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Linking Bioturbation and Sensory Biology: Chemoreception Mechanisms in Deposit-Feeding Polychaetes

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Final Report for Period: 09/2005 - 08/2006**Submitted on:** 12/04/2006**Principal Investigator:** Lindsay, Sara M.**Award ID:** 0221229**Organization:** University of Maine**Title:**

Linking Bioturbation and Sensory Biology: Chemoreception Mechanisms in Deposit-Feeding Polychaetes

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

Supervisor of project, guided graduate student and undergraduate student research; collected and maintained all laboratory cultures polychaetes; lead author and collected approximately half of the data reported in Biological Bulletin publication (see rest of report).

Name: Rawson, Paul**Worked for more than 160 Hours:** No**Contribution to Project:**

Co-PI Paul Rawson provided overall guidance for the molecular biological portion of the study (isolation and characterization of g proteins from spionid polychaete palps), completed the sequencing of g-protein subunits initiated by graduate student M. Tsie, and created a subtractive hybridization library to begin screening for genes specific to regenerating feeding appendages (which presumably will be enriched in candidate chemoreceptors)

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

David Forest is a graduate student who completed his Master of Science in Marine Biology in May, 2005. He worked on identifying the structure of the central and peripheral nervous system in several spionid polychaete species using confocal laser scanning microscopy.

Name: Tsie, Marlene**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Marlene Tsie is a graduate student who completed her Master of Science in Marine Biology in August, 2006. She investigated the molecular mechanism of chemoreception in spionid polychaetes. She isolated 3 genes encoding 3 subunits of g-proteins from the feeding appendages of polychaetes, used immunohistochemistry to examine the localization of a g-protein alpha subunit protein in the sensory structures of polychaetes, and attempted to isolate polychaete chemoreceptors using a biochemical assay.

Undergraduate Student**Name:** He, Si Qing**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Si Qing He began her involvement in this project as a high school student summer intern. She then enrolled as an undergraduate at Brown University and continued her participation as a summer intern for two years, studying anterior regeneration in spionid polychaetes, assisting with specimen collection, immunolocalization experiments, molecular biological studies of g-protein expression and and general lab duties.

Name: Jackson, Jennifer

Worked for more than 160 Hours: Yes

Contribution to Project:

Jennifer Jackson was an undergraduate student who assisted with specimen collection, behavioral experiments, and microscopy. She also conducted related work as part of her honors thesis comparing the rates of regeneration among different spionid polychaetes. This work was relevant because it addressed how quickly worms could regenerate lost sensory structures.

Name: Babineau, Matthew

Worked for more than 160 Hours: Yes

Contribution to Project:

Matthew Babineau's senior capstone research project examined the effect of different chemicals on feeding behavior of a spionid polychaete with and without feeding appendages.

Technician, Programmer

Other Participant

Research Experience for Undergraduates

Organizational Partners

Other Collaborators or Contacts

Activities and Findings

Research and Education Activities:

Like many other deposit-feeding invertebrates, spionids exert a profound effect on the ecology, biology, geology, and chemistry of their habitats simply by the sediment disturbance created as they feed. In this research, we investigated the process of chemoreception and its coordination of deposit feeding in *Dipolydora quadrilobata*. In the first of three objectives, we described the ultrastructure and innervation of putative sensory structures on spionid polychaete palps using electron and confocal microscopy (Lindsay et al 2004). Forest (2005) extended this work to describe the central and peripheral nervous systems in three spionid species and identified FMRF-amide and 5HT-immunoreactive nerves and sensory cells in the feeding palps of each species. His work was presented at two meetings (Forest & Lindsay 2003, 2004) and two manuscripts are in preparation. Second, we described the effect of several particle-bound chemical cues on feeding behavior of *D. quadrilobata* (Riordan & Lindsay 2002), and then showed that the same cues activated sensory cells on the feeding palps in activity-dependent cell-labeling studies (Lindsay et al 2004). Third, we began investigating the molecular basis of chemoreception in *D. quadrilobata*. The chemoreception signal transduction pathway appears to involve G-protein coupled receptors. We have isolated partial gene sequences coding for several g-protein subunits (Tsie et al 2003) and recently demonstrated g-protein alpha subunit immunoreactivity in the nuchal organs and sensory cilia on the feeding palps of *D. quadrilobata*. The manuscript reporting these results is in preparation. Co-PI Rawson recently isolated full-length coding sequence for the g-protein alpha subunit, which is the subunit that interacts with receptor proteins. The broader impacts of this research included mentoring three graduate students, three undergraduate research students and three high school summer interns. During her research, one undergraduate student discovered that *D. quadrilobata* regenerated anterior segments as well as feeding appendages, and her resulting undergraduate honors thesis comparing regenerative capacity in spionids yielded two manuscripts (Lindsay et al 2006 and submitted). With Dr. J. Fegley, we are creating several internet inquiry activities for high school students that focus on marine sensory biology. The first of these WebQuests focuses on marine mammal acoustics and was recently launched (<http://www.marine.maine.edu/~slindsay/Whaleweb/whalehome.htm>).

Findings:

(2003-2004) Sensory structures on the feeding appendages have been identified using acetylated alpha tubulin as an indicator, and confocal laser scanning microscopy. These cells appear to synapse with one of the palp nerves, and these cells are the same that are activated by chemical cues that also elicit a behavioral response. Partial gene sequences for three g-protein subunits have been identified from *Dipolydora quadrilobata* feeding structures. Two species of spionid polychaetes regenerate lost anterior tissue at different relative rates, depending on the

amount lost, but they appear to reach morphological milestones of regeneration at about the same time. Ciliation on the palps that is necessary for feeding does not appear until about 9 days post-ablation.

(2004-2005) The peripheral sensory structures on feeding palps appear to be associated with FMRF-amide reactive cells. Some cells in the feeding appendages are serotonin immunoreactive, suggesting that serotonin may influence feeding behavior or mucus production in spionid polychaetes. *Dipolydora quadrilobata*, one of our focus species, does not feed following feeding appendage loss, and does not respond to waterborne chemical cues for several days following feeding appendage loss. This supports our previous work that concluded the feeding appendages also had a sensory function.

(2005-2006): The full coding sequence for a gene encoding a g-protein alpha subunit has been isolated from *D. quadrilobata* feeding palps. We used a commercial antibody to a G-protein alpha subunit to visualize the patterns of this subunit's localization in spionid polychaete feeding appendages and sensory structures. Immunoreactivity to the antibody is high in feeding appendage cilia and the nuchal organs, further suggesting that the palp cilia serve a sensory function and that the chemical signal transduction pathway involves g-protein coupled receptors.

Training and Development:

(2003-2004) David Forest has developed significant skills in immunohistochemistry and use of the confocal laser scanning microscope. He also attended the Chemosensory Neurobiology in the Marine Environment course at the Bermuda Biological Station for Research. Marlene Tsie has completed her required course work in Biological Oceanography and Chemical Oceanography, and also took the Chemosensory Neurobiology in the Marine Environment course at the Bermuda Biological Station for Research. Undergraduate Jennifer Jackson and high school student Si Qing He were trained on the scanning electron microscope.

(2004-2005): David Forest completed his M.S. degree in May 2005; he will participate in a Marine Science & Anthropology training program in the Solomon Islands this summer and then begin working at the Mount Desert Island Biological Laboratory as a technician beginning in the fall. Marlene Tsie completed her required coursework for her M.S. degree, including training in electron and confocal microscopy. Undergraduate Si Qing He began training in immunohistochemistry and confocal microscopy. Undergraduate Matthew Babineau learned how to do behavioral experiments and video analysis.

(2005-2006): Undergraduate Si Qing He continued training in immunohistochemistry and microscopy techniques, and also began learning RNA isolation, PCR, and cloning methods. Graduate Student Marlene Tsie completed her M.S. degree and is now employed as a technician at the Jackson Laboratory in Bar Harbor, ME.

Outreach Activities:

(2003-2004) Delivered two presentations and conducted activities on intertidal ecology at local middle school; Conducted workshops on marine bioluminescence with two local 4th grade classes as part of the UMaine NSF-Graduate K-12 program; hosted interactive display on sandy shore ecology at Maine Beaches Conference; participated in 'Sixth-Grade Science Day' in the College of Natural Sciences, Forestry and Agriculture at the University of Maine; presented an invited seminar on marine bioluminescence as part of ocean education strand at the Maine Science Teachers Association Annual Conference; published article on marine soft sediment ecology in the Gulf of Maine Marine Educators Newsletter

(2004-2005): Presented bioluminescence activity to local Montessori school; with Jill Fegley, began developing marine sensory biology web-based inquiry activities that will be part of my faculty website (expected launch by end of June, 2005). Served as field guide for Old Town Elementary School third grade field trip to the Darling Marine Center.

(2005-2006): Marine Mammal acoustics/sensory biology webquest launched (<http://www.marine.maine.edu/~slindsay/Whaleweb/whalehome.htm>) and submitted to the National WebQuest clearinghouse. The activity was also submitted for review to the BRIDGE, a nationally recognized clearinghouse for ocean science information and lesson plans, has been accepted and will be added to their website.

Journal Publications

Lindsay, S.M., T.J. Riordan, Jr., and D. Forest, "Identification and activity-dependent labeling of peripheral sensory structures of a spionid polychaete", *Biological Bulletin*, p. 65, vol. 206, (2004). Published

Forest, S. and S.M. Lindsay., "Comparative morphology of the nervous system and peripheral sensory cells in three species of spionid polychaetes (Published Meeting Abstract)", *Integrative and Comparative Biology*, p. 1035, vol. 43 (6), (2003). Published

Forest, D.; Lindsay, S., "Serotonin may influence escape response in a polychaete tube-worm (Published Abstract)", *Integrative and Comparative Biology*, p. 696, vol. 44 (6), (2004). Published

Tsie, M; Rawson, P; Lindsay, S., "Molecular Cloning of Putative Chemosensory G proteins from a Spionid Polychaete (Published Abstract)", *Integrative and Comparative Biology*, p. 756, vol. 44 (6), (2004). Published

Lindsay, S.M.; Jackson, J.L.; He, S.Q.; Forest, D., "Losing heads & making connections: anterior regeneration in spionid polychaetes (Published Abstract)", *Integrative and Comparative Biology*, p. 593, vol. 44(6), (2004). Published

Lindsay, S.M. , J.L. Jackson, and S.Q. He., "Anterior Regeneration in the spionid polychaetes *Dipolydora quadrilobata* and *Pygospio elegans*", *Marine Biology* DOI 10.1007/s00227-006-0431-0, p. see DOI, vol. online, (2006). Published

Lindsay, Sara M., Jackson, Jennifer L. and Forest, David L.

, "Morphology of anterior tissue and nervous system regeneration in spionid polychaetes", *Invertebrate Biology*, p. , vol. , (). Submitted

Forest, D. and S. Lindsay

, "Structure and composition of the spionid polychaete nervous system: A survey using immunohistochemistry and CLSM", *Journal of Morphology*, p. , vol. , (). in preparation

Tsie, M., Rawson, P, and Lindsay, S., "Immunolocalization of a putative chemosensory G-protein alpha subunit in a spionid polychaete", *Cell & Tissue Research*, p. , vol. , (). In preparation

Books or Other One-time Publications

Jackson, Jennifer L., "A study of anterior regeneration in three spionid polychaete species.", (2003). Thesis, Published
Bibliography: Undergraduate Honors Thesis, University of Maine, Orono ME

Forest, David L., "The nervous systems of spionid polychaetes: structure, composition, and effects of serotonin on behavior", (2005). Thesis, Published
Bibliography: M.S. Thesis, University of Maine, Orono ME

Tsie, Marlene S., "Cloning and immunolocalization of G-proteins in the spionid polychaete *Dipolydora quadrilobata*", (2006). Thesis, Published
Bibliography: M.S. Thesis, University of Maine, Orono ME

Web/Internet Site

URL(s):

<http://www.marine.maine.edu/~slindsay/Whaleweb/whalehome.htm>

Description:

This website is a product of the educational outreach component of the award that focused on creating internet resources and activities for K-12 students and teachers that highlight sensory biology in a marine context.

Other Specific Products

Contributions

Contributions within Discipline:

Our findings represent the first integrative description of chemoreception in a polychaete worm, showing first behavioral responses to known

chemical cues, next that these same cues activate possible sensory structures on the worms, next that these structures are indeed sensory, as illustrated by their innervation, and finally that the chemical signal transduction pathway in these worms may indeed involve g-protein coupled receptors, as suggested by the presence of g-protein subunits expressed in the sensory and feeding appendage cilia. Our findings provide the basis for continued work examining the evolution of chemoreception mechanisms in metazoans, the sensitivity of chemoreception in polychaetes, and the link between chemoreception and sediment disturbance rates.

Contributions to Other Disciplines:

Our work describing the peripheral and central nervous systems in spionid polychaetes is of interest to other researchers studying the evolution of nervous systems in the Annelida. Our work identifying putative chemosensory g-proteins in spionid polychaetes is relevant to researchers studying the evolution of chemoreception mechanisms among the metazoa.

Contributions to Human Resource Development:

Two graduate students were trained in immunohistochemistry and microscopy techniques, both have entered the scientific research workforce as laboratory technicians, and one is applying to Ph.D. programs. One undergraduate student who worked on the project graduated and entered a PhD program in an IGERT program combining training in physics and chemical ecology.

Contributions to Resources for Research and Education:

Contributions Beyond Science and Engineering:

Categories for which nothing is reported:

Organizational Partners

Any Product

Contributions: To Any Resources for Research and Education

Contributions: To Any Beyond Science and Engineering