Caribbean wide survey of PaV1 prevalence in adult lobsters in lobster fishing grounds from 2006-2007 and 2010-2011 (Lobster disease connectivity project)

Website: https://www.bco-dmo.org/dataset/556371
Version: 4
Version Date: 2021-05-04

Project
» Connectivity of disease in marine Ecosystems: multi-scale dynamics of a viral disease infecting caribbean spiny lobster (Lobster disease connectivity)

Contributors	Affiliation	Role
Butler, Mark	Old Dominion University (ODU)	Principal Investigator
Behringer, Donald	University of Florida (UF)	Co-Principal Investigator
Paris-Limouzy, Claire B.	University of Miami Rosenstiel School of Marine and Atmospheric Science (UM-RSMAS)	Co-Principal Investigator
Shields, Jeffery	Virginia Institute of Marine Science (VIMS)	Co-Principal Investigator
Copley, Nancy	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

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Dataset Description

Related References:

The results of these data have been reported in the following publication:

Moss, J., D. Behringer, J. D. Shields, A. Baeza, A. Aguilar-Perera, P. G. Bush, C. Dromer, A. Herrera-Moreno, L. Gittens, T. R. Matthews, M. R. McCord, M. T. Schärer, L. Reynal, N. Truelove, and M. J. Butler. 2013. Distribution, prevalence, and genetic analysis of Panulirus argus Virus I from the Caribbean Sea. Diseases of Aquatic Organisms 104: 129–140

Methods & Sampling

From February 2006- February 2007 and from September 2010 through October 2011, adult *Panulirus argus* tissues were obtained directly from fishermen as fresh or recently frozen tissue samples (fifth walking leg) from locations throughout the Caribbean. In most cases, approximately 100 lobsters were sampled from each country or sample location and carapace length and sex were taken for each lobster. Samples consisted of a 1 – 2 cm piece of a walking leg preserved in clear rum (40% ethanol, 80 proof), shipped to the Virginia Institute

of Marine Science and transferred upon receipt into 95% ethanol for long-term storage in preparation for genetic analysis.

Molecular diagnostics and DNA sequencing
Genomic DNA was extracted using Chelex resin (Biorad, Hercules, CA). Using sterile methods, aliquots of ~15mg of tissue or 150µl of hemolymph in anticoagulant, were incubated with 50µl of 10% Chelex resin (w/v) and 12µl of proteinase K (Qiagen, Valencia, CA) at 60ºC for approximately 4 hrs. Once tissue lysis was complete, samples were vortexed for 15 s, heated at 100ºC for 10 min, vortexed briefly again and then centrifuged at 13,600 rpm for 1 min. The DNA (supernatant) was removed and stored at either -20ºC or 4ºC prior to genetic analysis. For quality control, genomic DNA from each lobster was assessed by amplifying the small subunit ribosomal RNA (SSU) using 'universal' SSU primers. The amplified target DNA fragment was approximately 1800 bp in length.

Diagnosis of PaV1 was done using PCR primers published previously with modifications to reagent concentrations, thermocycling parameters, and reaction volume as described in Moss et al. (2012). The concentrations of the assay reagents were 1X PCR buffer (20mM Tris-HCl (pH 8.4), 50mM KCl), 0.2 mg ml-1 bovine serum albumin (BSA), 1.5 mM MgCl2, 0.2 mM dNTPs, 0.5 uM 45aF (forward primer), 0.5 uM 543aR (reverse primer) and 1.0 unit Taq polymerase (Invitrogen, Carlsbad, CA). Aliquots of 2.5 uL of lobster genomic DNA were added to 22.5 uL of PCR reagents for a final PCR volume of 25 uL. An additional control reaction containing 2.5 ?l of ddH20 plus PCR reagents in the above concentrations was included as a separate control (no DNA). The thermocycling parameters were an initial denaturation step at 94°C for 5 min followed by 40 cycles of 94°C for 45 s, 63°C for 45 s, 72°C for 1 min, all followed by a final elongation step at 72°C for 10 min. Aliquots of 10 ?L of the PaV1 PCR product or 6 ?L of the SSU product were loaded onto a 2% agarose gel (w/v), electrophoresed at 100V, stained with ethidium bromide and examined under UV light. Images were recorded using the Alpha Innotech FluorChem? (San Leandro, CA) imaging system.

Amplification products from all spiny lobsters found to be PCR positive for PaV1 were cloned and sequenced. Briefly, between 4 and 8 clones were sequenced per sample. PCR products of the correct size (~500 bp) were cloned into the plasmid pCR4-TOPO and then transformed into Escherichia coli using a TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA) using half the manufacturer's recommendation of vector and chemically-competent cells. Selection was based on ampicillin resistance. Transformed bacterial colonies were screened for inserts using a boil-prep method followed by PCR amplification using the M13 vector primers. Before sequencing, PCR products were treated with shrimp alkaline phosphatase (SAP) and exonuclease I (Exo I) (Amersham Biosciences, Piscataway, NJ) to remove excess oligonucleotides and dNTPs. Bidirectional sequencing was performed using the Big Dye Terminator Kit v3.1 (Applied Biosystems, Norwalk, CT) with M13 sequencing primers in 5 uL reactions with one eighth the concentration of Big Dye recommended by the manufacturer's protocols. Sequence products were re-suspended in 20 uL of Hi-Di formamide (Applied Biosystems, Foster City, CA) and 10 uL of each were electrophoretically separated on an ABI 3130 Prism Genetic Analyzer.

Vector trimming and sequence editing were performed using CodonCode Aligner v3.7.1.1. Sequences were aligned using the ClustalW algorithm in MacVector v.12.5.1. Settings for pairwise alignment were an open gap penalty of 10.0 and an extend gap penalty of 0.1. Settings for the multiple alignment stage were an open gap penalty of 10.0 and an extend gap penalty of 0.05. Following ClustalW alignment, the resulting alignment was edited by hand. Sequences were examined for unique alleles using Collapse v.1.2. Measures of genetic distance and nucleotide diversity were performed using MEGA v.5.0.

Data Processing Description

Rarefaction analysis: For cost effectiveness, efficiency in sample processing, and quality control, we examined how the presence of alleles (allele richness) varied with the number of infected lobsters examined and the number of clones analyzed (4 vs. 8 bacterial clones sequenced per infected lobster). Rarefaction analysis on a preliminary data set was used to examine allele richness. Values for Cole's rarefaction were estimated using EstimateS8.0 (Colwell 2006). In the first analysis, common and rare alleles from all of the clones (4 or 8 clones per infected lobster, see above) were used to examine allele richness. In the second analysis, we asked whether common alleles were represented in both the 4-clone and 8-clone datasets. Common alleles were defined as those present in three or more infected lobsters using 8 clones from each, or two or more lobsters using 4 clones from each. For each analysis the number of clones (4 or 8 clones) examined were run separately, and the presence of an allele was counted as one when one or more clones from an infected lobster had it.

BCO-DMO Processing:

- added conventional header with dataset name, PI name, version date, reference information
- renamed parameters to BCO-DMO standard
- added lat/lon
- changed sex ? to nd
- changed blanks to nd
- changed lat/lon to decimal degrees
- changed all-caps to lower case/title case
- change sex=H to sex=M

Version 4 (2021-05-04) replaced version 3 (2015-04-22)
* non-utf8 characters were causing incompatibility issues. Converted source file carib_survey_v3.dat to utf8.

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Data Files

File
caribbean_survey.csv (Comma Separated Values (.csv), 194.57 KB) MD5:23eb652903d69aa9bf2e11e776f02028 Primary data file for dataset ID 556371

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Parameters

Parameter	Description	Units
year	year of survey	yyyy
date	date of survey	yyyy-mm-dd
site	survey site	unitless
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
sex	sex of lobster	unitless
length_carap	carapace length of tethered lobster	millimeters
molt_cond	visual assessment of molt condition: I = intermolt; PR = premolt; PO = postmolt	unitless
leg_old_new	old and new injuries number of old/new leg injuries if any	unitless
ant_old_new	number of old/new antennae injuries if any	unitless
other_old_new	number of old/new other injuries if any	unitless
visible_disease_flag	signs of PaV1 infection in lobster obvious to naked eye; 0 = no; 1 = yes	unitless
blood_sample	hemolymph sample vial label if any	unitless
PCR_result	positive or negative PCR test for presence of PaV1 virus DNAa	unitless
comments	comments	unitless

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	ABI 3130 Prism Genetic Analyzer
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	pCR
Generic Instrument Name	Thermal Cycler
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

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Deployments

Butler_Caribbean

Website	https://www.bco-dmo.org/deployment/556145
Platform	Lobster habitat
Start Date	2006-02-11
End Date	2011-10-19
Description	lobster disease studies

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Project Information

Connectivity of disease in marine Ecosystems: multi-scale dynamics of a viral disease infecting caribbean spiny lobster (Lobster disease connectivity)

Coverage: Florida Keys, USA and Carrbbean

Scientists are struck by how different terrestrial epidemiology is from that in marine ecosystems, a crucial difference being the more rapid spread of diseases in the ocean due to the presumed absence of barriers to waterborne dispersal. Yet, the movement of pathogens in the sea and its importance to disease dynamics in marine metapopulations is virtually unstudied. Marine pathogens do spread among distant host populations, as demonstrated by dramatic epizootics, but is this common or demographically relevant? Nearly all studies of marine diseases treat such events as transitory, focusing instead on local disease dynamics. This approach

suggests either that small-scale phenomena normally trump the influence of large-scale pathogen connectivity or, alternatively, that the dispersal of marine pathogens by highly motile adults or free-living waterborne pathogens is simply too intractable for empirical investigation. Yet, there is another perhaps unappreciated mechanism – dispersal by infected larvae. Most marine animals have life histories that include planktonic larvae, many of which are highly dispersive. If infected by pathogens, these “larval vectors” would provide an efficient mechanism for distributing pathogens at high concentrations directly into habitats where hosts dwell. More so than passive, waterborne pathogens that are subject to rapid dilution and have no means of targeting distant hosts.

We have evidence that long-distance pathogen dispersal in the sea via infected meroplanktonic larvae may be possible. The pathogen in question is an often lethal, pathogenic virus (PaV1; Panulirus argus virus 1) that infects the Caribbean spiny lobster, Panulirus argus – a species broadly distributed throughout the Caribbean where it supports the most valuable fishery in the region. We described the PaV1 virus in 1999 and since then have studied its pathology, epidemiology, transmission, and effects on juvenile lobster populations in the Florida Keys. Like others, our focus has been on local pathogen-host dynamics, but PaV1 infections in lobsters are now confirmed in distant areas of the Caribbean (Belize, Mexico, St. Croix) in regions that are demographically linked only by dispersing larvae that spend >6 mos. in the open ocean. We recently discovered that many lobster postlarvae recruiting to coastal nurseries in Florida are infected with PaV1, providing novel evidence for pathogen connectivity among distant host populations.

Focusing on the spiny lobster-PaV1 virus association as a case study, we propose an ambitious program of laboratory, field, and modeling research whose broader implications will better our understanding of the importance of dispersal by infectious agents on the spread and maintenance of disease in marine populations. The project builds upon data and techniques developed with prior NSF sponsorship, and brings together partners in developing Caribbean nations with a multidisciplinary group of scientists with long-standing research programs in larval biology, biophysical and ecological modeling, crustacean biology, molecular biology, and the study of marine diseases.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0928930
NSF Division of Ocean Sciences (NSF OCE)	OCE-0929086

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