Text S1. Methods

Brd Bacteria

Photobacterium angustum cultures were grown for 1.5 days in the dark with and without BrdU (800µM final concentration) in marine broth 2216. The bacteria were washed by centrifuging at 3,000 rpm for 10 min at 4C. The supernatant was removed without disturbing the bacterial pellet. 10 mL of sterile 1xPBS was used to resuspend pellets and they were centrifuged again. This was repeated three times and after the final wash bacterial pellets of the same treatment were resuspended in a total of 20 ml sterile 1xPBS and cell concentration determined by flow cytometry.

Immunoprecipitation

Prior to use with BrdU labeled DNA, the antiBrdU antibody (Sigma B8434; clone BU-33) was blocked using bacterial DNA. 125ng of *P. angustum* DNA per sample, in 50 µl 1xPBS, was denatured for 5 minutes at 95C. The tubes were transferred to an ice bath for 2 minutes, then the denatured bacterial DNA was mixed with 0.5 µl of the anti-BrdU antibody in 50 µl of 2xIP buffer (2xPBS, 0.1% TritonX-100) in a 1.5 ml microfuge tube and incubated at 4C on a rotating mixer for 30 minutes.

1-2 µg of each BrdU-labeled DNA was denatured in a total of 10 µl 1xPBS at 95C for 5 minutes, then placed on ice for 2 minutes. Each denatured BrdU DNA was combined with 100 µl of the blocked antibody mix and incubated on a rotating mixer at 4C for 45 minutes. 25 µl of DynabeadsTM ProteinG (Invitrogen) was added to the mixture and continued on the rotator at 4C for 30 minutes. To wash the beads, tubes were transferred to a magnet, then the solution removed and disposed of once it was clear. The beads were fully resuspended in 1 ml of cold IP Wash Buffer (1xPBS, 0.05% TritonX-100), then the solution allowed to clear on the magnet, followed by removal and disposal. This step was repeated two more times and then the beads were resuspended in 1 ml of cold TE pH 7.6. After the solution cleared on the magnet, it was removed and disposed. To elute the DNA, 100 µl of 65C elution buffer (TE, 0.5% SDS) was added to each sample and incubated at 65C for 10 minutes. The solution was cleared using a magnet and the liquid transferred to a new tube. Qiagen MinElute was used to concentrate the IP DNA, and was eluted from the column using 10 µl EB that had been heated to 65C.

Amplicon sequence

These are the basic qiime2 steps that were used to analyze our MiSeq data.

Demultiplex

qiime tools import --type 'SampleData[SequencesWithQuality]' --input-path <manifest file name> --output-path <name.qza> --input-format SingleEndFastqManifestPhred33V2

Denoise, dereplicate, quality filter & remove chimeras (also removes singletons)

qiime dada2 denoise-single --i-demultiplexed-seqs <.qza file from above> --p-trim-left 7 --p-trunc-len 200 --p-trunc-q 10 --p-chimera-method pooled --o-table <name.qza> --o-representative-sequences <name.qza> --o-denoising-stats <name.qza>

Merge table and repseq files

qiime feature-table merge --i-tables <list tables> --o-merged-table <new table name>
qiime feature-table merge-seqs --i-data <list of repset files> --o-merged-data <new file name>

Cluster sequences based upon identity

qiime vsearch cluster-features-de-novo --i-table <name.qza> (table created by dada2) --i-sequences <name.qza> (rep-seqs file created by dada2) --p-perc-identity 1.0 --o-clustered-table <name.qza> --o-clustered-sequences <name.qza>

Assign taxonomy

qiime feature-classifier classify-consensus-vsearch --i-query <name.qza> (this is the rep-set of sequences just created by cluster) --i-reference-reads silva132_99.qza --i-reference-taxonomy taxonomy_all_levels.qza --p-maxaccepts 1 --o-classification <name.qza>

Table S1. Active heterotroph/parasite ASVs identified by the same methods as the active mixotrophs.

	Menauhant						Childs River					
ASV	**6/8/19**	**6/21/19**	**7/5/29**	**7/20/19**	**8/3/19**	**8/16/19**	**6/8/19**	**6/21/19**	**7/5/19**	**7/20/19**	**8/3/19**	**8/16/19**
Jakobida	x	x	x	x	x	x	x	x		x		
Telonema	x			x		x	x		x		x	x
Pirum gemmata									x			
Pseudoperkinsus tapetis	x											
Lankesteria metandrocarpae				x								
Novel Apicomplexa Class 1		x						x	x	x		
Novel Apicomplexa Class 2			x									
Parauronema longum	x											
Askenasia sp. LWW2010032604	x											
Parauronema cf. virginianum								x				
Cryptocaryon uncultured marine eukaryote						x						
Favella sp. A type I							x					
Pelagostrobilidium uncultured ciliate	x						x					
Rimostrombidium veniliae							x					
Rimostrombidium uncultured alveolate	x					x	x	x				
Choreotrichia uncultured marine eukaryote				x								
Strombidium basimorphum							x					
Strombidium chlorophilum							x					
Strombidium uncultured alveolate						x						
Strombidium uncultured eukaryote						x						
Oligotrichia uncultured ciliate	x											
Amoebophrya sp. ex Gymnodinium instriatum		x										
Amoebophrya sp. ex Prorocentrum minimum				x					x	x		
Amoebophrya uncultured eukaryote					x							
Syndiniales Group I uncultured marine eukaryote	x	x	x				x	x			x	x
Syndiniales Group I uncultured marine picoplankton	x						x					
Syndiniales Group I uncultured marine alveolate						x						
Syndiniales Group II uncultured marine eukaryote	x		x	x	x	x	x					
Syndiniales Group II uncultured dinoflagellate				x								
Syndiniales Group II uncultured eukaryote										x		
Alveolata uncultured eukaryote	x	x	x	x	x	x	x	x	x	x	x	x
Uncultured Cercozoa	x											
Nudifila uncultured marine picoeukaryote	x											
Peregriniidae D2P04A09 uncultured Rhizaria	x											
Peregriniidae D2P04A09 uncultured marine eukaryote	x											
Peregriniidae unculturedmarine eukaryote				x								
Thaumatomastix sp. CC002-Boundary Bay	x											

Thaumatomastigidae sp. 1 JMS-2012							x					
Cercozoa Novel Clade 2 uncultured eukaryote								x				
Cercozoa Novel Clade 2 uncultured marine picoeukaryote	x											
Cercozo; Novel Clade 2 uncultured marine eukaryote				x								
Cryothecomonas uncultured Rhizaria						x						
Cryothecomonas uncultured eukaryote						x						
Cryothecomonas uncultured marine cercozoan	x											
Protaspis uncultured eukaryote;			x		x	x		x	x	x		x
Ebria uncultured eukaryote			x	x		x					x	x
Cercozoa sp. WHOI LI1-14							x					
Uncultured Cercozoa								x				
Mataza hastifera							x					
Thecofilosea uncultured Rhizaria					x							
Thecofilosea uncultured eukaryote				x	x				x			
Thecofilosea uncultured marine eukaryote	x					x					x	
Thecofilosea cultured Cercozoa			x	x	x	x			x		x	
Aplanochytrium uncultured eukaryote	x											
Aplanochytrium uncultured marine eukaryote							x					
Thraustochytriaceae uncultured eukaryote	x			x	x							
Thraustochytriaceae uncultured labyrinthulid	x		x	x	x	x	x	x	x	x	x	
MAST-12A uncultured eukaryote	x							x		x		
MAST-12B uncultured eukaryote								x				
MAST-12B uncultured phytoplankton				x					x			
MAST-12 uncultured marine eukaryote	x										x	
MAST-1C uncultured stramenopile	x	x		x		x		x			x	x
MAST-6 eukaryote marine clone ME1-24	x						x					
Paraphysomonas foraminifera							x					
Paraphysomonas imperforata							x					
Paraphysomonas uncultured marine eukaryote						x						
Ochromonadales uncultured marine eukaryote	x											
Chrysophyceae marine metagenome	x											
Spumella sp.	x						x					
Chrysophyceae uncultured marine stramenopile				x								
Peronosporomycetes uncultured Lagenidales	x			x			x		x			
Peronosporomycetes uncultured eukaryote						x				x		