NSF Final Project Report for Award No. OCE-0003884

Project Title:
ZooGene, a DNA sequence database for calanoid copepods and euphausiids: an OBIS tool for uniform standards of species identification

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NSF Grant No. OCE-0003884
September 1, 2000 – August 31, 2004
http://www.ZooGene.org

ACTIVITIES

The ZooGene partnership includes four lead investigators and thirteen expert taxonomic consultants from seven countries. Zooplankton samples have been sorted from existing archival collections, obtained in coordination with planned oceanographic research efforts, and/or collected during National Marine Fisheries Service field surveys. Taxonomic experts have confirmed species' identifications; DNA sequencing has been done at the University of New Hampshire and, in some cases, in other partners' laboratories. For each species, a DNA type sequence has been determined for a portion of the mitochondrial cytochrome oxidase I (mtCOI) gene; multiple mtCOI sequences are included as necessary to reflect intraspecific variation. For selected species, a portion of the slowly-evolving, nuclear small-subunit (18S) rRNA has been sequenced to examine evolutionary relationships among genera and families. The ZooGene database has been designed, maintained, and distributed as part of the proposed work; the data are being integrated into the Ocean Biogeographical Information System (OBIS).

New ZooGene collections: ZooGene partners have collected and shipped to UNH comprehensive sets of samples for molecular analysis using ships of opportunity. Specimens have been analyzed for many copepod genera, based on samples collected and identified by Susumu Ohtsuka (Hiroshima University, Japan); Shuhei Nishida (University of Tokyo); David McKinnon (Australian Institute of Marine Science); Janet Bradford-Grieve (National Institute of Water & Atmospheric Research, New Zealand); Bruce Frost (University of Washington); Peter Wiebe and Nancy Copley (Woods Hole Oceanographic Institution); and Fredrica Norrbin (Tromsö University). Euphausiid samples are in hand from Shozo Sawamoto (Tokai University, Japan) and Jaime Färber Lorda (Centro de Investigación Cientifica y de Educación Superior, Mexico). ZooGene samples have been collected in association with Ecosystem Monitoring Surveys by the NOAA/NMFS Northeast Fisheries Science Center (NEFSC), and used to assess spatial and temporal variation in gene frequencies in selected taxa.

Taxonomic analysis of samples: Janet Bradford-Grieve (NIWA, NZ); David McKinnon (Australian Institute of Marine Science); Shuhei Nishida (University of Tokyo); Susumu Ohtsuka (Hiroshima University, Japan); and other taxonomic experts have identified specimens of diverse copepod species from collections throughout the world. Shozo Sawamoto (Tokai University, Japan); Jaime Färber Lorda (Centro de Investigación Cientifica y de Educación Superior, Mexico); and Peter Wiebe and Nancy Copley (WHOI) served as taxonomic experts for euphausiids. Nancy Copley (WHOI) identified specimens of 90 copepod species and 25 euphausiid species for genetic analysis from samples collected from across the N. Atlantic and N. Pacific Oceans, Black Sea, and Southern Ocean.

DNA reference sequences: To date, considering only the molecular analyses done in Ann Bucklin's laboratory at UNH, the ZooGene project has determined and submitted to GenBank 150 DNA sequences for 81 species of calanoid copepods and 44 DNA sequences for 38 species of euphausiids. These numbers will increase with continued work at UNH and elsewhere, as part of follow-on research efforts. MtCOI sequences have proven to be diagnostic molecular systematic characters for both copepods and euphausiids. The sequences are also useful to reconstruct phylogenetic relationships among congeneric species, resolve large-scale population genetic structure and taxonomically-significant geographic variation, and reveal cryptic species (Hill et al., 2001; Bucklin et al., 2003).

Educational activities: A number of students received partial support from ZooGene, including:

- NH high school students (2): Fiona He and Anupama Vasudevan (2004 Oyster River HS, Durham, NH)

- UNH undergraduate students (8): Lisa D. Allen (Work-Study and Senior Honors Thesis, 1998-1999); Foster Kerrison (SURF award, 1999); David T. Camire (Laboratory Assistant 1998-2000); Naomi Cameron (Work-Study 1999-2000); Benjamin Curran (Work-Study 2000-2003); Meredith A. Bailey (Independent Study and Senior Honors Thesis, 2001-present; SURF award 2003); Elsa Rodrigues (Work-Study 2002-present); and Fiona He (Laboratory Assistant 2004-present)

- UNH graduate students (5): Christopher A. Manning (M.S. Zoology, 8/2003); R. Sean Hill (Ph.D. Genetics, 12/2004); Kaitlin Bonner (M.S. Zoology, exp. 8/2005); Brian D. Ortman (Ph.D. Zoology, exp. 8/2007); Ebru U. Yigiterhan (Ph.D. Zoology, exp. 8/2007)

- UW graduate students (1): Mikelle Rasmussen (M.S. Oceanography, 12/2004)

Outreach activities: A variety of informal education activities have been completed:

- A ZooGene molecular kit and curriculum module were designed and implemented for use in New Hampshire high schools. An informative brochure for teachers was produced and introduced in one-day workshops at UNH. Five teachers participated in August, 2000; six teachers participated in July, 2004. A NH Sea Grant Marine Docent is taking the lead in this effort, which is now being tested by students in NH high schools. One student is doing a ZooGene-related project funded by the GK PROBE initiative.

- A project web site, http://www.ZooGene.org, was established. The web site provides general information on the partnership, as well as technical information to guide colleagues in collecting and preserving zooplankton for molecular analysis. The website links to the ZooGene database, with specimen collection information, DNA sequences, and links to GenBank via accession numbers.

- The ZooGene database, http://www.ZooGene.org, was created. The database includes detailed collection information for the individual copepod or euphausiid used to determine the mtCOI type sequence and 18S rRNA sequence. Also included are the DNA sequences with links to the GenBank database via accession numbers. The database is linked to and searchable from the Ocean Biogeographical Information System (OBIS) portal/server (http://www.iobis.org/OBISPortal). To achieve the desired integration, ZooGene scientists and database managers participated in a number of meetings, including: the NOPP – OBIS VODHUB Workshop in Narragansett RI (September 13-14, 2000); OBIS Technical Working Group meetings at the University of Kansas (December 4-5, 2001) and Rutgers University (March 5-6, 2003); and other national meetings.

International Exchanges: ZooGene implemented plans for an international partnership through international exchanges between researchers. Examples of this successful approach to building an international partnership include:

- Sabine Grabbert (Alfred Wegener Institute for Polar and Ocean Science, Germany), a Ph.D. student of Dr. Sigrid Schiel (AWI) spent six months in Ann Bucklin's laboratory at UNH during two visits in 2001 and 2002, carrying out genetic analysis of copepods that will be included in her doctoral dissertation for the University of Oldenburg (Germany).

- David McKinnon (AIMS, Australia) visited UNH during December 2002 to collaborate on the analysis of a comprehensive set of samples of *Acartia* spp. collected and identified from Australian coastal waters. Analysis of mt16S rRNA sequence variation supported McKinnon's descriptions of cryptic species based on ultrastructural analysis by SEM.

- So-Young Soh (Yosu University, Korea) visited UNH during May 2003, working on mtCOI and 18S rRNA sequencing of the complex copepod super-family, the Arietelloidea. These integrated molecular and morphological analyses of this difficult group provide an excellent example of ZooGene's usefulness for phylogenetic analyses.

- Ann Bucklin (UNH) and Peter Wiebe (WHOI) spent several weeks in Japan during July 2003 at the invitation of Prof. Yoshinari Endo (Tohoku University, Sendai, Japan). They also visited ZooGene partner, Shuhei Nishida (University of Tokyo, Japan).

- Hiroshi Ueda (Ehime University, Japan) visited Ann Bucklin's laboratory at UNH during October 2003. Topics of mutual interest included phylogeography of several species of *Acartia*, in coastal regions of the USA and Japan. Another focus was cryptic species within *Paracalanus parvus* s.l.

- Ryuji Machida (University of Tokyo, Japan) visited UNH during March 2004, as part of his postdoctoral studies with Dr. Shuhei Nishida on the molecular phylogenetics of calanoid copepods and euphausiids.

New research stimulated: The ZooGene partnership helped lead to the Census of Marine Zooplankton (CMarZ), a global survey of zooplankton biodiversity sponsored by the Census of Marine Life and launched in November 2004 with funding from the Alfred P. Sloan Foundation. CMarZ plans a taxonomically comprehensive, geographically extensive survey of all holozooplankton groups by 2010.

FINDINGS

1. Species identification: ZooGene approach was shown to be both conceptually sound and practically useful. MtCOI sequences can be used to identify and discriminate even very closely related species of copepods and euphausiids.

- Molecular systematics of calanoid copepods (A. Bucklin, B.W. Frost, J. Bradford-Grieve (NIWA), et al.). DNA sequences for a 639 bp region of mitochondrial cytochrome oxidase I (mtCOI) were determined for 34 species of 10 genera of calanoid copepods, including: *Calanoides, Cosmocalanus, Meoscalanus, Nannocalanus, Neocalanus* and *Undinula* (Superfamily Calanidae); and *Clausocalanus, Ctenocalanus, Drepanopus*, and *Pseudocalanus* (Family Clausocalanidae). MtCOI gene sequences proved to be diagnostic molecular systematic characters for accurate identification and discrimination of the species (Bucklin et al., 2003). MtCOI sequence variation resolved evolutionary relationships among species of *Clausocalanus, Neocalanus*, and *Pseudocalanus*, although there was evidence of saturation at some variable sites. Phylogenetic relationships among 11 copepod genera (adding *Calanus* to the list above) were reconstructed using a 660 bp region of nuclear small-subunit18S rRNA, a slowly-evolving gene that showed no variability within a species and differed by <1% to 6% among the genera.

- Genetic structure of *Calanus pacificus* (B.W. Frost, A. Bucklin, et al.). The subspecies of *Calanus pacificus* were genetically differentiated at the population level. MtCOI sequence variation was determined for samples of *C. pacificus oceanicus* from the Gulf of Alaska and *C. pacificus californicus* from off the west coast of North America. MtCOI haplotype frequencies were heterogeneous among geographic locales, and haplotype diversity was high for all populations. F_{ST}-values between four of the populations indicated significant genetic structure, and supported the subspecies designation for *C. p. oceanicus* and *C. p. pacificus*.

- Molecular systematic examination of subgenera within *Tortanus* (Copepoda, Calanoida) (S. Ohtsuka (Hiroshima Univ., Japan), H.-Y. Soh (Yosu Univ., Korea), A. Bucklin, et al.). MtCOI was sequenced for specimens of five species of *Tortanus*, representing four of five subgenera. The molecular mtCOI gene tree did not correspond to the hypothesized morphological relationship among the subgenera, and this portion of the study awaits confirmation with another gene. A related study is comparing populations of *T. derjugini* from Korean waters and the Ariake Sea, based on mtCOI sequences. Haplotype divergence is low (~1.3% between populations), but does not preclude recent reproductive isolation between the populations.

- Molecular systematics of euphausiids (A. Bucklin, P.H. Wiebe, J. Färber Lorda (CICESE), S. Sawamoto (Tokai Univ., Japan), N.J. Copley (WHOI), et al.). Phylogenetic relationships among 35 species of eight euphausiid (Crustacea; Euphausiacea) genera, *Euphausia, Meganyctiphanes, Nematobrachion, Nematoscelis, Nyctiphanes, Stylocheiron,* *Thyssanoessa*, and *Thysanopoda*, were reconstructed based on a 653 base-pair (bp) region of nuclear 18S rRNA. This molecular phylogeny differed from previous morphologically-based studies, and suggested that eye morphology (rather than thoracic limb structure) may most accurately parallel evolutionary relationships within and among euphausiid genera and species.

- Copepods of the N. Atlantic (A. Bucklin, P.H. Wiebe, M.J. Fogarty, N.J. Copley, et al.). Samples from ships of opportunity across the N. Atlantic have been examined for copepod species. Identified specimens were removed and sent to UNH for DNA sequencing of a portion of the mtCOI gene. A voucher collection has been established at UNH. To date more than 50 copepod species have been analyzed.

2. Molecular phylogeography: Cosmopolitan species of copepods and euphausiids have received particular attention under ZooGene. Samples were taken across each species' range, in order to evaluate evidence for geographic isolation and genetic differentiation within the species. MtCOI has provided preliminary evidence of population genetic structure within some geographically widespread species.

- *Calanoides carinatus* ZooGene Project (CCZP): Species and subspecies identification (M.D. Viñas (INIDEP, Argentina), H. Verheye (Marine and Coastal Management, SA) J.G. Bersano (Brazil), A. Bucklin). CCZP goals include the creation of mtCOI type sequences for *Calanoides carinatus* based on verified identification of the species in the type-locality; genetic comparison among populations of *C. carinatus* from different oceanographic regions; and incorporation of mtCOI sequences into the ZooGene and GenBank databases.

- Phylogeography of *Acartia* spp.: (A. Bucklin, H. Ueda (Ehime University, Japan), J. Bradford-Grieve (NIWA, NZ), D. McKinnon (AIMS, Australia), et al.). Taxonomically-significant variation has been observed in a number of species of *Acartia* collected from Japan, Australia, Europe, and the USA. Molecular systematic analysis has revealed marked genetic differentiation within putative species, with strong evidence of multiple cryptic species. Studies in coastal estuaries along the Atlantic coast of the Northeast USA indicated that populations in different estuaries, even closely adjacent estuaries, are geographically isolated and genetically divergent, and are likely cryptic species. Comprehensive, global-scale analysis of *Acartia* species is needed to accurately assess species-level diversity and to determine the underlying dynamics determining geographic patterns of genetic patterns, including rapid speciation and/or transport by ocean current and/or human activities.

- Cryptic species within *Paracalanus parvus* s.l. (H.Ueda (Ehime University, Japan), H.-Y. Soh (Yosu Univ., Korea), S. Ohtsuka (Hiroshima Univ., Japan), D. McKinnon (AIMS, Australia), et al.). Molecular systematic analysis of samples from the USA, Japan, Korea, and elsewhere have provided phylogeographic evidence of at least four morphologically indistinguishable, genetically distinct clades within *P. parvus* (Fig. 1). The clades exhibit mtCOI sequence divergences of ~25%, suggesting that they are actually distinct species.

- <u>Molecular systematics of the *Metridia lucens* group</u>: A. Bucklin (UNH), B.W. Frost (UW), J. Bradford-Grieve (NIWA, NZ), M. Galbraith and D. Mackas (IOS, Canada). Collections of *Metridia lucens* and *M. pacifica* from throughout the world have been sequenced for both mt16S and mtCOI. The phylogeographic analysis indicated that there are two groups of *Metridia* in the central N Pacific and along NE Pacific coastline. Based on genetic distances, the two groups are two sibling species. One species grouping includes *M. pacifica* from Station PAPA, La Perouse Bank, and Effingham Inlet. The other species group includes *M. lucens* from the N. Atlantic and New Zealand. There was ~25% sequence difference between species, but only 3% sequence difference between distant populations within each group, similar to other circumglobal copepod species for mtCOI. The mtCOI differences were sufficient for design of SS-PCR protocols to discriminate the species.

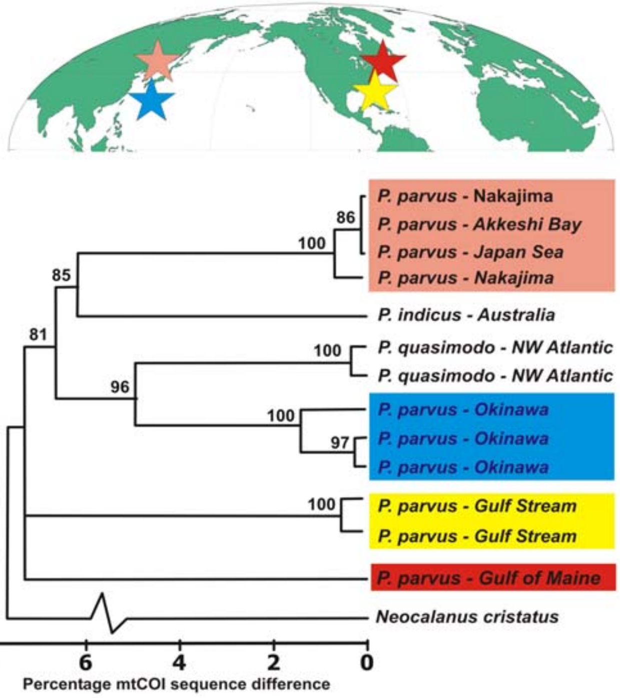

Figure 1. Phylogeographic analysis of *Paracalanus parvus* s.l. based on mtCOI sequence variation.

- <u>Morphological and molecular phylogenetic analysis of *Clausocalanus* spp.</u> A. Bucklin (UNH) and B.W. Frost (UW). Phylogenetic relationships among 13 species of *Clausocalanus* (Crustacea; Copepoda; Calanoida) were examined based on morphological, quantitative, and molecular characters, in order to examine evolutionary relationships among the species, including evidence that three described species groups are monophyletic evolutionary lineages, and to investigate further hypotheses of the tempo and mode of speciation in this systematically complex genus. This study builds upon the monographic analysis by Frost and Fleminger (1968) and molecular phylogenetic analysis using mitochondrial cytochrome oxidase I (mtCOI) by Bucklin et al. (2003).

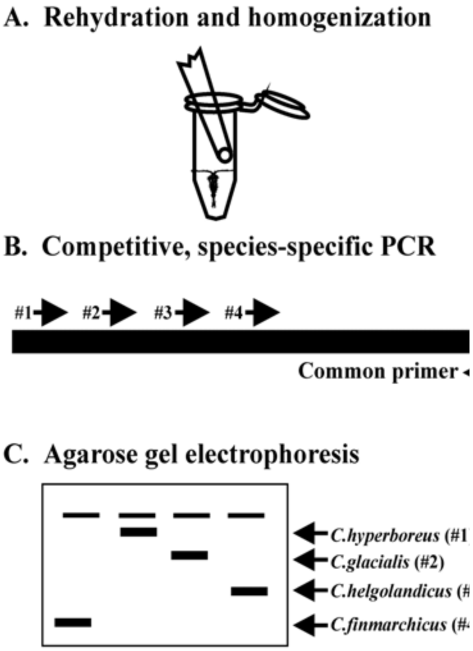

Figure 2. Diagram showing steps in the molecular identification of copepods using a competitive, multiplexed SS-PCR reaction. See Hill et al., 2001.

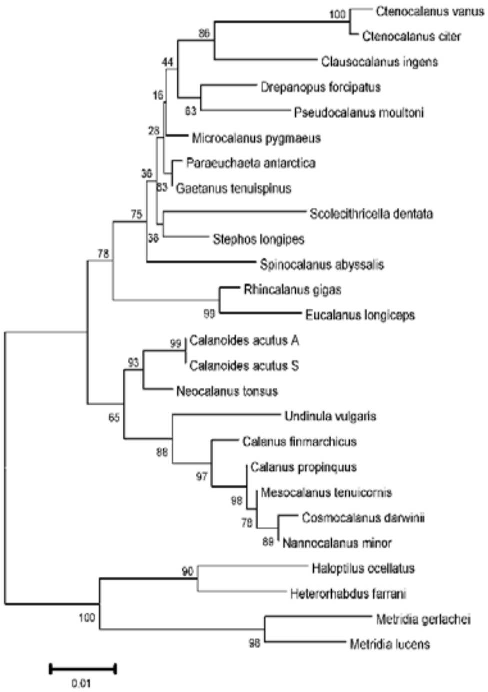

Figure 3. Gene tree of evolutionary relationships among calanoid copepod species, genera, and families based on a 660 base-pair region of nuclear 18S rRNA. Data from Bucklin et al (2003) and S. Grabbert (Univ. Oldenburg,

***3. Rapid molecular protocols for species identification*:** We have designed molecular protocols based on species-specific PCR (SS-PCR) that can discriminate cryptic, co-occurring copepod species: multiplexed SS-PCR protocols are available for two species of *Pseudocalanus* (Bucklin et al., 1999, 2001); four species of *Calanus* (Bucklin et al., 1999; Hill et al., 2001; Fig. 2); and two species of *Metridia* (Bucklin et al, in preparation).

4. Phylogenetic reconstruction: Evolutionary relationships among congeneric copepod and euphausiid species are typically well-resolved by mtCOI sequence variation. MtCOI gene trees were largely concordant with morphological phylogenetic analyses for species of both copepods (Bucklin et al., 2003) and euphausiids (Bucklin, in preparation). Phylogenetic relationships among genera and families of calanoid copepods and euphausiids have been examined using 18S rRNA sequences, which provide accurate resolution (Bucklin et al., 2003, unpublished; Grabbert et al., in preparation; Fig. 3), and are useful for comparison with morphological analyses (Bradford-Grieve et al., unpublished).

PUBLICATIONS AND PRODUCTS

ABSTRACTS and PRESENTATIONS

Bucklin, A. (2000) *Calanus finmarchicus in Icelandic waters: population genetics and ecology at the Norwegian Sea / N. Atlantic boundary*. ICES Annual Science Meeting, Brugge, Belgium. *Best Paper of the Conference* Award. (September, 2000)

Bucklin, A. (2001) *Transport of planktonic copepods between Atlantic and Arctic waters near Iceland: rapid assays of genetic diversity and structure for the copepod Calanus finmarchicus*. Invited talk at the ICES Annual Science Meeting, Oslo, Norway (September, 2001)

Bucklin, A. (2002) *Zooplankton genomic database (ZooGene) project: integrating molecular, taxonomic, and oceanographic data*. American Geophysical Union / Ocean Sciences, Honolulu HI (February, 2002)

Bucklin, A. (2002) *Molecules and morphology: understanding evolution within and among calanoid copepod species*. Invited speaker for the Eighth International Conference on the Copepoda, Keelung, Taiwan (July, 2002)

Bucklin, A. (2002) *Molecules and morphology: integrating information in biodiversity research*. Invited panelist for Marine Biodiversity in the Present (Known, Unknown, and Unknowable) Conference, La Jolla, CA (December, 2002)

Bucklin, A. (2002) *ZooGene: integrating molecular, taxonomic, and oceanographic data*. Invited speaker for 1st Annual Meeting, CORONA Project, Shoals Marine Laboratory, NH (August 20 - 23, 2002).

Bucklin, A. (2002) *Integrating information to gain new understanding: molecules and morphology in mari2e biodiversity*. Invited speaker for Census of Marine Life Roundtable, Marine Biodiversity in the Present, La Jolla CA (December 6-9, 2002)

Bailey, M.A., B. Curran, E. Rodrigues, J.G. Beaudet, and A. Bucklin (2003) *Distribution and abundance of cryptic species of the copepod Pseudocalanus on Georges Bank (Northwest Atlantic).* Poster presentation at COLSA Undergraduate Research Conference, UNH (May 3, 2003)

Bucklin, A., D.J. McGillicuddy, and M.A. Bailey and B. Curran (2003) *SS-PCR discrimination of morphologically cryptic, ecologically distinct species: seasonal evolution of Pseudocalanus spp. on Georges Bank.* Poster presentation at the Third International Zooplankton Production Symposium, Gijon Spain (May 20 - 23, 2003)

Bucklin, A., B.W. Frost, J. Bradford-Grieve, P.H. Wiebe, and M.J. Fogarty (2003) *ZooGene: molecular systematic and phylogenetic analysis of calanoid copepods and euphausiids.* Presentation at the Third International Zooplankton Production Symposium, Gijon Spain (May 20 - 23, 2003)

Bucklin, A. (2003) *Molecular population genetics of marine zooplankton* and *Global-scale patterns of biodiversity of planktonic calanoid copepods*. Invited seminars at Tohoku University and Tokyo University, Japan (July 15-25, 2003)

Bucklin, A. et al. (2003) *Biological-physical processes determining Pseudocalanus spp. (Crustacea; Copepoda) distribution and abundance on Georges Bank in the Northwest Atlantic*. ICES Annual Science Conference, Tallinn, Estonia (22-30 September 2003)

Bucklin, A. (2003) *Integrating morphological and molecular systematics in the pelagic realm*. Census of Marine Symposium, Ocean Life: The Known, Unknown, and Unknowable. Washington DC (October 24, 2003)

Bucklin, A., et al. (2004) *Population dynamics of cryptic species of Pseudocalanus in the Northwest Atlantic: biological and physical coupling at small- to mesoscales*. AGU Ocean Sciences Meeting, Portland OR (25-29 January 2004)

Bailey, M.A., R.S. Hill, and A. Bucklin (2004) *Geographic isolation and genetic divergence of Acartia tonsa (Crustacea; Copepoda) populations among NW Atlantic coastal estuaries: how do they do that?* AGU Ocean Sciences Meeting, Portland OR (25-29 January 2004)

Bucklin, A. (2004) *Barcoding marine zooplankton*. Invited seminar, New England Biolabs, Beverly MA (21 April 2004)

Bucklin, A. (2004) *Barcoding marine zooplankton*. Invited talk, Barcode of Life Initiative workshop, Washington DC (25 May 2004)

Bucklin, A. (2004) *DNA barcoding: a new tool for species identification and discovery*. Invited talk, Natural Science Collections Alliance meeting, Washington DC (26 May 2004)

Ortman, B. D. A. Bucklin, and M. Youngbluth (2004) *Linking molecular and morphological characters to assess gelatinous zooplankton diversity*. ASLO, Athens, Georgia (June 2004)

Bucklin, A., E. Ünal, P.H. Wiebe, and N.J. Copley (2004) *Interannual variation of population genetic diversity of Calanus finmarchicus (Copepoda, Calanoida) in the Gulf of Maine, Northwest Atlantic*. ICES Annual Science Conference, Vigo, Spain (September 23-25, 2004)

Ortman, B.D., and A. Bucklin (2005) *Integration of morphological and molecular characters to facilitate species identification and biodiversity within the Ctenophora*. SCIB, San Diego, CA (January 5-8, 2005)

Rasmussen, M., B.W. Frost, B.W., and A. Bucklin (2005) *Genetic structure of the planktonic copepod Calanus pacificus in the Eastern North Pacific*. ASLO, Salt Lake City, UT (February 22-25, 2005).

Ünal, E., P.H. Wiebe, D.J. McGillicuddy, A. Bucklin (2005) *Basin-scale spatial patterns and climate-related temporal patterns of population genetic diversity structure of Calanus finmarchicus (Copepoda, Calanoida) in the N. Atlantic Ocean*. International Marine Biotechnology Conference (IMBC), St. Johns Nova Scotia CA (June 8-12, 2005)

THESES and DISSERTATIONS (partially supported by ZooGene)

Bailey, M.A. (2003) *Molecular population genetic analysis of possible cryptic species within Acartia tonsa (Copepoda: Calanoida) in New England estuaries*. Senior Honors Thesis, University of New Hampshire, Durham.

Manning, C.A. (2003) *Temporal and spatial variation in copepod community structure in the western Gulf of Maine coastal region*. M.S. Thesis, University of New Hampshire, Durham.

Hill, R.S. (2004) *Genetic diversity and structure of calanoid copepods: molecular evolutionary patterns in coastal estuaries (Acartia tonsa) and the open ocean (Calanus spp.)*. Ph.D. Dissertation, University of New Hampshire, Durham.

Rasmussen, M. (2004) *Genetic structure of the planktonic copepod Calanus pacificus in the eastern North Pacific*. M.S. Thesis, University of Washington, Seattle.

PUBLICATIONS

Bucklin, A. (2000) Methods for Population Genetic Analysis of Zooplankton. Chapter 11 in: *The Zooplankton Methodology Manual*, International Council for the Exploration of the Sea. Academic press, London. Pp. 533-570.

Hill, R.S., L.D. Allen, and A. Bucklin (2001) Multiplexed species-specific PCR protocol to discriminate four N. Atlantic *Calanus* species, with a mtCOI gene tree for ten *Calanus* species. Marine Biology 139: 279-287.

Bucklin, A., M. Guarnieri, D.J. McGillicuddy, and R.S. Hill (2001) Spring evolution of *Pseudocalanus* spp. abundance on Georges Bank based on molecular discrimination of *P. moultoni* and *P. newmani*. Deep-Sea Res. 48: 589-609.

Bucklin, A., P.H. Wiebe, S. B. Smolenack, N.J. Copley, and M.E. Clarke (2002) Integrated biochemical, molecular genetic, and bioacoustical analysis of mesoscale variability of the euphausiid *Nematoscelis difficilis* in the California Current. Deep-Sea Res. 49: 437-462.

Bucklin, A., B.W. Frost, J. Bradford-Grieve, L.D. Allen and N.J. Copley (2003) Molecular systematic assessment of thirty-four calanoid copepod species of the Calanidae and Clausocalanidae using DNA sequences of mtCOI and nuclear 18S rRNA. Mar. Biol. 142: 333-343.

Caudill, C.C. and A. Bucklin (2004) Molecular phylogeography and evolutionary history of the estuarine copepod, *Acartia tonsa,* on the Northwest Atlantic coast. Hydrobiol. 511: 91-102.

Bucklin, A. and L.D. Allen (2004) MtDNA sequencing from zooplankton after long-term preservation in buffered formalin. Molec. Phylog. Evol. 30: 879-882.

Manning, C.A. and A. Bucklin (2005) Multivariate analysis of the copepod community of near-shore waters in the western Gulf of Maine (Northwest Atlantic). Mar. Ecol. Prog. Ser. (in press)

Papetti, C., L. Zane, E. Bortolotto, A. Bucklin, and T. Patarnello (2005) Genetic differentiation and local temporal stability of population structure in the euphausiid *Meganyctiphanes norvegica*. Mar. Ecol. Progr. Ser. (in press)

PUBLICATIONS IN PREPARATION

Ueda, H. and A. Bucklin (in preparation) Description of *Acartia* (Odontacartia) new species from Ariake Bay, Japan

Bucklin, A., P.H. Wiebe, S.B. Smolenack, N.J. Copley, J.G. Beaudet, J. Färber Lorda, and J.J. Pierson (in preparation) Comparative molecular and morphological assessment of 35 species and eight genera of euphausiids (Euphausiacea, Crustacea)

Bucklin, A., J. Bradford-Grieve, S. Grabbert, H.Y. Soh, S. Nishida, S. Ohtsuka, N.J. Copley, and J.G. Beaudet (in preparation) Molecular phylogeny of the Calanoida: evidence for family and superfamily relationships.

Bucklin, A., B.W. Frost, and J.G. Beaudet (in preparation) Morphological and molecular phylogenetic analysis of evolutionary lineages within *Clausocalanus* (Crustacea, Copepoda, Calanoida).

Bucklin, A., J.G. Beaudet, B.W. Frost, M. Galbraith, and D. Mackas (in preparation) Molecular systematic analysis of the *Metridia lucens* group, with a multiplexed PCR protocol to discriminate the sibling species.

Bucklin, A., H. Ueda, D. McKinnon, J. Bradford-Grieve, et al. (in preparation) Molecular phylogeographic analysis of *Acartia*: evidence for rapid evolutionary divergence and cryptic speciation in coastal and estuarine waters.

Bucklin, A. H. Ueda, and J. Bradford Grieve (in preparation) Cryptic species within the coastal, cosmopolitan copepod, *Paracalanus parvus s.l.*

WEB MATERIALS

A project web site, http://www.ZooGene.org, provides technical information to guide colleagues in collecting and preserving zooplankton for molecular analysis. The website links to the ZooGene database, with specimen collection information, DNA sequences, and links to GenBank via accession numbers.

The ZooGene database, with molecular systematic, taxonomic, and biogeographical data, is accessible at http://www.ZooGene.org. The database includes detailed collection information for the individual copepod or euphausiid used to determine the mtCOI type sequence and 18S rRNA sequence. Also included are the DNA sequences; links to the GenBank database will be implemented shortly (via the GenBank accession number); and to environmental data in the U.S. GLOBEC Data and Information Management System (via the collection location). The database is linked to and searchable from the Ocean Biogeographical Information System (OBIS) portal/server (http://www.iobis.org/OBISPortal).

CONTRIBUTIONS

Principal discipline(s) of the project

ZooGene will allow rapid and accurate identification of planktonic species and estimation of marine biodiversity, which are essential for understanding, assessing, and predicting ecosystem health, and facilitating wise use of marine resources and sound coastal zone planning. We anticipate that biologists, ecologists, and systematists will increasingly use DNA sequences as additional characters for taxonomic identification. The molecular information may be used to ensure uniform standards of species identification, evaluate the taxonomic significance of geographic variation, reveal cryptic species, accurately estimate species diversity, determine evolutionary relationships among species, develop rapid molecularly-based species identification protocols, and identify morphologically-indistinguishable species at any life-stage. Despite the challenges, it is important that ecologists and oceanographers identify species accurately. Zooplankton species are of considerable importance in biogeochemical cycles, because of their numerical abundance, biomass predominance, and/or critical position in coastal and ocean trophic webs.

Development of human resources

Professional training: Nancy Copley (WHOI) participated in the ICES Zooplankton Taxonomic Workshop held at the Sir Alistair Hardy Foundation for Ocean Science, Plymouth, UK.
She presented the WHOI Silhouette Digitizer image analysis software, a new method of analyzing the abundance, size, and identification of zooplankton, and furthered her knowledge of copepod and euphausiid taxonomy, especially for euphausiid larval stages and the complex copepod genus *Clausocalanus*, a subject of ZooGene investigations.

Students supported by ZooGene

Secondary school students: Fiona He (2004 Oyster River HS, Durham, NH) and Anupama Vasudevan (2004 Oyster River HS, Durham, NH) received support from ZooGene for work during Summer 2004.

UNH Undergraduate Students: Lisa D. Allen (Work-Study and Senior Honors Thesis, 1998-1999); Foster Kerrison (SURF award, 1999); David T. Camire (Laboratory Assistant 1998-2000); Naomi Cameron (Work-Study 1999-2000); Benjamin Curran (Work-Study 2000-2003); Meredith A. Bailey (Independent Study and Senior Honors Thesis, 2001-present; SURF award 2003); Elsa Rodrigues (Work-Study 2002-present); Fiona He (Laboratory Assistant 2004-present).

UNH Graduate Students: Christopher A. Manning (M.S. Zoology, 8/2003); R. Sean Hill (Ph.D. Genetics, 12/2004); Kaitlin Bonner (M.S. Zoology, exp. 8/2005); Brian D. Ortman (Ph.D. Zoology, exp. 8/2007); Ebru U. Yigiterhan (Ph.D. Zoology, exp. 8/2007)

UW Graduate Student: Mikelle Rasmussen (M.S. Oceanography, 12/2004)

NH Secondary School Curriculum: A ZooGene molecular kit and curriculum module were designed and implemented for use in New Hampshire high schools. An informative brochure for teachers was produced and introduced in one-day workshops at UNH. Five teachers participated in August, 2000; six teachers participated in July, 2004. A NH Sea Grant Marine Docent is taking the lead in this effort, which is now being tested by students in NH high schools. One student (Melissa Durham of Manchester, NH) is doing a ZooGene-related project, with additional funding from the GK PROBE initiative.