

5-16-2012

Sea Slug—Algal Chloroplast Symbiosis: Towards an Integrated Understanding of Long-Term Chloroplast Functioning in an Animal

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Recommended Citation

Rumpho, Mary E., "Sea Slug—Algal Chloroplast Symbiosis: Towards an Integrated Understanding of Long-Term Chloroplast Functioning in an Animal" (2012). *University of Maine Office of Research and Sponsored Programs: Grant Reports*. 308.
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Annual Report for Period:08/2011 - 07/2012**Submitted on:** 05/16/2012**Principal Investigator:** Rumpho, Mary E.**Award ID:** 0726178**Organization:** University of Maine**Submitted By:****Title:**

Sea Slug - Algal Chloroplast Symbiosis: Towards an Integrated Understanding of Long-Term Chloroplast Functioning in an Animal

Project Participants**Senior Personnel****Name:** Rumpho, Mary**Worked for more than 160 Hours:** Yes**Contribution to Project:****Post-doc****Name:** Pelletreau, Karen**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Karen joined my lab April 1, 2008 as a Post-doctoral research associate. She is working on characterizing the uptake of chloroplasts by the sea slug, especially focused on identifying the recognition factor(s). She will be using various microscopy approaches. Karen also helps direct the graduate and undergraduate students in the lab. She is being supported 100% by the NSF grant.

Graduate Student**Name:** Warful, Jared**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Jared is completing his MS in biochemistry working on this project, focusing on horizontal gene transfer. He also established the in-lab culture system to raise photosynthetic sea slugs and study their development and acquisition of chloroplasts. Jared was supported by the Univ. of ME on a teaching assistantship.

Name: Soule, Kara**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Kara is in the first year of her MS program in biochemistry. She is examining chloroplast gene expression in the alga and sea slug to better understand the role of the nucleus in regulating chloroplast gene expression. Kara was supported by the Univ. of ME on a teaching assistantship.

Name: Clegg, Katie**Worked for more than 160 Hours:** No**Contribution to Project:**

Graduate student in the Master of Science in Teaching program for math and science. Working on assessing the teaching of photosynthesis in junior and senior high school and the possibility of using the sea slug system as a new way to deliver information on photosynthesis.

Name: Moustafa, Ahmed**Worked for more than 160 Hours:** No**Contribution to Project:**

Ahmed assisted with DNA sequence analysis and phylogenetic analysis on the project. He is a graduate student at the Univ. of IA.

Name: Devine, Susan

Worked for more than 160 Hours: Yes

Contribution to Project:

Susan is focused on identifying the bacterial species symbiotically associated with two populations of sea slugs.

Undergraduate Student

Name: Pekrul, Jordon

Worked for more than 160 Hours: Yes

Contribution to Project:

Jordon worked on the sea slug development system as part of her senior research project. She received work merit funding from the Univ. of ME.

Name: Devine, Susan

Worked for more than 160 Hours: Yes

Contribution to Project:

Susan also worked on the sea slug development system and will look at bacterial symbionts of the sea slug next year as part of her senior research project. She received some funding from the Univ. of ME.

Name: Fournier, Craig

Worked for more than 160 Hours: Yes

Contribution to Project:

Craig completed his senior research working on this project, specifically measuring the activity of the chloroplast enzyme, phosphoribulokinase in sea slugs and algae.

Name: Mattsson, Helen

Worked for more than 160 Hours: Yes

Contribution to Project:

Helen carried out her Honors' capstone research on this project attempting to identify symbiotic bacteria associated with the sea slug. She received financial support from the Honors' College and a private departmental scholarship to support research costs.

Name: Doucette, Christopher

Worked for more than 160 Hours: Yes

Contribution to Project:

Chris is carrying out his senior capstone research in my lab focused on sea slug/alga recognition.

Name: Varney, Margaret

Worked for more than 160 Hours: Yes

Contribution to Project:

Meg is using molecular tools to quantify the time it takes for algal organelles (nuclei and mitos.) to clear out of the sea slug after feeding. Meg is paid with funds from this NSF grant.

Name: Cusack, Siobhan

Worked for more than 160 Hours: Yes

Contribution to Project:

Siobhan is working on developmental biology aspects related to the sea slug project. She is partially supported by this NSF grant.

Name: Montesano, Noelle

Worked for more than 160 Hours: No

Contribution to Project:

Noelle is working on developmental biology of the sea slug *Elysia chlorotica* and recording photographic images and making measurements related to development of kleptoplasty.

Name: Saraver, Kara

Worked for more than 160 Hours: Yes

Contribution to Project:

Kara carried out her senior honors' thesis project on this study analyzing gene expression in individual animals. She continued on

the project during the summer after her senior year.

Name: Davis, Geoffry

Worked for more than 160 Hours: Yes

Contribution to Project:

Geoff was supported by a supplemental NSF-REU grant to work on developing a FISH technique to use with the sea slug and alga. He completed an Honors' thesis based on this work and is now entering graduate school at Michigan State University for fall 2012.

Technician, Programmer

Name: Kozlowski, Ron

Worked for more than 160 Hours: Yes

Contribution to Project:

Constructed and maintains project web site at <http://sbe.umaine.edu/symbio/>. Assists with video productions.

Name: Price, Dana

Worked for more than 160 Hours: No

Contribution to Project:

Bioinformaticist specialist. Assists with analyzing genomic and transcriptomic data. Funded by Rutgers University through Prof. Bhattacharya.

Other Participant

Name: Bhattacharya, Debashish

Worked for more than 160 Hours: No

Contribution to Project:

Dr. Bhattacharya of the Univ. of IA assisted on the project to analyze genome sequence data.

Name: Muhlin, Jessie

Worked for more than 160 Hours: No

Contribution to Project:

Jessie collaborates with us related to ecological studies through her senior capstone research projects. Jessie is a faculty member at Maine Maritime Academy.

Research Experience for Undergraduates

Name: Davis, Geoff

Worked for more than 160 Hours: Yes

Contribution to Project:

Geoff is working on sea slug developmental biology. He will be supported during the summer of 2010 partially by an NSF-REU connected to this project.

Name: Fisher, Elizabeth

Worked for more than 160 Hours: Yes

Contribution to Project:

Beth is also using molecular tools to quantify the time it takes for algal organelles (nuclei and mitos.) to clear out of the sea slug after feeding. She is also working on culturing the alga *Vaucheria litorea*. She will be supported during the summer of 2010 partially by an NSF-REU connected to this project.

Organizational Partners

Texas A&M University Main Campus

Dr. James Manhart (Professor) and Dr. Jungho Lee (Post-doc) collaborated on the project to sequence and map the chloroplast genome of *Vaucheria litorea*. We also collaborated in writing two manuscripts, one published in PNAS (Nov. 2008) and a second submitted to Molecular Plant, June 2009.

University of Iowa

Dr. D. Bhattacharya and Mr. Ahmed Moustafa, then of the Univ. of IA, collaborated with us to help analyze the mitochondrial genome of the sea slug *Elysia chlorotica*. They are co-authors on a paper published in PNAS (Nov. 2008) and Dr. Bhattacharya is a co-author on a manuscript published in *Molecular Plant* in 2009.

University of Mississippi

My graduate student, Jared Worful, traveled to the Univ. of MS to collaborate with Dr. Mark Hamann in Jan. 2008 on the production of natural products by the kleptoplastic sea slug. A manuscript is in preparation. We continue to collaborate on natural product biosynthesis in the sea slug.

Maine Maritime Academy

We are collaborating with Dr. Jessie Muhlin and her undergraduate research students on senior capstone projects. We have also used the sea water table at MMA to carry out experiments. This collaboration will continue and expand in 2009-2010.

Rutgers University New Brunswick

Prof. Bhattacharya moved his lab from the Univ. of IA to Rutgers Univ. in the summer of 2009. We continue to collaborate with Prof. Debashish Bhattacharya on high-throughput genomic and transcriptomic sequencing of the sea slug *Elysia chlorotica*. His lab provides the computational analysis and also now conducts sequencing with a new Illumina instrument. His former student, Ahmed Moustafa, and new technician, Dana Price, provide computational analysis for the project.

Heinrich-Heine-University

We are collaborating with Andreas Webber at Heinrich-Heine-University to carry out lipid analysis and proteomics. To date, this has included sending samples to him for analysis.

Indiana University

Profs. Murray and Hu and Indiana Univ. are assisting us with visualization approaches to better tease out the mechanisms associated with establishment of the chloroplast symbiosis within the digestive cells of the sea slug by applying new technologies and instrumentation in their state-of-the-art live cell imaging facilities at IU. Research Associate Karen Pelletreau spent one week in their lab during 2011 using their equipment and expertise.

Other Collaborators or Contacts

We collaborated with personnel at Michigan State University related to high-throughput gene sequencing during 2008 and with the Univ. of Iowa during 2009 and 2010.

We are also using the Genome Sequencing Center at Washington University, St. Louis, MO, for lower-throughput DNA sequencing.

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

The molluscan sea slug *Elysia chlorotica* has evolved the means to sustain itself by carrying out photosynthesis as a result of acquiring chloroplasts from its algal prey *Vaucheria litorea*. The overall goal of this project is to combine cellular and molecular analysis with studies of organelle uptake and organismal biology towards an integrated understanding of how such an endosymbiotic association can form and be sustained, and also influence the evolution of photosynthesis in an animal.

The specific objectives include: 1) using PCR, rtPCR and genome walking to convincingly demonstrate integration of targeted algal nuclear genes (*prk* and *psbO*) into the sea slug, 2) using high-throughput sequencing of the partial transcriptome from a normalized photosynthetic sea slug cDNA library and sea slug egg DNA as a more global approach to identifying HGT, 3) completing the sequencing and analysis of the mitochondrial DNA of *E. chlorotica* to determine if algal genes have been transferred into the more typically insert-friendly mitochondrial genome, and 4) exploiting laboratory-reared kleptoplastic sea slugs and laser-scanning confocal microscopy (with organelle-specific fluorescent dyes) to characterize feeding specificity, uptake and establishment of the kleptoplastic association, and the functional longevity of

kleptoplasts.

With additional seed grant support from Maine SeaGrant, we have also initiated a metagenomics study of the microbiont associated with two populations of *Elysia chlorotica*, one from Martha's Vineyard, MA, USA, and the other from Halifax, Nova Scotia, Canada. We are interested in identifying microbial symbionts that may be aiding in providing nutrition (N, P, S, etc.) to the sea slug and possibly also linked to the production of secondary products.

PI Rumpho spent a sabbatical leave partially in the lab of Dr. Debashish Bhattacharya at Rutgers University during the fall of 2009 collaborating on analysis of high-throughput DNA sequencing. In addition, she and Post-doctoral Associate Karen Pelletreau met with Dr. Bhattacharya and his bioinformatics technician, Dana Price, for three days in May, 2010, to further analyze transcriptomic and genomic data in preparation for a manuscript submission.

Select National and International Meeting Speaking Invitations from 2007 - present:

American Society for Microbiology (ASM), 'Photosynthetic Sea Slugs: Endosymbiotic Association between Algal Chloroplasts and a Marine Mollusc,' May 2011

International Society for Endocytobiology (ISE). 'Genomics of a Sea Slug ? Algal Plastid Symbiosis.' Tr?msø, Norway, August 2010

The Waller Memorial Lecture, Plant Cellular and Molecular Biology Department, The Ohio State University, 'The Symbiotic Making of a Photosynthetic Animal ? Developmental Biology to Genomics.' and 'Symbiosis and Biological Novelty,' May 2010

JEB Symposium ? The Biology of Energy Expenditure. 'Solar-Powered Sea Slugs ? Evolution of Photosynthesis in an Animal.' M?rren, Switzerland, March 2010

Tsujimoto Lecture, Plant and Microbial Biology Department, University of California, Berkeley, 'The Symbiotic Making of a Solar-Powered Sea Slug.' March 2010

7th Okazaki Biology Conference on The Evolution of Symbiotic Systems. 'The Symbiotic Making of a Solar-Powered Sea Slug.' Kakegawa, Japan, January 2010

International Symposium ? Microbial Interactions Leading to Novel Biological Functions. 'The Symbiotic Making of a Solar-Powered Sea Slug.' University of Tsukuba, Japan, January 2010

International Symbiosis Society. Symposium on 'Agents of dependence ? from viruses to organelles.' Madison WI, August 2009.

American Society of Plant Biologists. Symposium on 'Genomics approaches for systematics, energy metabolism and acclimation.' Honolulu, HI, July 2009.

Gordon Research Conference on Mitochondria and Chloroplasts. 'Horizontal gene transfer in a sea slug - algal chloroplast endosymbiosis.' Biddeford, ME, August 2008.

University/Departmental Invited Seminars (2007 - present)

Indiana University, Molecular, Cellular, & Developmental Biology Program, May 2011

Cornell University, Dept. of Molecular Biology and Genetics, February 2011

Bowdoin College, ME, Biology Dept., January 2011

University of Maine, Dept. of Molecular & Biomedical Sciences, April 2010

Rutgers University, Dept. of Ecology, Evolution, and Natural Resources and The Ecology & Evolution Graduate Program, November 2009

Michigan State University, Dept. of Plant Biology, March 2008

Washington State University, Biology Dept., February 2008

University of Maine, School of Marine Science, November 2007

Findings:

Findings include:

Obj. 1) PCR, RT-PCR and qRT-PCR (for *prk* and *psbO*), Northern blotting (*prk* only) and genome walking (*psbO* only) supported the presence of the algal nuclear genes *prk* and *psbO* in the adult sea slug, as well as aposymbiotic sea slug eggs.

In 2010-2011 we examined the longevity of algal nuclear-encoded plastid transcripts in individual sea slugs. RNA was extracted during a feeding time-course of adult *E. chlorotica* and RT-PCR and qRT-PCR were used to examine for presence and expression of 11 genes for algal nuclear-encoded chloroplast-proteins over a feeding time course. Surprisingly, a mosaic pattern of algal gene presence was observed among individual animals coupled with no apparent correlation between gene presence and expression or any indication of uptake of stable transcripts from the algal food. These results do not support the possibility of *E. chlorotica* co-opting stable transcripts from the algal food when feeding, but they do suggest a more complex explanation than massive HGT alone is needed to explain long-term kleptoplast function.

Obj. 2) We initially obtained a rough estimate for the size of the slug genome of 587 Mb by the Gregory Lab at the University of Guelph. More recently, using more sophisticated approaches, this estimate has been increased to 2.5 to 2.8Gbp. Using 454 pyrosequencing we generated 148

Mbp of cDNA sequence data from starved, but actively photosynthesizing adult *E. chlorotica* (n=5). From this partial transcriptome, 13,978 assembled unigenes and 99,873 unassembled singletons were analyzed using BLASTx (e-value cut off 10^{-10}) to generate putative gene annotations and to assign the taxonomic origin of ESTs. As expected, at least 95% of the predicted proteins had top hits to Metazoa. The putative non-metazoan top hits returned by BLASTx analysis of the *E. chlorotica* unigenes (123) and singletons (354) were used as queries in a phylogenomic pipeline specifically designed to identify genes of foreign origin from the host. This approach identified 20 ESTs of potential foreign origin derived from different prokaryotes, eukaryotes, and viruses, as well as several plastid-derived transcripts primarily from *V. litorea*, indicating plastid activity. None of these 20 ESTs, however, have a direct involvement in photosynthesis. In addition, specific BLASTn searches of both the contig and singleton data for genes previously identified as HGT candidates (*lhcv* 1,2,3,4; *fcp*; *psbO*; *prk*; *uroD*; *chlD*, *H*, *G*), failed to identify homologs. Over 90% of these genes were found in a preliminary assembly (4.29X genome coverage) of the egg genome of *E. chlorotica* validating their presence in the nuclear germline DNA of the sea slug. In 2011-2012, we extended our sequencing of the sea slug genome and now have 22.5 Gbp of data in hand. We blasted all 421 genes/gene fragment ESTs (including 52 nuclear encoded genes; 27 of which are involved in PS) that had been reported by the Pierce lab to be present in their sea slug transcriptome against our genomic data. We recovered no positive hits for any of the 421 gene (EST) fragments in the sea slug egg DNA.

Altogether these data suggest that under the prevailing model of how the sea slug is able to conduct photosynthesis with the stolen chloroplasts, the algal-derived genes are very poorly or transiently expressed and/or contained on extra-chromosomal material and not captured with our approach. Alternatively, the sea slug is utilizing algal genes of photosynthetic function in a fashion that is yet to be understood. We are continuing to carry out both genomic and transcriptomic sequencing on the sea slug and algal to arrive at a final genome sequence for both organisms to use as a basis for transcriptome analysis as it relates to development while also coming to final terms with how much (if any) horizontal gene transfer has or is taking place.

Obj. 3) The mitochondrial genome (mtDNA) of the sea slug was sequenced and mapped in its entirety and published. The genome was found to encode the standard 37 genes found in other typical animal mitochondria. No introns were identified and only 0.0125% of the DNA was non-coding. By measuring the GC-content over adjacent windows of 500 nucleotides (nt) with 200 nt overlaps, the values were found to be uniformly distributed across the windows suggesting homogeneity in GC-content of the mitochondrial genome and not supporting the existence of a chimeric region. In order to further assess the possibility of HGT in *E. chlorotica* mitochondria, we did phylogenetic analyses with nucleotide data generated using a sliding window approach with the genome data (i.e., DNA sequences that are independent of gene structure) and using the complete translated open reading frames. The maximum likelihood phylogenetic trees inferred with these alignments showed that the *E. chlorotica* sequences are monophyletic with molluscs, consistent with a vertical evolutionary history for *E. chlorotica* mtDNA. These analyses point to an intact and 'typical' animal mitochondrial genome in *E. chlorotica*. We, however, do not argue absolutely against the possibility of a partial DNA insertion from an algal or other source in this genome. Rather, that if such an insertion exists it is not detectable using the approaches described here. In addition, we obtained the complete mtDNA sequence through high-throughput genomic sequencing of sea slug egg DNA; this confirmed our earlier analyses that no gene transfer has taken place into this genome.

Obj. 4) We succeeded in culturing sea slugs in the laboratory and establishing the kleptoplastic association using laboratory-cultured algal prey, *Vaucheria litorea*. The plastids in *E. chlorotica* are not transmitted vertically; rather, they must be acquired with each generation early in development to ensure maturation to the adult sea slug. We succeeded in laboratory co-culturing studies to establish that the alga *Vaucheria litorea*, a derived stramenopile that contains 'secondary' plastids of red algal origin, was the sole source of plastids in our sea slugs. Veliger association with *V. litorea* is required for metamorphosis, and ingestion of chloroplasts is required for development to the adult stage. During 2010-2011, specificity of the alga for feeding and metamorphosis were investigated using bio- and settlement-assays, while a comparative surface composition (glycans) of various algae was investigated using confocal microscopy. Adult *E. chlorotica* and veliger larvae showed distinct preference patterns for *Vaucheria*, and the surface composition of *Vaucheria* proved unique when compared to other morphologically similar algae.

Through our in-lab culture experiments, we determined that the plastid association in *E. chlorotica* is reversible for the first 6 to 7 days and thereafter, becomes permanent. Multiple generations of a limited number of sea slugs have been produced in the laboratory. As a result of obtaining a UMaine Instrumentation grant, during 2010-2011, we were able to fix *E. chlorotica* samples throughout the initial developmental cycle focusing on the switch between reversibility and irreversibility at 6 to 7 days. Triplicate samples were fixed on a daily basis from 0 to 14 days and these have been analyzed by transmission electron microscopy (TEM) to better understand how the algal chloroplasts are taken up by the sea slug and retained. Our TEM and live and fixed confocal observations reveal that the permanent establishment of the kleptoplasty correlates with massive lipid accumulation around the chloroplasts. We are analyzing the composition of these lipids in collaboration with Prof. Weber at Heinrich-Heine-Universität, Düsseldorf. Profs. J. Murray and K. Hu, Indiana University, are assisting us with the confocal microscopy studies.

Other research: We extracted sea slugs (collected from Halifax, NS) and algae in collaboration with Dr. Mark Hamann at the Univ. of MS in an attempt to identify any natural products which might exhibit anti-cancer activity. Initial analysis revealed no kahalalide products (found in other sea slugs), but a small bio-active molecule (loliolide) which we are continuing to explore. Since this original study, Dr. Hamann has published

that the synthesis of kahalalide F is linked to the presence of *Vibrio* symbionts of the sea slug. We have since identified *Vibrio* symbionts (using 16s rDNA amplicon sequencing) with Martha's Vineyard sea slugs, but not those collected from Halifax. Hence, we are now collecting more sea slugs from Martha's Vineyard to carry out the natural products analysis to see if the microbiome population is linked to production of specific secondary compounds.

We also utilized 16S rDNA-based metagenomic analyses to characterize the microbial diversity associated with two populations of *E. chlorotica* from Halifax, Nova Scotia, Canada, and from Martha's Vineyard, MA, USA. Whole, entire adult animals were examined immediately after collection from their native environments, after being starved of their algal prey for several months in the laboratory, and after being bred in the laboratory (second-generation sea slugs) to characterize the effect of varying environmental and culturing conditions on the associated bacteria. Additionally, the microbiome of the algal prey, laboratory-cultured *V. litorea*, was analyzed to determine whether the lab-bred sea slugs obtained bacteria from their algal food source during development. Bacterial profiles varied between populations and among all conditions except for the F2 lab-bred samples, which were similar in diversity and abundance, but not to the algal microbiome. Alpha-, beta-, and gamma-proteobacteria dominated all of the samples along with Actinobacteria, Bacilli, Flavobacteria, and Sphingobacteria. Bacteria capable of polysaccharide digestion and photosynthesis, as well as putative nitrogen fixation, vitamin B12 production, and natural product biosynthesis were associated with the sea slug and algal samples. One paper has been accepted for publication on the bacterial diversity and a master's level student is now further characterizing the diversity at the level of the gut tissue and external mucus layer.

Training and Development:

The PIs have incorporated aspects of this research project into their teaching of a graduate course in plant biochemistry-photosynthesis, topic-focused seminar and one-credit classes (PI Rumpho), and both a graduate and undergraduate course in developmental biology (PI Tyler). In the fall of 2010, spring of 2010 and 2011 and summer of 2011, PI Rumpho taught a graduate level special topics course on Symbiosis in collaboration with Post-doctoral Associate Karen Pelletreau.

Research training was provided to one female Post-doctoral Research Associate (now Research Associate as of April 1, 2011), Karen Pelletreau, who joined the project April 1, 2008, and students including: graduate students (3; 2 female), undergraduate senior capstone projects (6; 4 female) including 2 female Honors' undergraduates in spring 2009 who also completed Honors theses on this project; undergraduate researchers (5; 3 female), and one high school female minority student through the the Univ. of ME Upward Bound program for minority high school students. Our student (who was with us for two summers) was awarded with the overall best student award at the end of her project period. The PI is also involved in co-advising 1 female student in the Master of Science Teaching program. The Post-doc and graduate students are intimately involved in training the undergraduate students and high school student and also incorporate the sea slug project into their teaching assistant positions in the biochemistry department. MS student, J. Worful, completed his degree in Dec. 2008 and also had the opportunity to present his research to a biology class at Maine Maritime Academy and at the Phycological Society of America meeting. In addition, he trained at the Univ. of Mississippi for about three weeks learning how to extract natural compounds from the sea slugs and algae. The Post-doctoral Associate also presented an annual lecture from 2009-2012 to the marine biology class at the Maine Maritime Academy and mentored an undergraduate from that institution on the sea slug project for one year.

During 2009-2010, 1 Post-doctoral Research Associate (K. Pelletreau), 2 MS students (K. Soule [graduated Dec. 2009] and S. Devine), and 7 undergraduates (4 females: M. Varney, E. Fisher, N. Montesano, S. Cusack and 3 males: Geoff Davis, Kyle Duca and Chris Doucette) contributed to this project.

During 2010-2011, 1 Post-doctoral Research Associate (K. Pelletreau), 1 MS student (S. Devine), and 8 undergraduates (5 females: M. Varney, E. Fisher, S. Cusack, E. Keim, K. Sarver) and 3 males: Geoff Davis, Craig Harrison and Chris Doucette) contributed to this project. E. Keim, K. Sarver and C. Harrison completed their senior honors' thesis research projects studying various aspects of the *Elysia/Vaucheria* symbiosis. K. Sarver and G. Davis will present their work this summer (2011) at the American Society of Plant Biologists meeting in Mpls, MN. Six of the eight students also presented oral talks or posters at the UMaine Center for Undergraduate Research Symposium in the spring of 2011. K. Sarver won third place for her oral presentation at this symposium and first place at our departmental senior capstone day symposium.

During 2011-2012, 1 Research Associate (K. Pelletreau), 1 MS student (S. Devine), and 1 post-undergraduate (Kara Sarver) and 1 undergraduate (Geoff Davis) contributed to this project. Undergraduate Geoffry Davis completed an Honors' Thesis in Biochemistry spring 2012 after developing a FISH protocol for both the sea slugs and alga. Geoff presented his work at the UMaine Undergraduate Research Expo April, 2012 and won an Honorable Mention. Kara is working as a Research Technician at Oregon State University and Geoff is now beginning a PhD program in Plant Molecular Biology at Michigan State University.

Mentoring activities for the Post-doc (Karen Pelletreau) included attending and presenting at the International Symbiosis Society meeting (summer 2009) and attending a Post-doctoral advisory session at the meeting. In addition, Karen spent one week in Prof. Bhattacharya's lab during fall 2009 learning bioinformatic tools and met with his lab again in May 2010. Karen was also mentored in writing a successful internal

education proposal and will attend a 'new media' course this fall to learn new techniques in delivering scientific information. She was mentored in the classroom through her participation in teaching graduate level special topics courses. Finally, she is being supervised and advised in training undergraduates and presenting science to visiting student and parent groups. During the summer of 2011, Karen presented her work at the Phycological Society of America and the American Society of Plant Biologists meetings. She also spent a week in the fall 2011 at Indiana University conducting confocal visualization studies with Profs. Murray and Hu in the biology dept. Karen will present her research at the International Symbiosis Society Congress in Krakow, Poland, in July 2012.

Outreach Activities:

We reach out to students of all ages around the world through our educational web site: <http://sbe.umaine.edu/symbio/>

Through the Masters in Science Teaching (MST) program and an MS student that we help advise at UMaine, we are able to reach out to K-12 teachers and promote education on photosynthesis-related topics, focusing on the sea slug system.

The PIs provided images and text for several public science publications including 'The Smithsonian,' 'New Scientist,' 'Science Digest,' 'Science World,' 'Maine Today,' among others and several foreign publications. Videos of sea slug development were provided on request for educational purposes. Live sea slugs were also sent to a high school teacher in Upper Arlington, OH.

PI Rumpho presented a general talk to the newly organized Maine Melanoma Foundation group in 2008 focused on the possibility of isolating anti-cancer compounds from marine organisms such as the kleptoplastic sea slug.

2009-2011

We developed a hands-on teaching/recruiting experience using the sea slugs and algal feeding experiments and used it with several visiting student groups. MS student Kara Soule also tested the 'program' at Mt. Desert Island High School in the honors biology course. We also had an Upward bound high school student in our lab during the 2009-2010 summers and she worked on developing these educational tools to bring science to the public in our lab and over the internet.

Yarmouth High school student Sam Coleman (completing his junior year) shadowed for 3 days in PI Rumpho's lab to gain hands-on experience in a research setting during May 2010.

PI Rumpho participated in a cell biology course at the Univ. of WA via live phone discussion with students (Fall 2009).

PI Rumpho presented her research to a Univ. of Maine 'Women in Leadership' group consisting of about 150 women during fall 2009. Out of this, she wrote a successful proposal for \$5000 to support undergraduate research and training for women in her laboratory.

A public seminar, 'The Waller Memorial Lecture,' was presented by PI Rumpho at The Ohio State University in May 2010 entitled, 'Symbiosis and Biological Novelty.' PI Rumpho also presented a public lecture on 'Eukaryotic Cell Evolution' in a special Darwin course at UMaine, fall 2009.

We took our sea slugs into the 11th grade chemistry classroom of Brewer High School, Brewer, ME, April 2011, and conducted a hands-on experiment with the students. We also provided live sea slugs to Ms Cindy Langdon, Vivian E. Hussey School in Berwick, ME, and Dr. Michele Prun, Dept. of Biol. Sci., Plymouth State Univ. Cindy has worked with the sea slugs with various classes for several years. She recently wrote, 'It is my hope that they [the sea slugs] can become something of a mascot for the science department. I also remain firmly convinced that they can serve to help facilitate a conversation between grade levels about how we think about living systems.' Michelle wrote, 'It is often difficult to get students jazzed about plants, so the slug acts as a great seg-way toward discussing photosynthesis, evolution of photosynthetic organisms, etc. Mary has been extremely helpful. She sent us 12 slugs last year and both Karen (Post-doc) and Mary suggested experiments we might try and have offered countless advice. It was so encouraging that Mary responded to my emails, and has been willing to support this collaboration.'

The PI was the recipient of a Women in Leadership & Philanthropy grant from UMaine entitled, 'Mentoring undergraduates in scientific research and development of educational materials.' This grant provides undergraduates in the sciences experience in scientific educational material development and 'delivering' science to the public. The PI was also the recipient of a UMaine Center for Excellence in Teaching and Assessment Active Learning Grant this past year entitled, 'Science in minutes: Using a community-based contributory research project as a dual recruitment and learning tool.' This proposal arose out of a hands-on exercise using the sea slugs and developed in our lab for visiting school groups and prospective students.

PI Rumpho has had talks with Carolina Biological about the commercialization potential of the sea slug system. This will continue to be pursued in 2012-2013, after the PI relocates to the Univ. of CT in Storrs.

Journal Publications

Rumpho, ME; Worful, JM; Lee, J; Kannan, K; Tyler, MS; Bhattacharya, D; Moustafa, A; Manhart, JR, "Horizontal gene transfer of the algal nuclear gene psbO to the photosynthetic sea slug *Elysia chlorotica*", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, p. 17867, vol. 105, (2008). Published, 10.1073/pnas.080496810

Rumpho, ME; Pochareddy, S; Worful, JM; Summer, EJ; Bhattacharya, D; Pelletreau, KN; Tyler, MS; Lee, J; Manhart, JR; Soule, KM, "Molecular Characterization of the Calvin Cycle Enzyme Phosphoribulokinase in the Stramenopile Alga *Vaucheria litorea* and the Plastid Hosting Mollusc *Elysia chlorotica*", MOLECULAR PLANT, p. 1384, vol. 2, (2009). Published, 10.1093/mp/ssp08

Rumpho ME, KN Pelletreau, A Moustafa and D Bhattacharya, "Making of a photosynthetic animal.", Journal of Experimental Biology, p. 303, vol. 214, (2011). Published,

Pelletreau KN, D Bhattacharya, DC Price, JM Worful, A Moustafa and ME Rumpho, "Sea slug kleptoplasty and plastid maintenance in a Metazoan.", Plant Physiology, p. 1561, vol. 155, (2011). Published,

Soule KM and ME Rumpho, "Light-regulated photosynthetic gene expression and phosphoribulokinase enzyme activity in the heterokont alga *Vaucheria litorea* and its symbiotic molluscan partner *Elysia chlorotica*.", Journal of Phycology, p. 373, vol. 48, (2012). Published, 10.1111/j.1529-8817.2012.01111.x

Pelletreau KN, JM Worful, G Davis, KE Sarver and ME Rumpho, "Observation of the sacoglossan *Elysia chlorotica* in an optimized laboratory culture??reveals a shift from transient to permanent kleptoplasty.", Journal of Experimental Marine Biology and Ecology, p. , vol. , (2012). Submitted,

Devine SP, KN Pelletreau and ME Rumpho, "16S rDNA-based metagenomic analysis of bacterial diversity associated with two populations of the kleptoplastic sea slug *Elysia chlorotica* and its algal prey *Vaucheria litorea*.", Biological Bulletin, p. , vol. , (2012). Accepted,

McFall-Ngai M, MHadfield, T Bosch, H Carey, T Domazet-Loaso, A Douglas, N Dubilier, G Eber, T Fukami, S Gilbert, U Hentschel, N King, S Kjelleberg, AH Knoll, N Kremer, S Mazmanian, J Metcalf, K Neelson, N Pierce, J Rawls, A Reid, E Ruby, M Rumpho, J Sander, "Animals in a Bacterial World: A New Imperative for the Life Sciences.", Science, p. , vol. , (2012). Submitted,

Books or Other One-time Publications

Gross J, KN Pelletreau, A Reyes-Prieto, ME Rumpho and D Bhattacharya, "Secondary and Tertiary Endosymbiosis and Kleptoplasty.", (2012). Book, Published

Editor(s): R Bock and V Knoop

Collection: Advances in Photosynthesis and Respiration - Genomics of Chloroplasts and Mitochondria.

Bibliography: Springer Science+Business Media B.V. 35, DOI 10.1007/978-94-007-2920-9_2

Web/Internet Site

URL(s):

<http://sbe.umaine.edu/symbio/>

Description:

This web site is an educational site focused on the kleptoplastic sea slugs. It discusses these unusual molluscs and their ability to form an endosymbiotic association with algal chloroplasts, their ability to photosynthesize, their developmental biology, evolutionary biology, and other related topics.

Other Specific Products

Product Type:

Data or databases**Product Description:**

Gene or protein sequences deposited in GenBank database:

1. Worful JM and ME Rumpho 2008 Protein O from photosystem II of the heterokont alga, *Vaucheria litorea*. EU621881.
2. Worful JM and ME Rumpho 2008 *Vaucheria litorea* like-protein O of photosystem II from *Elysia chlorotica*. EU621882.
3. Kannan K and ME Rumpho 2008 *Elysia chlorotica* mitochondrion complete genome. EU599581.
4. Kannan K, ME Rumpho and JR Manhart 2007 *Vaucheria litorea* 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence. EF441743.

Sharing Information:

Available through GenBank

Product Type:**Data or databases****Product Description:**

Rumpho, M.E., Worful, J.M., Lee, J., Kannan, K., Tyler, M.S., Bhattacharya, D., Moustafa, A. and Manhart, J.R. 2008 EU912438 *Vaucheria litorea* chloroplast, complete genome.

Sharing Information:

It is shared on the public GenBank website.

Product Type:**Audio or video products****Product Description:**

Videos of developing sea slugs.

Sharing Information:

Two videos are available through the PNAS web site and link to our Nov. 2008 publication. Lower resolution videos are available on our Symbio web site. High resolution video files have also been sent on disc upon request.

Product Type:**Physical collection (samples, etc.)****Product Description:**

We have made live sea slugs (*Elysia chlorotica*) available upon request and availability in our lab.

Sharing Information:

We do not advertize this, but respond to individual requests and ship sea slugs when we have them available.

Product Type:**Teaching aids****Product Description:**

Images of the sea slug *Elysia chlorotica*.

Sharing Information:

These images are shared on our Symbio web site and also sent out upon individual requests. These images are used in teaching, general publications, other web sites, and the back cover of a book.

Contributions**Contributions within Discipline:**

We are examining the potential for the evolution of photosynthesis in an animal through experiments aimed at understanding both the uptake and establishment of the foreign organelles in an animal cell as well as what contributes to the long-term maintenance of these organelles in the animal.

Photosynthesis generates the oxygen needed for life on earth as well as the biomass for food and biofuel production. This process is driven by the absorption of the sun's energy by chloroplasts in plants and algae. The sea slug *Elysia chlorotica* has fascinated scientists for years because of its ability to retain 'stolen' chloroplasts and carry out photosynthesis. These emerald green molluscs feed early in their life-cycle by sucking

out the cellular contents of their algal prey (*Vaucheria litorea*). As a result of retaining the green plastids in cells lining their digestive gut, the animals survive for months on only sunlight and air by carrying out photosynthesis as if they were a plant. This is perplexing because chloroplast activity requires the nuclear genome to make most of the chloroplast proteins and there are no algal nuclei in the sea slug.

By sequencing and publishing the complete chloroplast genome of the algal prey, we showed that the chloroplasts cannot make all of the proteins necessary to support photosynthesis. By using polymerase chase reaction (PCR), RT-PCR, qRT-PCR, and biochemical studies, we presented indirect evidence for horizontal gene transfer (HGT, the exchange of DNA between unrelated organisms) from the algal nucleus to the mollusc. More recent Next-Gel high-throughput transcriptomic and genomic sequencing of *Elysia chlorotica* has not supported the presence of foreign genes in the sea slug. However, other labs have suggested that foreign genes are found in the transcriptome. Needless-to-say, research is continuing in this area, especially at the genome level to come to a conclusion about the extent of HGT. This model system is allowing us and others to advance our knowledge of photosynthesis and energy capture in a photosynthetic animal and also pursue the role of gene transfer in facilitating these key processes.

Our sequence data are being deposited in public databases and significantly adding to the sacoglossan mollusc database as well as heterokont algae.

The results of our research contribute to our fundamental understanding of symbiosis, evolution, photosynthesis, secondary endosymbionts, mollusc genomics, and the development of chloroplast-containing (kleptoplastic) molluscs. We are exploiting 'solar-powered sea slugs' through multimedia educational materials disseminated through an interactive Web site.

Contributions to Other Disciplines:

Kleptoplastic sea slugs potentially have a direct bearing on human health, through their production of anti-cancer/tumor compounds, as models for immuno-therapy and drug delivery, and for their glaring absence of an immune-rejection response. We initiated studies to determine if *E. chlorotica* produces any anti-cancer compounds including the kahalalides discovered in other related molluscs. We did not find any kahalalides, but we have found a small, toxic metabolite, loliolide, which we are characterizing at this time.

We developed genetic resources including the sequence and map of the mitochondrial genome of the sacoglossan mollusc, *Elysia chlorotica*, which will be of value to others studying molluscs and their evolution.

We have succeeded in obtaining and analyzing high-throughput sequence data from normalized cDNA from adult *Elysia chlorotica* and genomic data from sea slug egg DNA using GS-FLX 454 and Illumina sequencing. These data will be of value to others looking to use these techniques, interested in studying symbiotic systems, identifying horizontal gene transfer, and/or interested in mollusc sequence information.

Further understanding into how the chloroplasts are recognized, where they are incorporated in the diverticula, and how quickly other algal cellular materials are digested, will allow greater insight into how these chloroplasts enable photosynthesis by an animal. In addition, our progress in culturing the sea slugs and establishing the chloroplast endosymbiosis may provide a supply of this unusual organism for classrooms and laboratories, marine aquaria hobbyists, and contribute to protection of a rare species and its native habitat.

Contributions to Human Resource Development:

Research training was provided to one Post-doctoral Research Associate (female; joined the project April 1, 2008) and students including: graduate students (3; 2 female), undergraduate senior capstone projects (5; 4 female); undergraduate researchers (5; 3 female), and one high school female minority student.

In 2009-2010, the Post-doctoral Research Associate continued to work on the project as well as 2 graduate and 7 undergraduate students. In addition, one high school student job shadowed in the lab and a second will carry out research during the summer of 2010.

During 2010-2011, 1 Post-doctoral Research Associate (K. Pelletreau), 1 MS student (S. Devine), and 8 undergraduates (5 females: M. Varney, E. Fisher, S. Cusack, E. Keim, K. Sarver) and 3 males: Geoff Davis, Craig Harrison and Chris Doucette) contributed to this project.

During 2011-2012, 1 Research Associate (K. Pelletreau), 1 MS student (S. Devine), 1 post-undergraduate (K. Sarver) and 1 Honors' Thesis undergraduate (G. Davis) contributed to this project. The undergraduate accepted an RA position to pursue his PhD in Plant Molecular Biology at Michigan State University.

Contributions to Resources for Research and Education:

We are continuing to develop our educational web site at <http://sbe.umaine.edu/symbio/>. PI Rumpho also participates in the International Symbiosis Society and helped to organize one session on enigmatic symbioses at the meeting in Vienna in 2006. The PIs have deposited several gene and protein sequences in GenBank for public access and all of their high-throughput sequence information. In addition, we provided educational materials related to the sea slug project to other students and educators who have contacted them from around the world.

Contributions Beyond Science and Engineering:

Two of the videos we produced of developing sea slugs were purchased by a German scientific team for inclusion in a larger educational video. We are in communication with Carolina Biological about possibly commercializing our sea slug system for teaching, hobbyist and research purposes.

Conference Proceedings

Special Requirements

Special reporting requirements: None

Change in Objectives or Scope: None

Animal, Human Subjects, Biohazards: None

Categories for which nothing is reported:

Any Conference