imaging equipment, most of which is quite robust. The department has a regular maintenance program for its microscopes, which is conducted by an outside contractor. The college Heating Ventilation and Refrigeration department will maintain the incubators. The departmental supplies and equipment allocation will cover other repairs.

Broader Impacts:

The activity proposed here is directly designed to foster the development of intellectual curiosity and problem solving skills in the students it targets. Furthermore it is designed to provide opportunities for students to use cutting edge approaches to answer research questions using an experimental model organism that has proven merit. Since at least 50% of our students will become biology teachers we expect that these teachers should be able to utilize *C. elegans* in their secondary school laboratory exercises. Furthermore, *C. elegans* is an ideal organism for independent research projects for high school students. We plan to post on our website information gained from implementation of our project. This information should serve as a resource for both college faculty and secondary school

educators in implementing *C. elegans*-based exercises in their curricula. We will also publish the outcomes of this project, reporting on the formative process of implementing the project, upon the exercises implemented and on the extent to which we met our goals.

We also expect that undergraduates in our department will begin using the worm system in their undergraduate research projects. Many of the students pursuing independent research and honors projects go on to graduate and professional schools and should contribute to the science and technology work force.

Finally, this project can provide ideal demonstrations of the linkage between scientific discovery and societal benefit by demonstrating that studies on a "simple" worm reveal that at the molecular, cellular and behavioral level, this worm serves as an excellent model for gaining fundamental information about these aspects of human biology.