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Collaborative Research: Determinants of male reproductive success in natural spawns

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Annual Report for Period:01/2003 - 01/2004**Submitted on:** 11/24/2003**Principal Investigator:** Yund, Philip O.**Award ID:** 0117623**Organization:** University of Maine**Title:**

Collaborative Research: Determinants of male reproductive success in natural spawns

Project Participants**Senior Personnel****Name:** Rawson, Paul**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Rawson is no longer the PI, but FastLane will not let Yund change this information in the annual report. Rawson continues to participate as a co-PI, and has supervised development of new molecular markers for paternity analysis.

Post-doc**Graduate Student****Name:** Johnson, Sheri**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Sheri is developing and implementing the molecular techniques used for paternity analysis. She is directly supported by this award.

Name: Phillippi, Aimee**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Aimee is a senior grad student who has developed a couple of independent projects within the framework of the award. She is not directly supported by this award, but is funded through a private fellowship.

Undergraduate Student**Name:** Gensheimer, William**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Summer undergraduate assistant from Cornell University, directly supported by this award. Bill developed an independent project on the rate at which ascidian colonies take up sperm.

Name: Pinho, Dan**Worked for more than 160 Hours:** No**Contribution to Project:**

Dan is a Univ. of Maine undergrad who has recently started working on this project part time (supported by this award). His primary duties are DNA extraction and PCR amplification. As of the date of this report, he has not yet worked 160 hours.

Technician, Programmer**Other Participant****Name:** Yund, Philip**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Yund believes that he is now the PI, and oversees all aspects of this project.

Research Experience for Undergraduates**Name:** Murdock, Kaitlin**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Kaitlin's second summer of participation in the project was funded by an REU site award, not this award (as in the first project year). Kaitlin developed an independent project that looked at sperm dispersal in three dimensions; that work is currently in preparation for publication.

Years of schooling completed: Junior**Home Institution:** Other than Research Site**Home Institution if Other:** Brown University**Home Institution Highest Degree Granted(in fields supported by NSF):** Doctoral Degree**Fiscal year(s) REU Participant supported:** 2003**REU Funding:** REU site award**Organizational Partners**

University of New Orleans

Other Collaborators or Contacts

John Stewart-Savage, University of New Orleans, has a collaborative award to work on related aspects of this project (gamete compatibility among individuals). Because John's award is subject to separate reporting requirements, his work is not described in detail in this report.

Activities and Findings**Research and Education Activities:**

This project is testing a series of hypotheses about the various ecological factors that determine male reproductive success (i.e., the ability of a male to fertilize a batch of eggs) in a brooding, sessile invertebrate (the colonial ascidian *Botryllus schlosseri*). In the second year we continued development work on a suite of molecular markers (microsatellites) to be used for paternity analysis, and also conducted initial field studies in which colony locations in discrete quadrats were mapped, fertilization allowed to occur, and then embryos collected for paternity analysis. DNA is currently being extracted from all the adult colonies and embryos from these experiments, and PCR amplification of a suite of microsatellite markers will proceed this winter.

Two graduate and two undergraduate students also conducted independent project that explored aspects of self incompatibility (the subject species is a hermaphrodite), assayed 3-dimensional sperm dispersal patterns in the field, tested the effects of temperature and mixing on sperm longevity, and assessed the rate at which colonies acquire sperm from the water column. All of these projects will contribute to our overall goal of understanding mating patterns in nature.

Findings:

Development work on the molecular markers is largely complete, but we are still experimenting with various optimization procedures prior to starting large scale PCR amplification. Because of the number of samples to ultimately be processed, relatively small increases in the information return per unit effort will have a large effect on total costs. We expect to start obtaining paternity results from the initial field studies early in the third project year, once optimization procedures are complete.

Student projects have established that sperm in this species are extremely long-lived (3-4 days). In the field, sperm availability is high even many tens of meters from natural populations, but at different sites sperm accumulate either near the substratum or near the surface. These differences appear to be driven by local circulation patterns rather than any intrinsic biological attributes of sperm. Ascidian colonies are highly efficient at collecting dilute sperm from seawater, and process the sperm from many times their body volume of water in less than an hour (much as sponges do with food particles).

Training and Development:

In the second year of the project this award provided direct support for one graduate student and two undergraduates; an additional graduate student and an undergraduate participated via support from a private fellowship and an REU site, respectively. The additional graduate student is in the writing phase of her PhD and plans to finish in May, 2004. She is a co-author on one submitted paper, and will be submitting several

of her own. One of the undergraduates is a co-author on a manuscript currently in preparation. Both undergrads are completing their BS degrees.

Outreach Activities:

Interview with a local public radio station to discuss invasions by non-native colonial ascidians.

Journal Publications

Murdock, K., and P.O. Yund., "Horizontal and vertical dispersal patterns of sperm in a colonial ascidian.", *Mar. Ecol. Prog. Ser.*, p. , vol. , (). in preparation

Yund, P.O., and S.K. Meidel., "Sea urchin spawning in benthic boundary layers: Are eggs fertilized before advecting away from females?", *Limnol. Oceanogr.*, p. 795, vol. 48, (2003). Published

Newlon, A.W. III, Yund, P.O., & J. Stewart-Savage., "Phenotypic plasticity of male, female, and asexual reproduction in a colonial ascidian, *Botryllus schlosseri*.", *J. Exp. Zool.*, p. 180, vol. 297A, (2003). Published

Phillippi, A., Hamman, E., and P.O. Yund, "Fertilization levels in an egg-brooding colonial ascidian do not vary with population density.", *Biol. Bull.*, p. , vol. , (). Submitted

Johnson, S. and P.O. Yund., "Exceptional sperm longevity in an egg-brooding free-spawning invertebrate.", *Biol. Bull.*, p. , vol. , (). Will be submitted for review by Dec. 15, 2003

Yund, P.O., and A. Stires, "Spatial variation in population dynamics in a colonial ascidian (*Botryllus schlosseri*).", *Mar. Biol.*, p. 955, vol. 141, (2002). Published

Books or Other One-time Publications

Web/Internet Site

URL(s):

<http://www.ume.maine.edu/~marine/yund.htm>

Description:

Other Specific Products

Contributions

Contributions within Discipline:

The microsatellite development work is technical in nature, and so will produce few contributions until the samples are fully analyzed. The student projects have demonstrated that sperm are surprisingly long lived, and can produce successful matings across unexpectedly large distances. Both of these results challenge the conventional wisdom that sperm are short-lived and fertilization only possible if individuals are in close proximity.

Contributions to Other Disciplines:

The sperm longevity data present a bit of a challenge to physiologists; how can a tiny single cell with scant energy reserves remain active for days? Our results raise questions in this field, but do not answer them.

Contributions to Human Resource Development:

The graduate student directly supported by this award (Johnson) is making excellent progress on her PhD thesis and developing independent questions about male reproductive success that can be tested with the molecular tools we are developing. The grad student supported with private funds (Phillippi) is entering the writing phase of her PhD and applying for jobs. One undergraduate (Murdock) is writing up her independent project as her senior honors thesis at Brown University; Yund is modifying this draft effort for journal publication. Another undergrad (Gensheimer) is completing his BS degree and applying to medical school.

Contributions to Resources for Research and Education:

This project has not been designed to provide much enhancement to infrastructure.

Contributions Beyond Science and Engineering:

Special Requirements

Special reporting requirements: None

Change in Objectives or Scope: None

Unobligated funds: less than 20 percent of current funds

Animal, Human Subjects, Biohazards: None

Categories for which nothing is reported:

Any Book

Any Product

Contributions: To Any Beyond Science and Engineering