

# EDEN Funding Application Part I



## SECTION A: Funding Type

This section is required of all applicants.

### 1. Program you are applying for (choose one)

Research Exchange Funding

Undergraduate Internship

Workshop/Symposium  
Attendance

## SECTION B: Applicant Information

This section is required of all applicants.

### 1. Last Name

### 2. First Name

### 3. Position

Undergraduate  
Student

Graduate Student

Postdoc

Faculty

### 4. Home Institution

### 5. Applicant Contact Details

Address 1:

Address 2:

City/Town:

State/Province:

ZIP/Postal Code:

Email Address:

Phone Number:

# EDEN Funding Application Part II

## SECTION C: Research Proposal Information

This section is required of applicants for Research Exchanges or Undergraduate Internships.

### 1. Proposal Title

### 2. Host Laboratory PI

### 3. Host Laboratory Institution

### 4. Host Laboratory Contact Details

Address 1:

Address 2:

City/Town:

State/Province:

ZIP/Postal Code:

Email Address:

Phone Number:

## SECTION D: Workshop/Symposium Application Information

This section is required of applicants for Workshop/Symposium Attendance funding.

### 1. Name of Workshop/Symposium

### 2. Date of Workshop/Symposium

## EDEN FUNDING APPLICATION CHECKLIST

Make sure that your application contains all of the following information:

### 1. For all applicants:

- € This application form, completed
- € Recent CV

### 2. For research exchange and undergraduate internship applicants:

- € A two-page research proposal \*
- € A letter of support from the director of the host lab \*\*

### 3. For workshop/symposium attendance applicants:

- € A one-page explanation of why you wish to attend the workshop/symposium

\* Proposals from graduate students postdocs, and faculty applicants must address scientific merit, community building, and dissemination plans for protocols developed. Proposals from undergraduate applicants should address both specific scientific goals for the internship and general career goals.

\*\* The letter from the director of the host lab should indicate a willingness to host the visitor, and when appropriate, an agreement to allow public release of the technique protocol.

ALL REQUIRED DOCUMENTS SHOULD BE UPLOADED AS A SINGLE PDF DOCUMENT AT

<http://www.edenrcn.com/funding/>

Graduate student and postdoc applicants must have a letter of support submitted by their main research adviser (for graduate students and postdocs). These letters must reach EDEN by the application deadline.

Undergraduate applicants must have two letters of support submitted on their behalf. These letters should be from people who can comment on your scientific or academic interests and abilities. Letters must reach EDEN by the application deadline.

ALL REFERENCE LETTERS SHOULD BE EMAILED TO EDENRCN@FAS.HARVARD.EDU OR POSTED TO

EDEN  
c/o Barbara Perlo  
Harvard University  
Department of Organismic and Evolutionary Biology  
16 Divinity Avenue  
BioLabs Building Room 4105  
Cambridge, MA 02138

# Temporal analysis of gene expression from RNA-Seq data – EDEN 2013

Bruno C. Vellutini

30/10/12

Uncovering the mechanisms of embryonic development is essential to nurture insights about the evolution of animals. The candidate gene approach has been successful in revealing the role of genes in developmental processes, but it depends on a priori knowledge about gene functions. Comprehending the dynamics of complex gene regulatory networks requires a reliable method to identify the full set of active genes and to quantify expression levels during development.

RNA-Seq is a deep sequencing technology for obtaining the transcriptome of samples (eg, specific tissue or developmental stage) that brings several advantages when compared to other profiling methods such as microarrays. It is not limited to previous genetic information, has low background signal, and has a large dynamic range of expression levels, thus allowing accurate quantification with single-base resolution for the entire transcriptome (Wang, Gerstein, and Snyder 2009).

I am a PhD student in Andreas Hejnol's group at Sars International Centre for Marine Molecular Biology in Bergen, Norway working with the evolution of larval body patterns in understudied marine invertebrates such as bryozoans, brachiopods, and nemertean. We use microscopy and molecular techniques including confocal, time-lapse 3D imaging, gene cloning, and in situ hybridization to investigate development in a comparative manner using a candidate gene approach. Some of the studied subjects are the evolution of the nervous system, digestive system, coeloms, and larval tissues.

In order to improve our knowledge about these organisms it is crucial to advance to a high-throughput analysis of development. Transcriptome data of relevant embryonic stages will yield important information about transcript dynamics and reveal previously unknown genes, potentially important for developmental studies. Such data combined with our established in situ hybridization protocols is a promising approach for evo-devo studies.

Although we are skilled in RNA extraction methods, we have no experience in processing and analyzing temporal data sets of high-throughput data. There are several bioinformatics challenges related to these large data sets such as

removal of low-quality reads and assembly/mapping of high-quality reads. In addition, time-series analyses require specific statistics and data processing to extract relevant transcripts according to the sampled developmental stages.

Our goal with this proposal is to establish a collaboration with Casey Dunn's laboratory at Brown University in Providence, US to get experience in processing and analyzing gene expression of different embryonic stages from RNA-Seq data. Dunn has vast expertise in next-generation sequencing and computational work for the analysis of differential gene expression (Siebert et al. 2011; Howison, Sinnott-armstrong, and Dunn 2012).

The organism of choice is *Priapulius caudatus*, a conservatively evolving marine worm with a key phylogenetic position within the Ecdysozoa. Our group has successfully collected and fertilized embryos of the species and established in situ hybridization protocols. The complete description of *P. caudatus* development including expression patterns involved in gut formation was recently published, bringing deep implications for our understanding of animal evolution (Martín-Durán et al. 2012).

*P. caudatus* comes as a primer system for profiling the temporal expression of developmental genes. Mapping its gene regulatory networks will provide valuable information about priapulid morphogenesis and support further insights about the developmental diversity of ecdysozoans. We will collect samples of different stages and send to sequencing beforehand. Data will be taken to Dunn's laboratory to be processed and analyzed. We estimate 2 months for working with the data and building community resources from the project.

In summary, we want to foster collaboration between the two groups and learn the methodology for processing and analyzing time-series of RNA-Seq data. We chose *P. caudatus* because of its relevancy to the current evo-devo scene and also to assess challenges of this type of analysis in non-model marine invertebrates.

I will document the whole process and establish a reusable workflow for processing and analyzing gene expression data of developmental stages. It will contain a tutorial including the code and sample datasets used for the analysis. Source code will be available at [GitHub](#) (collaborative platform for source code) and the remaining documents and datasets made freely accessible at [figshare](#) (platform to publish research outputs).

The workflow established during the project will be integrated to the repertoire of techniques of the Hejnal group opening the possibility of analyzing time-series of gene expression in several other poorly studied organisms in our laboratory.

Next-generation sequencing is a valuable technique for comprehending the underlying mechanisms of development and specially attractive for organisms with no previous genetic information. However, since high-throughout data is computationally challenging, by building a robust and open bioinformatics workflow we hope to bring advances for this type of analysis, allowing researchers to apply these methods to other organisms and improve our understanding of the embryo.

## References

- Howison, Mark, Nicholas A. Sinnott-armstrong, and Casey W. Dunn. 2012. "BioLite, a lightweight bioinformatics framework with automated tracking of diagnostics and provenance." In *4th USENIX Workshop on the Theory and Practice of Provenance (TaPP12)*.
- Martín-Durán, José M, Ralf Janssen, Sofia Wennberg, Graham E Budd, and Andreas Hejnl. 2012. "Deuterostomic Development in the Protostome Priapulid *Priapulid caudatus*." *Current Biology* (oct): 1–6. doi:10.1016/j.cub.2012.09.037.
- Siebert, Stefan, Mark D. Robinson, Sophia C. Tintori, Freya Goetz, Rebecca R. Helm, Stephen a Smith, Nathan Shaner, Steven H. D. Haddock, and Casey W. Dunn. 2011. "Differential gene expression in the siphonophore *Nanomia bijuga* (Cnidaria) assessed with multiple next-generation sequencing workflows." *PLOS one* 6 (jan): 22953. doi:10.1371/journal.pone.0022953.
- Wang, Zhong, Mark Gerstein, and Michael Snyder. 2009. "RNA-Seq: a revolutionary tool for transcriptomics." *Nature reviews. Genetics* 10 (jan): 57–63. doi:10.1038/nrg2484.



BROWN

Dr. Casey W. Dunn  
Ecology and Evolutionary Biology  
Box G-W  
Providence, RI 02912  
401 863-9806  
Fax 401 863-2166  
casey\_dunn@brown.edu

OCTOBER 24, 2012

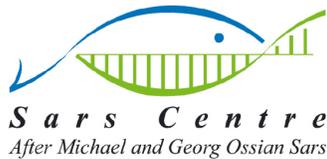
Dear Colleague,

I am writing to enthusiastically express my willingness to host Bruno Vellutini in my lab and to allow public release via the EDEN website of all protocols arising from the exchange. I met Mr. Vellutini on a recent visit to the Sars Institute in Norway. We share many similar interests, his project builds directly on many tools we have developed and worked with in my lab, and I was impressed by his progress in his own work.

Sincerely,

A handwritten signature in black ink, reading "Casey W. Dunn".

Dr. Casey William Dunn, Assistant Professor  
Department of Ecology and Evolutionary Biology, Brown University



Bergen, 30<sup>th</sup> October 2012

Dear EDEN committee,

**Letter of recommendation for Bruno Vellutini**

I am writing this letter in strong support for the application of Bruno Vellutini to the EDEN Research Exchange Funds.

Bruno is a doctorate student in my group at the Sars Centre since November 2011 and has been working on the evolution of larval body patterns in marine invertebrates. He has a broad background in zoology and in the past year has gained acquaintance with common molecular techniques for evo-devo studies such as gene cloning and in situ hybridization.

He is a dedicated learner and cooperative person always seeking to expand his abilities in biology. He gained experience in programming while developing a marine biology image database of his previous workplace. In the laboratory he has applied this knowledge for creating automated tasks and managing research notes for molecular cloning. Bruno is also familiarized with several scientific online tools and was involved in communication of science early on his career.

His proposal for working with time-series of gene expression data from RNA-Seq is a great opportunity for expanding his breadth of knowledge and applying the previous programming abilities to biological questions. The subject is directly related to his work interests and to the scope of my research group.

The collaboration with Casey Dunn laboratory will build important resources for characterizing gene expression dynamics during development and working with high-throughput transcriptome data in non-model organisms.

Sincerely Yours,

Dr. Andreas Hejnol  
Group Leader, Sars International Centre for Marine Molecular Biology  
Comparative Developmental Biology of Animals  
Thormøhlensgt. 55  
N-5008 Bergen, Norway

# Bruno C. Vellutini

## Curriculum Vitae

Øvre Blekeveien, 16  
5003 Bergen, Norway

☎ +47 4508-9464

✉ [bruno.vellutini@sars.uib.no](mailto:bruno.vellutini@sars.uib.no)

<http://organelas.com/>



---

### Research interests

- Evolutionary developmental biology of marine invertebrates
- Body patterning of larvae and evolution of life cycles
- Gene regulatory networks and developmental diversity
- Biodiversity and science outreach

---

### Education

2011–today **PhD**, *Sars International Centre for Marine Molecular Biology / University of Bergen*, Bergen, Norway, *PhD in Molecular Biology*.  
Comparative development of spiralian larvae.

2006–2008 **MSc**, *Department of Zoology of Biosciences Institute of University of São Paulo & Marine Biology Center of University of São Paulo*, São Paulo & São Sebastião, SP, Brazil, *Master in Zoology*.  
A morphological study on the development of the sea biscuit *Clypeaster subdepressus*.

2001–2005 **BSc**, *Biosciences Institute of University of São Paulo*, São Paulo, SP, Brazil, *Bachelor in Biological Sciences*.  
Undergraduate studies in biology.

---

### PhD thesis

title Comparative development of spiralian larvae.  
supervisor Andreas Hejnol  
summary I am investigating the embryonic origin and fate of larval tissues in bryozoans, brachiopods, and nemerteans using microscopy and gene expression patterns to better understand the developmental diversity of Spiralia.

---

### Master's thesis

title Development and reproductive cycle of the sea biscuit *Clypeaster subdepressus* (Echinodermata: Echinoidea) from São Sebastião, SP  
supervisor Alvaro E. Migotto  
summary I documented the embryonic, larval, and juvenile development of a sea biscuit with emphasis on the morphology of post-metamorphic juveniles. Histological analysis was conducted to evaluate the gonadal maturation of adults during an year.

---

## Work experience

### Positions

- 2011–today **PhD Student**, *Sars Centre & Faculty of Mathematics and Natural Sciences of University of Bergen*, Bergen, Norway.
- 2010-2011 **Editor, programmer, and front-end developer**, *Marine Biology Center of University of São Paulo*, São Sebastião, SP, Brazil.  
Cifonauta is a metadata-driven image database for marine biology photos and videos. Development is based on Python using Django web framework and PostgreSQL database. <http://cifonauta.cebimar.usp.br/>
- 2006-2008 **MSc Student**, *Department of Zoology of Biosciences Institute of University of São Paulo & Marine Biology Center of University of São Paulo*, São Paulo & São Sebastião, SP, Brazil.  
Research on developmental patterns of marine invertebrates. Artificial fertilization and maintenance of embryos and larvae of echinoderms, still and video documentation with optical microscopy, histological techniques for tissue analysis.
- 2007-2008 **Co-organizer and web developer**, *Marine Biology Center of University of São Paulo*, São Sebastião, SP, Brazil.  
Itinerant photographic exhibit of marine organisms. <http://www.usp.br/cbm/oceano/>
- 2001-2004 **Intern Researcher**, *Institute of Biomedical Sciences of University of São Paulo*, São Paulo, SP, Brazil.  
Innate immune response of Tropical and Antarctic animals at the Evolutive Histophysiology Laboratory.

### Courses

- 2012 **8th MIC Confocal Microscopy Course**, *Department of Biomedicine, University of Bergen*, Bergen, Norway, 24h.
- 2010 **Concepts and Model Organisms in Regenerative Biology**, *Universidad de Chile and Pontificia Universidad Catolica de Chile*, Santiago, Chile.  
Satellite course of Latin American Society for Developmental Biology 2010 meeting, 76h
- 2008 **Mini-course theoretical-practical on image processing and embryo manipulation**, *Institute of Biomedical Sciences (ICB) and Heart Institute (INCOR), University of São Paulo*, São Paulo, SP, Brazil.  
During the II Meeting of Developmental Biology Students, 8h
- 2006 **Comparative Invertebrate Embryology (BIOL536)**, *Friday Harbor Laboratories, University of Washington*, Friday Harbor, WA, USA.  
Summer Session A, 9 credits
- 2006 **Larval Biology (BIOL533)**, *Friday Harbor Laboratories, University of Washington*, Friday Harbor, WA, USA.  
Summer Session B, 9 credits

### Conferences, posters, and presentations

- 2012 **Bergen Marine Invertebrate Symposium**, *Bergen, Norway*, in English.
- 2012 **4th Euro Evo Devo meeting**, *Lisbon, Portugal*, in English, poster presentation.  
Germ cell development in non-spiralian lophotrochozoans: insights from a bryozoan and a brachiopod.

- 2010 **V International Meeting of the Latin American Society for Developmental Biology**, *Santa Cruz, Chile*, in English, poster presentation.  
Juvenile development of the sea biscuit *Clypeaster subdepressus* (Echinodermata: Clypeasteroidea)
- 2010 **Workshop on Marine Diversity, FAPESP**, *São Paulo, SP, Brazil*.
- 2008 **II Meeting of Developmental Biology Students**, *São Paulo, SP, Brazil*.
- 2008 **8th Larval Biology Symposium**, *Lisbon, Portugal*, in English, oral presentation.  
Larval and juvenile development of the sea biscuit *Clypeaster subdepressus* (Echinodermata: Clypeasteroidea).
- 2008 **XXVII Brazilian Congress of Zoology**, *Curitiba, PR, Brazil*, in Portuguese, poster presentation (2).  
Embryonic and larval development of the sea biscuit *Clypeaster subdepressus* (Echinodermata: Clypeasteroidea).  
Metamorphosis and post-metamorphic development of the sea biscuit *Clypeaster subdepressus* (Echinodermata: Clypeasteroidea).
- 2007 **III International Symposium of Developmental Biology**, *Uberaba, MG, Brazil*, in Portuguese, poster presentation.  
Life cycle of the sea biscuit *Clypeaster subdepressus* (Echinodermata: Clypeasteroidea).
- 2007 **I Meeting of Developmental Biology of University of São Paulo**, *Ribeirão Preto, SP, Brazil*, in Portuguese, oral presentation.  
Development of the sea biscuit *Clypeaster subdepressus*.
- 2005 **15th International Society of Developmental Biologists Congress**, *Sydney, Australia*.
- 2005 **2nd International Meeting of the Latin American Society for Developmental Biology**, *Guarujá, SP, Brazil*.

#### Teacher Assistant

- 2007 **Diversity and evolution of marine invertebrates (9200001)**, *Marine Biology Center of University of São Paulo*, São Sebastião, SP, Brazil.  
2nd Semester, 6 credits
- 2005 **Invertebrates I (BIZ0210)**, *Biosciences Institute of University of São Paulo*, São Paulo, SP, Brasil.  
2nd Semester, 8 credits

#### Honors, Awards, and Scholarships

- 2011–today **PhD Research Fellow**, *Faculty of Mathematics and Natural Sciences*, University of Bergen.  
Comparative development of spiralian larvae
- 2011 **Winner, 3rd Place (Category 4: Photomicrographs)**, *I Prêmio Fotografia-Ciência & Arte CNPq*.  
Photomicrograph “Sea biscuit juvenile”  
<http://www.cnpq.br/saladeimprensa/noticias/2011/0927b.htm>
- 2009 **Winner, 5th Place**, *Nikon Small World Photomicrography Competition*.  
Photomicrograph “Oral surface of a young sea star”  
<http://nikonsmallworld.com/detail/year/2009/5>

- 2009 **Honorable mention**, *Nikon Small World Photomicrography Competition*.  
Photomicrograph “Pluteus larva of a sea biscuit”  
<http://nikonsmallworld.com/detail/year/2009/32>
- 2008 **Honorable mention**, *Olympus BioScapes Digital Imaging Competition*.  
Photomicrograph “Larval skeleton of *Clypeaster subdepressus*”  
<http://www.olympusbioscapes.com/staticgallery/2008/hm58.html>
- 2007 **Honorable mention, 2nd place**, *III Simpósio Internacional de Biologia do Desenvolvimento*, Uberaba, MG, Brazil.  
Poster “Life cycle of the sea biscuit *Clypeaster subdepressus*”.
- 2006–2008 **MSc Scholarship**, *Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)*, 2006/01898-7.  
Development and reproductive cycle of the sea biscuit *Clypeaster subdepressus* (Echinodermata: Echinoidea) from São Sebastião, SP.
- 2002–2004 **Scientific Initiation Scholarship**, *Conselho Nacional de Desenvolvimento Científico e Tecnológico (PIBIC-CNPq)*.  
Characterization of the phagocytic activity of coelomocytes of the sea urchin *Lytechinus variegatus* (Lamarck - 1816) *in vivo* and *in vitro*.

#### Science Outreach

- 2010-today **Cifonauta**, *Co-author, programmer, and front-end developer*, CNPq.  
A metadata-driven image database for marine biology  
<http://cifonauta.cebimar.usp.br/>
- 2007-today **Ocean: hidden life**, *Co-organizer and web developer*, Fundo de Cultura e Extensão da Universidade de São Paulo.  
Itinerant photo exhibit of marine organisms  
<http://www.usp.br/cbm/oceano/>

---

#### Languages

Portuguese	<b>Native language</b>	
English	<b>Fluent</b>	<i>read/speak/write</i>
French	<b>Basics</b>	<i>read/speak</i>

---

#### Skills

microscopy	Light microscopy (advanced; Koehler, DIC, Polarization), Confocal and SEM (basics)
imaging	Photomicrography, video and timelapse capture, 3D reconstruction with ImageJ
histology	Rotary microtome sectioning and staining techniques
programming	Python and Bash (intermediate), ImageJ macros (basics), R (basics)

---

#### Publications

- Vellutini, B.C. and Migotto, A.E. Embryonic, larval, and juvenile development of the sea biscuit *Clypeaster subdepressus* (Echinodermata: Clypeasteroidea). *PloS ONE*, 5(3):e9654, **2010**. doi:10.1371/journal.pone.0009654.
- Vellutini, B.C. Bolacha no mar? Que história é essa? *Ciência Hoje das Crianças [Magazine]*, 204:8–11, **2009**.

Fukuzawa, A.H., Vellutini, B.C., Lorenzini, D.M., Silva, P.I., Mortara, R.A., Silva, J.R.M.C., and Daffre, S. The role of hemocytes in the immunity of the spider *Acanthoscurria gomesiana*. *Developmental and comparative immunology*, 32(6):716–25, **2008**. doi:10.1016/j.dci.2007.11.002.

Lindner, A., Migotto, A.E., Vellutini, B.C., and Silva-Neto, I.D. Vida escondida. *Telescópio [Magazine]*, pages 2–3, **2008**.

Vellutini, B.C. and Migotto, A.E. A Sea Biscuit's Life [*Film*], **2008**. doi:10.4016/8126.01.

Silva, J.R.M.C., Vellutini, B.C., Porto-Neto, L.R., Pressinotti, L.N., Ramos, M.D.C., Cooper, E.L., Hernandez-Blazquez, F.J., Jensch-Junior, B.E., and Borges, J.C.S. Resposta imune inespecífica de animais ectotérmicos antárticos sob temperaturas polares. *Oecologia Brasiliensis*, 11(1):110–121, **2007**.

Silva, J.R.M.C., Cooper, E.L., Sinhorini, I.L., Borges, J.C.S., Jensch-Junior, B.E., Porto-Neto, L.R., Hernandez-Blazquez, F.J., Vellutini, B.C., Pressinotti, L.N., and Costa-Pinto, F.A. Microscopical study of experimental wound healing in *Notothenia coriiceps* (Cabeçuda) at 0°C. *Cell and tissue research*, 321(3):401–10, **2005**. doi:10.1007/s00441-005-1139-z.

Silva, J.R.M.C., Sinhorini, I.L., Jensch-Junior, B.E., Porto-Neto, L.R., Hernandez-Blazquez, F.J., Vellutini, B.C., Pressinotti, L.N., Costa-Pinto, F.A., Cooper, E.L., and Borges, J.C.S. Kinetics of induced wound repair at 0°C in the Antarctic fish (Cabeçuda) *Notothenia coriiceps*. *Polar Biology*, 27(8):458–465, **2004**. doi:10.1007/s00300-004-0611-7.