Experimental results on the growth of Aplanochytrium (a sea fan parasite) cells over a temperature gradient conducted at the Harvell lab at Cornell University

Website: https://www.bco-dmo.org/dataset/3719

Data Type: experimental

Version: 1

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Project

» Influence of Temperature and Acidification on the Dynamics of Coral Co-Infection and Resistance (Climate_CoralDisease)

Contributors	Affiliation	Role
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Abstract

Experimental results on the growth of Aplanochytrium (a sea fan parasite) cells over a temperature gradient. Two types of assays were used in two trials to quantify Labyrinthulomycota cultures: cell counts using a hemocytometer and total protein concentration.

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Dataset Description

Two types of assays were used in two trials to quantify *Labyrinthulomycota* cultures: cell counts using a hemocytometer and total protein concentration.

Acquisition Description

Sampling and Analytical Methodology:
In the first trial, a *Labyrinthulomycota* culture held at 22 degrees C was divided into 18 sub-cultures that were incubated at 15 degrees C, 20 degrees C, 25 degrees C, 30 degrees C, and 32 degrees C in triplicate for three days. Culture temperatures and incubation period were based on previous visual observations of *Labyrinthulomycota* growth, where over-growth of culture flasks occurs after three days at temperatures of 25 degrees C and higher. In the second trial, nine sub-cultures were incubated at 20 degrees C, 25 degrees C, and 30 degrees C in triplicate for three days.

In the second trial, total protein was also assessed. After three days, the media was poured off, rinsed once with 3 mLs of 0.22 um-filtered artificial sea water, and replaced with 3 mLs of 0.22 um-filtered artificial sea water. With a sterile wooden dowel, the bottom of each culture was scraped until no *Labyrinthulomycota* growth was visible on the bottom of the culture. 700 uL of each culture was placed in a bead beater and mixed at 300 rpm for 30 sec; 400 uL was set aside for protein assays and 300 uL for cell counts and held on ice until use.

Prior to counting using a hemocytometer, cells were vortexed for about 20 sec. In each culture, cells were counted in triplicate.

Total protein was extracted from each sample by adding 400 uL of extraction buffer (0.15 ug mL-1 DTT in Tris-HCl) to each tube. The contents of the tube were mixed and lysed for 2 minutes with the Fisherbrand disposable pestle grinder system, and incubated for 45 minutes on ice for extractions. Protein was measured using the DC protein kit (Bio-Rad), and read in triplicate using the Synergy HT multi-Detection microplate reader with KC4 software (Biotek Instruments, Vermont) at 750 nm.

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Related Publications

Burge, C., Douglas, N., Conti-Jerpe, I., Weil, E., Roberts, S., Friedman, C., & Harvell, C. (2012). Friend or foe: the association of Labyrinthulomycetes with the Caribbean sea fan Gorgonia ventalina. Diseases of Aquatic Organisms, 101(1), 1â€“12. doi:10.3354/dao02487

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Parameters

Parameter	Description	Units
trial	Experimental trial number (1 or 2; see Acquisition Description).	unitless
temp	Incubation temperature.	degrees C
count_avg	Average of total cells per temperature.	# cells (unitless)
count_avg_se	Standard error of count_avg.	# cells (unitless)
count_log10_avg	Average of log 10 transformed cell counts per temperature.	# cells (unitless)
count_log10_avg_se	Standard error of count_log10_avg.	# cells (unitless)
protein_avg	Average protein concentration per temperature.	mg protein per culture
protein_avg_se	Standard error of protein_avg.	mg protein per culture
protein_log10_avg	Average of log 10 transformed protein concentration per temperature.	mg protein per culture
protein_log10_avg_se	Standard error of protein_log10_avg.	mg protein per culture
rep	Replicate.	unitless
count	Total number of cells per culture.	# cells (unitless)
protein	Protein concentration measured in milligrams of protein per culture.	mg protein per culture
count_log10	Total cells per culture, log 10 transformed.	# cells (unitless)
protein_log10	Protein concentration, log 10 transformed.	mg protein per culture

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Deployments

lab_Harvell

Website	https://www.bco-dmo.org/deployment/58856
Platform	Cornell
Description	Harvell Lab at Cornell University. See more information on the lab's website.

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Project Information

Influence of Temperature and Acidification on the Dynamics of Coral Co-Infection and Resistance (Climate_CoralDisease)

Coverage: Florida Keys & Puerto Rico

This award is funded under the American Recovery and Reinvestment Act of 2009 (Public Law 111-5). Coral reef ecosystems are highly endangered by recent increases in temperature and by projected increases in ocean acidification. Although temperature has been identified as a driver of some coral disease outbreaks, nothing is known about direct effects of acidification on host immunity and pathogen virulence, or the potential for synergism with temperature. Natural coral populations often suffer from simultaneous infection by multiple pathogens that can also influence host immune responses, but co-infection dynamics have not been investigated in invertebrate systems lacking classical adaptive immunity. Changing climate will very likely influence the outcome of single and co-infection. This project will investigate the influence of environmental stress on co-infection dynamics of the sea fan coral, Gorgonia ventalina, with a fungal pathogen, Aspergillus sydowii and a protist parasite, SPX. The goal is to identify the mechanisms through which multiple infections, temperature and acidification modify host resistance, leading to changes in within- and among-colony rates of disease spread. The objectives of this project are to: (1) Identify incidence and co-infection frequency of Aspergillus sydowii and SPX. Detailed field surveys of the two diseases will test the hypothesis that co-infection is significant, provide valuable information about drivers of aspergillosis, and will help to characterize an emerging new sea fan disease. (2) Investigate how co-infection influences sea fan susceptibility, resistance, and within host disease dynamics. Through manipulative lab inoculation experiments we will test the hypothesis that single infections increase susceptibility to a second pathogen. (3) Examine the effects of temperature increase and ocean acidification on pathogen virulence, on underlying host resistance, and on the dynamics of single and co-infections. The hypotheses that acidification will increase pathogen virulence and host susceptibility will be tested in a temperature and pH controlled experimental system. This system will also allow the potential synergistic effects of temperature and acidification on host immunity and co-infection dynamics to be explored. The primary intellectual merit of the proposed work will be a greater understanding of how changing climate mediates co-infection and immunity in a non-model invertebrate. While fungal pathogens are primarily opportunistic, labyrinthulid protozoans are recognized as primary pathogens in shellfish. Even in shellfish, little is known about co-infections involving labyrinthulids, and these protists are entirely unstudied in corals. Publications associated with this project: Burge CA, Douglas N, Conti-Jerpe I, Weil E, Roberts S, Friedman CS & CD Harvell. (May 2012) Friend or foe: the association of Labyrinthulomycetes with the Caribbean sea fan, Gorgonia ventalina. Dis Aquat Org. 101:1-12. doi: 10.3354/dao02487 Burge CA, Mouchka, ME, Harvell, CD & S Roberts. (In review) Immune response of the Caribbean sea fan, Gorgonia ventalina exposed to an Aplanochytrium parasite as revealed by transcriptome

sequencing.

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Funding

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