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Several species of bioluminescent earthworms are known and available in the US. However, little is known about their habitat requirements. We will research the appropriateness for use as a teaching aid in terms of the ease of raising and maintenance, and methods of stimulation of the bioluminesence display. Emphasis will be on development of observation and hands on experiments appropriate for use in integrated curricula for grades K-12.
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Worm Proposal

Because earthworm bioluminescence involves a hydrogen peroxide reaction, most studies in the literature have utelized chemical stimulation to elicite maximal bioluminescence. This will not be appropriate for the NIGHT-FARM educational discovery product. Bioluminescence is also stimulated by mechanical and electrical means, though there have not been detailed studies of these approaches.

Our major emphasis will be on mechanical stimulation, including the low frequency, low amplitude stimulation characteristic of large organisms walking on land. The worms can sense land surface vibration caused by walking and running animals. The Childrens' Inquiry Analogue of this would be a light tapping on the surface of the transparent NIGHT-FARM container. Higher frequency, low amplitude stimulation will be studied using acoustic means with a focused audio speaker and signal generator.

We will do some preliminary experiments with electrical stimulation with electodes placed on the walls of the container, and low voltage DC stimulation. This will, of course, be a strong function of the nature of the soil, the worm density in the soil, the humidity of the soil, etc.

Task C-2

The intensity and spatial distribution of the bioluminescence which results from stimulation will be recorded using high speed ASA 10,000 to 20,000 scientific polaroid film simply placed in contact with the transparent chamber. Given the dimensions of the worm, the intensity of the luminescence, and our chamber design, this contact print method of detection is very inexpensive and effective and, together with experimenter viewing of the results under dark-adapted conditions, will provide the qualitative information neede to select optimum stimulation perameters.

Once these more qualitative and optimization experiments are complete, then a more rigorous measurement and analysis of bioluminescence intensity, duration, recovery, will be done using a highly sensitive video tape camera system. PSI already has access to such a system through the University's Department of Bioengineering.

Dr. V. Hlady, Associate Research Professor of Bioengineering at the University of Utah, will conduct these experiments using a

photometrix CCD camera system [15].

Senior Personnel:

Trish Stoddart, Ph.D. is Assistant Professor of Educational Studies in the Graduate School of Education at the University of Utah. Dr. Stoddart's major research area is in the assessment of teacher knowledge, and the means by which incorrect concepts can be relearned. She sees bioluminescence as an ideal tool with which to study teacher pre-conceptions, and with which to motivate teachers to restructure their concept space. She will function as an advisor to the project, and will begin to play a significant participatory role in Phase II.

John Wampler is Professor of Biochemistry at the University of Georgia in Athens, Georgia. He is internationally recognized for his work on bioluminescence and earthworms, and related organisms (see reference 5). J. Andrade has discussed the use of bioluminescence as a motivational tool for science education with him [11]. Dr. Wampler will advise and assist us as required.

Barbara Andrade is a first and second grade teacher with twelve years of teaching experience. She also serves as secretary of Protein Solutions, Incorporated. She has worked with earthworms in her classroom, and has been involved with PSI's efforts in the use of bioluminescence for science education for several years. She will directly advise and assist in the development and formulation of the bioluminescent earthworm concept into a practical and effective science education tool.

Rene Stofflett, Ph.D. is Assistant Professor of Education at Northern Illinois University. She was formerly a graduate student working under Dr. T. Stoddart. Dr. Stofflett was also involved in the discussions on the development and use of bioluminescence in elementary education. She is eager to apply the materials and products developed by PSI to her research and education activities in Northern Illinois.

Vladimir Hlady, Ph.D. is Associate Research Professor of Bioengineering at the University of Utah. He is an expert on optics and particularly the measurement of luminescence, fluorescence, and adsorbance at soli/liquid interfaces. His optical spectroscopy and engineering laboratory is a major resource for the more detailed light intensity and light duration studies proposed earlier. Dr. Hlady will serve in a formal consulting capacity. His letter of interest and agreement follows.

Potential Problems:

As research on bioluminescent earthworms is not a well developed sub-discipline, we cannot anticipate every eventualilty and problem. We certainly expect to be able to select, collect, grow, and maintain the needed bioluminescence species. We are not sure how they will respond to the confinement in a narrow transparent container, which is necessary for the efficient observation and experimentation.

If for some unknown reason bioluminescent earthworms prove to be completely impractical for this project, then we will focus our attention on the marine literal worms, pontodrilus, bermudensis, and related species. These worms live at the high tide level, have readily been maintained in captivitiy in artificial seawater, and have a particulalry bright bioluminescence. This will require developing a container which includes a water and sand or soil component in which the worm is expected to live at the interface. We anticiapte no problem here becuase we have already some experience with such a geometry in the NIGHT-COLONY dinoflagellate bioluminescent product, which is PSI's major private product development effort.

We do not anticipate any particular problems with regulation of organism transport and shipping, toxicity or environmental hazards, or with maintenance of the product during shipping and storage or shelf life. Problems along hose lines have already been addressed and effectively solved by the Ant-Farm, Grow-a-Frog, and Sea Monkey for brine shrimp enclosed products which are widely known in toy stores, museum gift shops, and educational material suppliers.
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A. Identification and Significance of the OPPORTUNITY

1. Introduction

This project will develop hands-on integrated science educational materials based on the phenomenon of bioluminescence [1]. The problems of our current science curricula are outlined in Project 2061: Science for All Americans. This report concludes the problems in much of current science education include the "learning of answers more than exploration and questions, memory at the expense of critical thought, bits and pieces of information instead of understandings in context, recitation over argument, reading in lieu of doing" [2]. PSI intends to address this critical need by developing materials, "toys", discovery aids, and new curricula using the excitement of bioluminescence.

Nearly everyone who discovers and observes bioluminescence is impressed and motivated to see and learn more. In these times where children have their senses constantly stimulated to near exhaustion, bioluminescence is a relatively unknown, unexperienced phenomenon which can readily compete for a student's attention and interest. It provides an opportunity to discover something totally new, eliminating or minimizing any preconceived notions or attitudes, which have been demonstrated to inhibit comprehension and retention [3].

The materials will aid in the observation, discovery and learning of basic concepts in various science fields (Figure 1). Teachers and their students will develop new and expanded understanding of science concepts in a completely new domain-- bioluminescence. The concepts and understanding derived from bioluminescence are general and applicable to the full range of scientific and technical subjects (Figure 1). The emphasis will be on inquiry, discovery, conceptual learning, and the nature of the scientific endeavor.

This proposal specifically addresses research on a novel teaching aid, Night farm, a bioluminescent earthworm analogue to the well known Ant Farm. We propose to develop the science and technology necessary to produce NIGHT FARM, and to provide accompanying curricula which would be introduced in the classrooms for discovery by teachers and students alike.

Several books in the science and juvenile sections of the library discuss observation and experimentation of earthworms [4], but with no mention of the existence of bioluminescent species. Also, most "educational" earthworm books do not attempt to present the integrated concepts involved, such as the effect of earthworms on local ecology or the biochemistry of the worm. We believe that by using bioluminesence and by very careful development, an exciting inter- and multi-disciplinary tool can be developed to encourage inquiry, thereby motivating children in the discovery of science as a fascinating and challenging endeavor.

2. Applications

A new awareness is emerging that the 90's will be a decade of increased emphasis on education. Most states have already adopted programs to enhance education at all levels, in both public and higher education. The National Science Foundation's budget for science education has increased dramatically in recent years. This will result in a significantly increased demand for products which have an educational component, including gifts and novelties for children and more serious products purchased by parents and teachers to enhance the education of their children and their students. PSI is addressing this interest with products that stimulate a child's natural curiosity

and eagerness to learn.

PSI expects that products based on the technical innovations proposed here will lead to enhanced science education. Night Farm is a transparent container of bioluminescent terrestrial annelids and Night Sea will be a transparent container of bioluminescent marine worms. Both produce light upon stimulation, providing a captivating introduction to the relatively unknown phenomenon of bioluminescence.

Several species of bioluminescent earthworms are found in many parts of the world, several in the United States [5]. The use of bioluminescent earthworms will enhance the excitement children develop upon exploration, motivating them for more in depth discovery and exploration. More importantly, however, PSI intends to develop a teaching aid for an integrated approach to science, where bioluminescence provides a vehicle for discovery of the interplay between the various disciplines of science. For example, earthworms provide an important mechanism for the breakdown of dead plant and animal material in soil and forests and in the maintenance of soil structure, aeration and fertility. Today, because of the ability of some earthworms to concentrate toxic metals such as lead in non-toxic forms, they are being researched for use in environmental clean-up efforts [6]. The biology, physiology and life cycles of earthworms leads directly into ecology and environmental studies. The bioluminescence aspects of these creatures provide opportunities for exploration of chemistry and physics as well as biology, geology, and environmental sciences (Figure 1).

Successful development and manufacture of Night Farm and Night Sea will provide a new, exciting introduction to the joy of discovery and exploration, and the impetus for developing questions and hypotheses. Educational reform to integrate the various divisions of science is now recognized as a critical step which must be taken if the U.S. is to compete with the advances in other competing nations [7]. Night Farm and Night Sea provide excellent introductions to the interplay of biology, chemistry and physics.

A totally different commercial application of the research proposed here is the use of bioluminescence in biotechnology. A number of biotechnology companies and universities are using bioluminescence to study gene expression and developmental biology [8]. Luciferin and luciferases, the biochemicals responsible for bioluminescence [1], are needed in biomedical research and are currently extremely expensive. Earthworm luciferin and luciferase has not yet been applied because quantities are limited and therefore expensive. The development of methods of culture and reproduction of bioluminescent earthworms would be of great value in applying worm bioluminescence to other problems.

Thus, successful development and commercialization of Night Farm and Night Sea will have a major impact on science education and on the enthusiasm children develop through the process of discovery.

B. Background

Flashing fireflies on summer evenings, glowing ocean surf, and other forms of natural bioluminescence have always been a target of curiosity. Ancient scientists Aristotle and Pliny the Elder studied bioluminescence, but it was not until the 1670's that English chemist Robert Boyle described some of its fundamental properties.[8] Although investigated for many years, biologists have been slow to exploit bioluminescence in the laboratory. Now scientists are using bioluminescence to study gene expression and developmental biology [8]. Other applications in biology, medicine and agriculture are just now becoming possible [9].

Bioluminescence is the light produced by certain plants and animals. It is not only fascinating to observe, but can be packaged and discovered so as to entertain a child while teaching him/her basic principles of biology, chemistry and physics, as integrated science education [9].

Bioluminescence in earthworms has long been known; references to this property go back to 1670. Perhaps the best known case of luminescence in the Oligochaeta is that of Microscolex phosphoreus, a worm whose original home is probably in South America but which has been carried to many parts of the world and has established itself in the United States [10]. Greenish yellow light appears when the worms are stimulated, such as stepping on the ground next to them. The light is given off by a luminous slime which usually exudes from the anal aperture and sometimes from the mouth. The dried slime can be made luminous again by the addition of water [10].

Another species, Diplocardia longa, found in southern Georgia, is perhaps the most extensively studied bioluminescent earthworm [11]. A slime emitting a blue-green light is exuded from the dorsal pores of these worms. The complete luminescence system in D. longa is packaged in coelomocytes found in the slime. The luminescent reactions are dependent upon oxygen and limited cross reactivity with other luminescent species is known, suggesting a common substrate for bioluminescence[11].

C. PHASE I OBJECTIVES

The long range goal for this project is the application of bioluminescence to discovery teaching aids and curricula to enhance

integrated science education in grades K-12. This will involve research to develop the technical aspects of bioluminescence, especially the culturing (raising) of bioluminescent organisms and stimulation methods for producing light on demand. Development of curricula and teacher training workshops would then be initiated. The ultimate goal is for these to be incorporated into the normal teaching curricula of several local and out of state school districts.

1. Technical Approach

We propose to develop integrated science educational materials and toys using bioluminescence as the "discovery" and observation vehicles. Night farm, will consist of a transparent container of bioluminescent terrestrial earthworms, an owners manual and specially developed food packets for healthy maintenance of the animals.

The technology required for the development of Night farm requires R&D efforts to determine the appropriate species and selection of individuals for the highest degree of luminescence while minimizing the difficulty in maintenance of the organisms. The technology further includes means to stimulate the bioluminescence by physical and chemical means. One major advantage over other chemiluminescent products is the ability to control the light emission, essentially to induce light on command. This is "living light", dynamic and variable.

2. Objectives

The goal of this Phase I proposal is to establish feasibility of identifying, raising and stimulating "farms" of bioluminescent earthworms.

Specific objectives for this phase of the overall project are:

A. Identification, selection, and collection of bioluminescent earthworm species for use in Night farm

B. Establishment of long term stable populations of worms.

C. Development of stimulation methods (chemical, acoustical, electrical and/or mechanical) for producing the bioluminescent response.

D. PHASE I RESEARCH PLAN

Initial efforts will involve the identification and selection of appropriate species for use in Night farm. At least twelve species from six genera of bioluminescent annelids are known and have been studied [5] Many of these are marine or littoral in habitat.

Potential candidate species for a terrestrial farm include Diplocardia longa (found in Georgia), Diplocardia mississipiensis (found in Georgia and Louisiana), Diplocardia eiseni (found in Florida), and Microscolex phosphoreus (found in Alabama). Generally, the luminescence is reported in the fluid exuded from the dorsal pores or the mouth when the animal is stimulated mechanically, chemically, or electrically [5]. Criteria for selection will be made on the basis of: ease of culture, ease of stimulation, intensity and duration of luminescence, and reproductive cycles.

Although much is known about several species of bioluminescent earthworms [5], raising and maintenance of the organisms in adequately high densities for Night Farm, will require extensive efforts on the study of their biology, hardiness and ease of raising/reproduction. For example, while numerous investigations into the bioluminescence chemistry, mechanisms and physiology have been conducted, none of the investigators have actively attempted to grow and farm the bioluminescent species [12]. Most reported research deals with the isolation, purification, and properties of the luciferins and luciferases found in the bioluminescent species. However, numerous books are available for the beginner earthworm farmer (non-luminescent species) which will aid in the development of methods and techniques for bioluminescent species. Shields Publications (Box 472, Elgin, Illinois 60120) contains an entire library series offering help for earthworm growers. Similar books can often be found in fish & bait stores Additionally, the "Louisiana Pink" (Diplocardia mississipiensis), a common earthworm used as bait has demonstrated bioluminescent properties, although the preparation of the worms by vendors of fish bait generally destroys this capability [12].

Following selection of candidate species, collection will begin. Other than the "Louisiana Pinks," it is not known if other potential candidate worms are commercially available. Establishment of successful communities or farms of these species will be undertaken with much emphasis on the particular habitat conditions (i.e., pH, organic content and temperature) required by each species. We propose to successfully establish at least two different species for study as Night Farms.

Next, methods for stimulation will be explored. Ideally, this will be a simple vibrational or electrical disturbance, which will cause rapid and sustained luminescence. All responses will be recorded as to intensity, length of response. and recovery after various stimuli. These data will be used for future design considerations which will be built into Night farm.

1. Planned Tasks

The program objectives will be reached by the performance of the 8 specific tasks (keyed to Objectives A-C above):

Task A-1. Selection of earthworms with sufficient bioluminescence- There are several known species of bioluminescent earthworms. For practical reasons, potential candidate species for a terrestrial farm include Diplocardia longa and Diplocardia mississipiensis (both found in Georgia), Diplocardia eiseni (found in Florida), and Microscolex phosphoreus (found in Alabama). Another species which will be considered as a follow-on or backup include the marine littoral dwelling Pontodrilus bermudensis found in Australia and Florida. This species has been successfully kept in the laboratory with artificial sea water [5]. Table 1 presents a list of potential candidates with some of their bioluminescence characteristics [5]. A thorough investigation of the research literature [13], as well as discussions with earthworm growers and scientists, provide an initial knowledge base. The best species will be identified and selections made based on their availability, bioluminescent intensity/duration, ease of culture and long term viability.

Table 1*

Task A-2. Design, fabrication and preparation of containers for culture Earthworms generally prefer moist, slightly alkaline soil, however this is not true of all potential candidates. Additionally, several studies have been conducted on the role of pH and carbon to nitrogen ratios in the rate of cocoon formation and hatching [14]. Prior to worm acquisition, determination of C:N ratios and optimum food materials will be determined for each of the species identified in Task A-1. Growth chambers will be designed, built and installed in our laboratory. Several different environments will be needed to maintain the different species.

Task A-3. Collection of potential earthworm candidates- With our advisors and other experts in the field, bioluminescent worms will be purchased. Earthworm growers and distributors provide "starter packages" and technical support. These will be contacted for a possible source of the particular species of interest. However, several of the potential candidates listed above are not commercially available and will be collected by PSI's staff and advisors [15].

Task B-1. Establishment of bioluminescent earthworm farms- With the information available from earthworm distributors and from advisors, communities of each species will be established. We will attempt to culture and maintain at least two separate bioluminescent species. Key parameters include pH, humidity, density, and C:N ratios of the soil for routine growth and maintenance. Determination of reproductive cycles will be explored. Some of the species proposed as candidates (i.e. Microscolex phosphoreus) are known to reproduce parthenogenetically, i.e. using male sperm and female ova from the

same individual, while others are hermaphroditic and require the mating of two individuals [14]. Most species are considered continuous or semi-continuous breeders [14]. Investigations into which soil and nutrient conditions will provide the maximum cocoon laying and hatch rates for culture will be conducted. Knowledge of the life cycles of many species is scanty, but it is known that most worms double in population in 30-120 days [14]. Several species of worms are known to live up to 10 years, however most live less than two [14]. The life cycle of the bioluminescent species selected will be observed. Experiments will be conducted to determine the influence of temperature and humidity on reproduction rates.

Task B-2. Determination of maximum community density- Experiments will be conducted to optimize the number densities of individual species by optimization pH, nutrients and temperature. Published studies report field densities of many species; such studies have not been conducted for the species proposed here. Attempts to concentrate too many worms in a fixed volume may result in unhealthy conditions and even death of the entire community.

Task B-3. Influence of temperature and light on long term stability- Using the optimum worm densities determined above for each species selected, experiments will be conducted to determine the influence of varying light cycles and temperatures in order to consider long term stability and storage issues. These factors will be critical in the development of packaging and shipping containers in subsequent phases of the project to maintain a viable, dense culture of earthworms.

Task C-1. Survey of methods for bioluminescent stimulation- Because earthworm bioluminescence involves a hydrogen peroxide reaction, most studies in the literature have utilized chemical stimulation to elicit maximal bioluminescence. This will not be appropriate for the Night farm educational discovery product. Bioluminescence is also stimulated by mechanical and electrical means, though there have not been detailed studies of these approaches.

Our major emphasis will be on mechanical stimulation, including the low frequency, low amplitude stimulation characteristic of large organisms walking on land. The worms can sense land surface vibration caused by walking and running animals. The children's inquiry analogue of this would be a light tapping on the surface of the transparent Night farm container. Higher frequency, low amplitude stimulation will be studied using acoustic means with a focused audio speaker and signal generator.

We will do some preliminary experiments, using electrical stimulation with electrodes placed on the walls of the container and low voltage DC stimulation. This will, of course, be a strong function of the nature of the soil, the worm density in the soil, the humidity of the soil, etc.

Task C-2. Quantitation of intensity and duration of optimal methods of
stimulation- The intensity and spatial distribution of the bioluminescence which results from stimulation will be recorded using high speed scientific Polaroid film simply placed in contact with the transparent chamber. Given the dimensions of the worm, the intensity of the luminescence, and our chamber design, this contact print method of detection is very inexpensive and effective and, together with experimenter viewing of the results under dark-adapted conditions, will provide the qualitative information needed to select optimum stimulation parameters.

Once these more qualitative and optimization experiments are complete, then a more rigorous measurement and analysis of bioluminescence intensity, duration, and recovery will be done using a highly sensitive video camera system. PSI already has access to such a system through the University of Utah's Department of Bioengineering.

Dr. V. Hlady, Associate Research Professor of Bioengineering at the University of Utah, will conduct these experiments using a Photometrix, Inc. CCD camera system [16].

2. Anticipated Results

The proposed R&D project has been planned and organized to test the feasibility of developing and maintaining viable bioluminescent earthworm farms for use as a science educational toy. We anticipate successful demonstration of feasibility of growing and maintaining these species of bioluminescent earthworms. After feasibility is demonstrated in this Phase I project, we expect to proceed to Phase II. This phase of the project will concentrate on development of the teaching curricula for use with Night Farm and to further develop and optimize Night farm and demonstrate more definitively its commercial applicability. Phase II will include discovery experiments which will be field tested in classrooms by expert teachers. PSI has been actively working with educators and administrators from the Salt Lake City and Davis County school districts to introduce bioluminescence into an experimental science curriculum. The reception has been enthusiastic.

If new science curricula are to be effectively implemented, research and development of student conceptions and misconceptions as well as the teachers' knowledge and skills must be assessed. Teachers must be "turned on" to the phenomenon of bioluminescence and motivated and prepared to alter their traditional teaching styles. These will also be goals for Phase II of this project. PSI is actively working with the Center for Integrated Science Education at the University of Utah to develop these teaching aids in conjunction with expert

educators and subject matter specialists to optimize the effectiveness of the materials developed by PSI. Our materials will allow teachers to develop an understanding of important science concepts and science pedagogy within a novel domain. Inservice courses will provide valuable new science content knowledge, with the influence of prior knowledge being minimized. We anticipate that teachers will feel less threatened about restructuring their views.

It is emphasized that the Phase I objectives of the tasks described above, including testing to be carried out, is to establish feasibility of growing bioluminescent earthworms and designing and building a Night Farm. This project will be followed by Phase II and Phase III studies. Upon demonstration of the feasibility of Phase I Night Farm, we anticipate no difficulty in obtaining funding for the follow-on Phase II (curriculum development and optimization) and Phase III (commercialization) of the over-all project.

Several novelty products are currently on the market which generate light through chemiluminescence such as "Lite-Stiks" (an American Cyanimid Co. Product). However, these do not address the educational market or attempt to stimulate scientific interest. These products lack the flexibility and versatility available with bioluminescence. To our knowledge, no other product is available or under development to meet the objectives of Night Farm and related Night Life Toys being researched and developed by PSI.

As research on bioluminescent earthworms is not a well developed sub-discipline, we cannot anticipate every eventuality and problem. We certainly expect to be able to select, collect, grow, and maintain the needed bioluminescence species. We are not sure how they will respond to confinement in a narrow transparent container, which is necessary for efficient observation and experimentation.

If for some unknown reason bioluminescent earthworms prove to be completely impractical for this project, then we will focus our attention on the marine worms, Pontodrilus bermudensis, and related species. These worms live at the high tide level, have readily been maintained in captivity in artificial seawater, and have a particularly bright bioluminescence [5]. This will require developing a container which includes a water and sand or soil component in which the worm is expected to live at the interface. We anticipate no problem here because we have already some experience with such a geometry in the NIGHT-COLONY dinoflagellate bioluminescent product, which is PSI's major private product development effort.

We do not anticipate any particular problems with regulation of organism transport and shipping, toxicity or environmental hazards, or with maintenance of the product during shipping and storage or shelf life. Problems along those lines have already been addressed and effectively solved by the Ant-Farm, Grow-a-Frog, and Sea Monkey

(brine shrimp) products which are widely available in toy stores, museum gift shops, and educational material suppliers.

E. SUMMARY

Substantial evidence exists for the commercial feasibility of Night farm. There is national recognition of the current crisis in science education. Most states have already adopted programs to enhance science education at all levels. This will result in a significantly increased demand for products which have an educational component. The trend in this revolution is for an integrated approach to science education, to connect or merge the different scientific subjects via discipline bridging themes. Night farm provides a unique vehicle for this integrated approach. Not only does Night farm use principles of physics, chemistry and biology, but it can also teach students to approach problems in the way scientists do, to stimulate questions, to formulate hypotheses, to design experiments and deduce conclusions and generalizations.

F. RELATED RESEARCH

PSI is uniquely positioned for the introduction of novel educational materials based on bioluminescence. First, we have had extensive collaboration with experts in the field, not only with earthworms, but other bioluminescent organisms. Dr. Andrade has been deeply involved in the field of bioluminescence over the past 3 years. Members of our staff have attended a workshop on marine phytoplankton culture and techniques at the Provasoli Guillard Center for culture of Marine Phytoplankton. In addition, we have been actively working with educators and administrators from the Salt Lake City, Jordon and Davis County school districts to introduce an experimental science curriculum based on bioluminescence with very positive response.

PSI is currently working on the development of techniques for large scale production of bioluminescent dinoflagellates for another product, Night Colony . This experience produced a wealth of knowledge as to the temperature, salinity and nutrient requirements, including trace metals and vitamin supplements, of the Pyrocystis noctiluca and Pyrocystis lunula organisms. Much of this experience will help in the development of the Night farm.

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12. J. Wampler, in discussion with J. Andrade of Protein Solutions, Inc. in Athens, GA on 4/29/91.

13. Smithsonian Worm Project

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15. John Wampler has agreed to assist us with collection in the Athens and Atlanta, Georgia areas. Personal friends of S. Winters and J. Andrade in Jacksonville, Miami, Raleigh, Atlanta, and Birmingham will also assist.

The University of Utah's Center for Integrated Science Education (CISE) has an ongoing study to map the distribution of bioluminescent earthworms, fungi, and fireflies in Utah (The Utah Bioluminescence Project & Contest).

16. See J.D. Andrade

17. Toy Industry Fact Book: 1990-91, Toy Manufacturers of America (1991).

H. PERSONNEL & CONSULTANTS

Suzanne Winters, Ph.D., Vice President for Development, will serve as Project Manager and Principle Investigator. Dr.Winters has worked in technology development for the past 5 years with CardioPulmonics, Inc. as Director of Membrane Technology. She recently joined Protein Solutions, Inc. Her vita follows.

Joseph D. Andrade, Ph.D., President and founder of Protein Solutions, Inc. has been studying bioluminescence for the past three years, primarily for science motivation and education. He is internationally recognized for his inter and multi-disciplinary approaches to science and engineering. He is Chairman of the Department of Bioengineering at the University of Utah and Director of the Center for Integrated Science Education. He has taught high school general science, chemistry and biology and has assisted in elementary school science instruction on a regular basis for several years. Dr. Andrade has been working on biomaterials and biotechnology problems for the past 25 years. His vast experience with the nature and behavior of proteins is directly applicable to the complex proteins involved in bioluminescence . His vita follows.

Advisors/Consultants:

Trish Stoddart, Ph.D. is Assistant Professor of Educational Studies in the Graduate School of Education at the University of Utah. Dr. Stoddart's major research area is the assessment of teacher knowledge, and the means by which incorrect concepts can be relearned. She sees bioluminescence as an ideal tool with which to study teacher pre-conceptions and with which to motivate teachers to restructure their concepts. She will function as an advisor to the project.

John Wampler, Ph.D. is Professor of Biochemistry at the University of Georgia in Athens, Georgia. He is internationally recognized for his work on bioluminescent, earthworms and related organisms [5]. J. Andrade has discussed the use of bioluminescence as a motivational tool for science education with him [12]. Dr. Wampler will advise and assist us as required.

Barbara Andrade is a first and second grade teacher with twelve years of teaching experience. She also serves as secretary of Protein Solutions, Inc. She has worked with earthworms in her classroom, and has been involved with PSI's efforts in the use of bioluminescence for science education for several years. She will directly advise and assist in the development and formulation of the bioluminescent earthworm concept into a practical and effective science education tool.

Rene Stofflett, Ph.D. is Assistant Professor of Education at Northern Illinois University. She was formerly a graduate student working under Dr. T. Stoddart. Dr. Stofflett was also involved in the discussions on the development and use of bioluminescence in elementary education. She is eager to apply the materials and products developed by PSI to her research and education activities in Northern Illinois.

Vladimir Hlady, Ph.D. is Associate Research Professor of Bioengineering at the University of Utah. He is an expert on optics, particularly the measurement of luminescence and fluorescence at solid/liquid interfaces. His optical spectroscopy and engineering laboratory is a major resource for the more detailed light intensity and light duration studies proposed earlier. Dr. Hlady will serve in a formal consulting capacity. His letter of interest and agreement follows.

I. FACILITIES & EQUIPMENT

Most of the work on this project will be carried out by PSI, Inc. in its laboratories located at Northgate Business Center, 825 No. 300 West, Suite 145 in Salt Lake City and affiliated laboratories at the University of Utah (see letters from Caldwell and Andrade). Our laboratories are equipped to perform the necessary biology, chemistry, physics, and engineering studies and evaluation.

J. CURRENT AND PENDING SUPPORT

PSI has no current outside support. Its funds are generated via stock sales to its founders and selected private investors. However, 3 proposals in addition to this proposal have been submitted or are currently in preparation.

K. COMMERCIAL POTENTIAL

The initial market for PSI's bioluminescence products are children, their parents and their teachers. With the growing

recognition that our educational system must increase its emphasis on science and mathematics, an increased demand for products which have a significant educational component is expected during the 90's.

Although PSI has not had the resources to do a complete market analysis, it is clear that the market is very large. For example there have been over ten million Ant Farms sold by its original inventor and developer, Milton Levine. There are at least two other manufacturers of ant farms who have a significant fraction of the market. The estimated sales volume for these ant farms and accessories is $25 million.

Market assessments performed by Toy Manufacturers of America estimate sales of educational toys for 1989 to be $45 million [17].

A key target for PSI's marketing emphasis will be museum gift shops and science centers. According to a recent Association of Science and Technology Centers Survey, museum stores represent a substantial source of revenue in some institutions. Numerous articles are appearing in journals across the nation from flight magazines found in your seat pocket on the airplane to Business Week describing museum shopping and the "chic" emporiums for unique gifts. Smithsonian Museum Shops stock merchandise to sell with the philosophy that every item must include an educational component.

We have estimated that the national public education market (K-12) for science textbooks and materials is over $2B/year.

PSI is collaborating with the Children's Museum of Utah on an interactive exhibit based on bioluminescence.

PSI is now obtaining information as to the total volume and subcategories of sales in museum gift shops nationally. Additionally, PSI is investigating the total budget and budget subcategories for science-related supplies and equipment in elementary, junior high and high school throughout the nation.

In summary, the educational toy market is already very large and is likely to be expanding in the next decade, based on the recognition of need for emphasis in science education. The ability to produce bright light in various patterns and under various degrees of stimulation will attract potential buyers to PSI's bioluminescent products. That attraction and novelty, coupled with the educational potential of the product, should guarantee a strong and loyal clientele.

* Taken from J. Wampler (1981) [5].

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¸¸¸˘¸¸ÛÛÌ¸¸¸¸Í¸¸ÛÁÁ¸ÍÄÄ@@[m Ecology: From Darwin to Vermic11, (The Smithsonian National Museum of Natural History now has the library collection of the late W.E. Gates, renowned worm expert. This collection has been reviewed recently by S. Winters, 6/91. ms, Their Ecology, and RelationsThis is a highly sensitive digital camera with 600 pixels and can provide a high resolution image under low light conditions.

CURRENT AND PENDING SUPPORT

PSI has no current outside support. A second proposal has been submitted to the National Science Foundation SBIR program titled Light Crawlers: Bioluminescence-Based Discoveries for Science Education. That proposal does not overlap with this project.

PSI anticipates submitting an SBIR to NASA on August 7, dealing with closed system ecologies (ecospheres) [16].

In addition to the anticipated Federal SBIR funds, PSI has already invested $60,000 (provided by its founders and major stock holders) in the initial studies and product development. PSI expects to continue funding the project at the same level. PSI is now discussing equity investments by a number of local investors and investment groups.

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strate for bioluminescence[11].onclusions and generalizations.Night farm.ineering studies and evaluation.

L. EQUIVALENT PROPOSALS

No similar proposal has been funded, is pending, or is abut to be submitted by Protein Solutions, Inc. to the National Science Foundation or any other agency.

The University of Utah's Center for Integrated Science Education (CISE) recently (May 15, 1991) submitted a proposal to NSF's Materials Development Program. The proposal, Night Life: Integrated Science Education Materials Based on Bioluminescence, focused on curricular development and curriculum integration. There is a slight overlap (estimated at 20% of this SBIR effort) in the are of bioluminescent dinoflagellates.

L. Equivalent Proposals 16Light CrawlersLight CrawlersLight CrawlersLight CrawlersLight CrawlersLight CrawlersLight CrawlersLight CrawlersLight CrawlersLight CrawlersLight Crawlers
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