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Rapid turnover of ciliate community members in New England tide pools

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ABSTRACT: The rocky intertidal zone represents a dynamic habitat marked by considerable species richness, which has been well-documented for invertebrates and macroalgae. This high biodiversity exists in the context of extreme fluctuations in abiotic factors such as temperature, salinity and pH that occur during each tidal cycle. Despite these attributes, few studies have focused on microbial diversity in tide pools, including analyses of the ciliate communities that are the focus of this study. We investigated the spatial and temporal distributions of ciliate species across the intertidal environment at sites in Maine and Connecticut, USA. Our study used a DNA fingerprinting technique, denaturing gradient gel electrophoresis (DGGE), which allows for genetic analyses of abundant community members. We investigated how ciliate diversity changed across several spatiotemporal scales: (1) between the open ocean and tide pools, (2) among different tide pools at varying distances from the low tide mark and (3) at differing times within a tidal cycle. In addition, we examined the differences between active and non-active members in these extreme environments by investigating diversity of both ribosomal DNA and RNA. In both Maine and Connecticut, we found abundant ciliate taxa that are either rare or absent in the open ocean, and that appear to quickly dominate tide pools once they are isolated from the open ocean. We also found that ciliate distributions within the tide pool community are complex and variable across spatial and temporal scales.

KEY WORDS: Microbial diversity · Ephemeral habitats · Biogeography · Denaturing gradient gel electrophoresis · Extreme environments

INTRODUCTION

The rocky intertidal zone, the marine area that extends between the points of the highest and lowest tide mark, is among the most dynamic environments for an organism to inhabit. Twice a day the zone is flooded and drained by the oncoming tide, and as the tide ebbs, it leaves behind pockets of water called tide pools. These pools are exposed to sun, wind and rain, which causes extreme fluctuations in temperature, salinity and pH (Ganning 1971, Morris & Taylor 1983, Huggett & Griffiths 1986). As the tide rises, water surges into the zone, bringing with it nutrients, planktonic organisms and any other organic material from the surrounding waters. For planktonic organisms living in the tide pools, these daily tidal cycles present ecological extremes: the connectivity to the open ocean provides a potential avenue of gene flow in a relatively constant environment, while the highly variable abiotic conditions of the isolated tide pools may select for traits such as the ability to encyst. Thus, studying the distribution of planktonic species across the intertidal environment compared to near-shore waters provides insight into patterns of biodiversity in overlapping yet extremely different habitats.

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Ciliates are microbes that play pivotal roles in food webs as both herbivores and predators, particularly in marine systems. However, their distribution in many ecosystems is largely understudied (Berk et al. 1977, Calbet et al. 2005, Chen et al. 2012). Previous studies of ciliate community composition in tide pools have been based largely on microscopy or targeted studies of particular taxa using molecular tools, and have revealed both wide-spread and endemic species (Jonsson 1994, Montagnes et al. 2002, Katz et al. 2005, Esteban & Finlay 2007, McManus et al. 2010). In a 30 d survey of ciliate species, Esteban & Finlay (2007) identified 210 morphospecies across 3 tide pools in the Isles of Scilly, UK, which represents 20% of the described global marine interstitial ciliate species at this time. Studies of *Strombidium oculatum* suggest that some ciliate morphospecies are both endemic to tide pools and marked by cryptic genetic diversity (Katz et al. 2005, McManus et al. 2010). *S. oculatum* has an internal rhythm that allows it to persist across tidal cycles by encysting and attaching to substrate before the tide pool is flooded by the oncoming tide (Jonsson 1994, Montagnes et al. 2002). To our knowledge, there have been no studies that have investigated the genetic diversity of ciliate communities in the intertidal environment.

Molecular 'fingerprinting' techniques, including denatured gradient gel electrophoresis (DGGE) used in this study, can elucidate spatial and temporal patterns of diversity. DGGE provides a snapshot of community composition by separating amplicons from environmental samples across a gel gradient (Díez et al. 2001, Jousset et al. 2010, Grattepanche et al. 2014, 2015, 2016a). We assessed ciliate communities in tide pools by investigating abundant species with DGGE, and we distinguished between quiescent and active cells by comparing SSU-rDNA and SSU-rRNA, as has been done in other protist studies (Poulsen et al. 1993, Debroas et al. 2015, Hu et al. 2016). We investigated ciliate species biogeography across 3 different scales: (1) between near-shore ocean and rocky intertidal environments; (2) between tide pools at varying distances from the low tide mark; and (3) at differing times within a tidal cycle. We investigated these parameters at 2 different sites: South Portland, Maine, and Avery Point, Connecticut.

MATERIALS AND METHODS

Sampling strategy

We collected samples from the open ocean (i.e. from surface waters just off shore) and tide pool environment at 2 different locations in the New England rocky intertidal: at South Portland (ME, USA) and at Avery Point, Groton (CT, USA) on 21 August 2015 and 31 August 2015, respectively. In South Portland, Maine we collected from 6 different tide pools that were distributed at varying distances from the low tide mark: 2 high-shore tide pools farthest from the low tide mark that were isolated for the longest period of time (ME_TP1 and ME_TP2), 2 mid-shore tide pools that were intermediate distance from the low tide mark (ME_TP 3 and ME_TP 4) and 2 low-shore tide pools that were isolated for the shortest amount of time from the ocean (ME_TP 5 and ME_TP 6; Fig. 1, see Table 1). We started collecting water samples when the upper tide pools were first isolated from the ocean and repeated collections about every hour over the duration of time the tide pools were isolated, i.e. we started sampling at 06:00 h, to the time just before they were recovered at 14:00 h. We also collected 2 open ocean samples from the near-shore waters at the beginning and end of our collection duration (i.e. 06:00 and 14:00 h). At Avery Point, Connecticut we collected water samples every hour from 2 high-shore tide pools (CT_TP1 and CT_TP2) starting when the tide pools were first isolated from the ocean at 13:00 h until low tide at 17:15 h. In addition, open ocean samples were also collected in adjacent near-shore waters at 13:00 h and again at 17:15 h (see Table 1).

Sampling protocol

On site, a sample of 500 ml of seawater was taken from the water column of each tide pool and the near-shore open ocean, and prescreened using an 80 µm nylon mesh to remove potential PCR inhibitors such as sediment from the tide pools and metazoans. The samples were then filtered using 2 µm polycarbonate filters to collect microbial community members. Filters were split in half, with one half stored in 1 ml of DNA prep buffer (100 mM NaCl, 10 mM Tris, 25 mM EDTA, 0.5% sodium dodecyl sulphate [SDS]) and stored at 4°C prior to DNA extraction. The other half was stored in 600 µl of RNA lysis buffer (Qiagen), which, upon returning to our lab, was vortexed and stored in a −80°C freezer. Environmental measurements were taken from the tide pools and near-shore waters at the beginning and end of the sampling periods. The environmental parameters measured included salinity measured with a refractometer, temperature using a bulb thermometer and pH using pH strips.

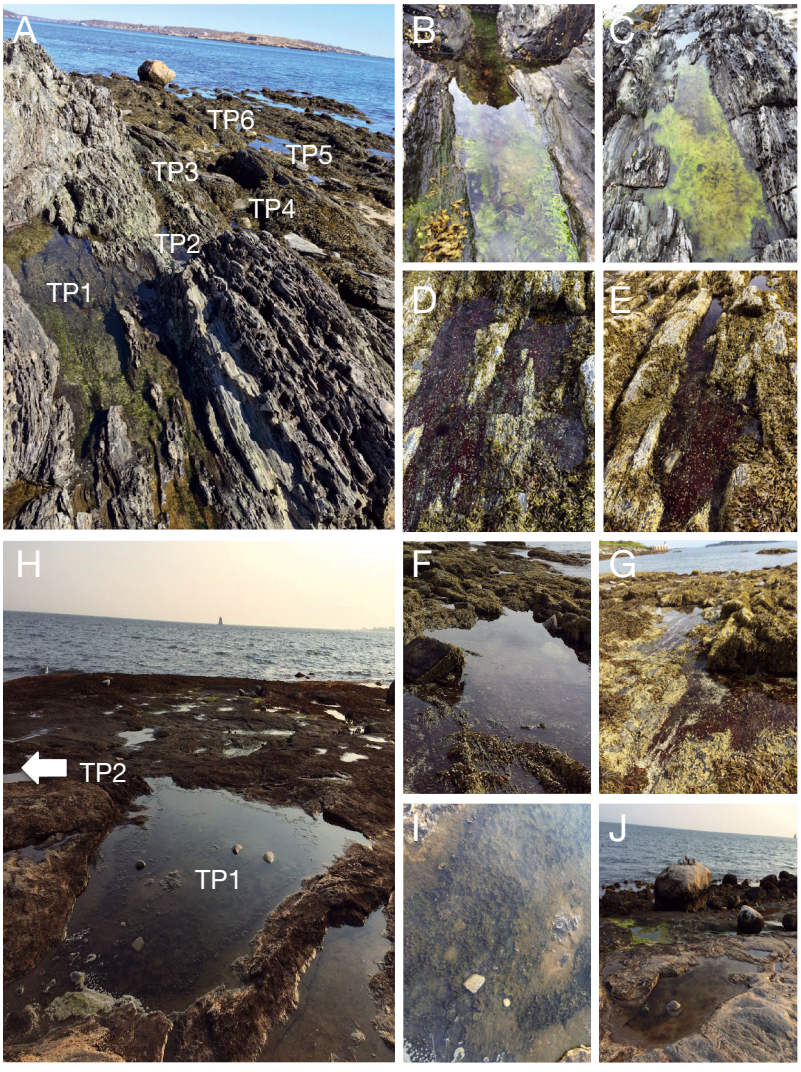

Fig. 1. Sampling sites in Maine and Connecticut. (A) All Maine tide pools exposed during low tide; tide pools sampled are indicated: (B) Maine tide pool 1 (ME_TP1), high-shore tide pool; (C) Maine tide pool 2 (ME_TP2), high-shore tide pool; (D) Maine tide pool 3 (ME_TP3), mid-shore tide pool; (E) Maine tide pool 4 (ME_TP4), mid-shore tide pool; (F) Maine tide pool 5 (ME_TP5), low-shore tide pool; (G) Maine tide pool 6 (ME_TP6), low-shore tide pool. (H) Connecticut tide pools exposed during low tide; tide pools sampled are indicated (CT_TP2 not pictured): (I) Connecticut tide pool one (CT_TP1); (J) Connecticut tide pool 2 (CT_TP2)

DNA and RNA extraction and processing

To enable characterization of both active and total community members, we isolated DNA and RNA from our communities. Genomic DNA was extracted using the ZR Soil Microbe DNA MiniPrep™ kit according to the instructions of the manufacturer (Zymo Research). Extracted genomic DNA was eluted using 100 µl of 10 mM Tris pH 8.0 and stored at −20°C until amplification. Total RNA was extracted using Qiagen RNeasy Mini Prep Kit and then purified of contaminant genomic DNA using the Ambion® TURBO DNA-free™ DNase Treatment and Removal kit (Life Technologies). The purified RNA was then translated into a single strand cDNA using the SuperScript III Cells-Direct cDNA Synthesis Kit (Invitrogen) with random hexamers (Thermo Fisher Scientific) and stored at −20°C until amplification.

DNA and cDNA amplification

We amplified DNA and cDNA samples from each tide pool using Q5 polymerase (NEB). The 20 µl PCR master mix consisted of Q5 buffer (NEB), 50 mM BSA, 50 µM of each dNTPs, 0.25 pM of each primer, and 1 unit of Q5 polymerase. We choose to use Spirotrichea-specific primers (Doherty et al. 2007) to target a region of the small subunit ribosomal RNA/DNA locus, though some non-target taxa were also amplified (e.g. a dinoflagellate sequence also reported in Grattepanche et al. 2014). We diluted genomic DNAs/cDNAs using 10 mM Tris pH 8.0 and amplified samples under the following cycling conditions: a hot start of 98°C for 1:30 min, 34 cycles of a denaturing temperature of 98°C for 15 s, an annealing temperature of 59°C for 15 s, and an extension temperature of 72°C for 30 s. This was followed by a final extension temperature of 72°C for 2 min.

DGGE analysis

In total, we pooled 5 replicates of PCR products generated using the Spirotrichea-specific primers with an additional GC clamp, an additional 39mer of G and C nucleotides that prevents strands from seperating during denaturation, attached to the reverse primer (Tamura et al. 2011). A total of 9 DGGE gels were produced for this study (see Figs. S1 & S2 in the Supplement at www.int-res.com/articles/

suppl/a080p043_supp.pdf). Each experiment also included oceanic samples taken at the beginning and end of sampling to allow comparisons of the community composition detected in the tide pools with those detected in the near-shore environments, and for comparison across DGGE gels. We also performed DGGE gel analysis for replicate samples amplified on different days to demonstrate the robustness of our methods (see Fig. S3). DGGE gel setup used the Dcode Universal Mutation system (Bio-Rad). Gels were composed of 6% acrylamide and had gradients from 35 to 55% (100% denaturant is composed of 7 M Urea and 40% deionized formamide). The gels were run at 245 V for 5 min and then incubated for at least 15 h at 45 V. After incubation, gels were stained for 30 min using 200 ml Tris-acetate-EDTA (TAE) buffer and 20 µl of SYBR gold (Invitrogen). After staining, the gels were photographed using Kodak molecular imaging software (Carestream Health).

Taxonomic assignment of operational taxonomic units

To identify specific taxa, we excised and sequenced bands from each DGGE gel. Excised bands were placed in 25 µl of 10 mM Tris pH 8.0 and incubated overnight at 4°C. The resulting elution was diluted at 1:100 using 10 mM Tris pH 8.0 and amplified under the previous cycling conditions for 30 cycles, using non-GC clamp versions of the primers (152+/528–; Tamura et al. 2011). Resulting PCR products were cleaned using the ExoSAP-IT® PCR product cleanup (Affymetrix) in duplicate reactions following the manufacturer's protocol. The cleaned PCR products were sequenced using BigDye® Terminator v.3.1 Cycle Sequencing Kit (Life Technologies Corporation) and Sanger sequencing at Smith College Center for Molecular Biology and at the Rhode Island Genomics and Sequencing Center. Sequences were aligned using SeqMan v.12.0 (DNASTAR) and first grouped at 80% similarity contigs to enable assessment of quality. Sequences that were too short or of too poor quality were discarded. Remaining sequences were then reassembled at 100% similarity in operational taxonomic unit (OTU). Under the conservative assumption that evolution had not occurred, discrepancies between the remaining sequences were examined by eye and ambiguities were resolved when possible. The OTU sequences were deposited in GenBank under accession numbers MF001091-MF001120.

Two approaches were used to assign taxonomy: a Basic Local Alignment Search Tool (BLAST) approach and a gene tree approach. We used BLAST against GenBank database in order to provide taxonomic identity for each OTU, and we report the top hit along with percent coverage and identity. For the phylogenetic approach, we used Mafft E (Katoh & Standley 2013) to align our sequences and morphospecies references from GenBank. We built phylogenetic trees using RAxML v.8.2.4 with the nucleotide substitution model GTR with the distribution rate gamma and proportion of invariable sites, as previously identified with jModelTest v.2.0 under Akaike's information criterion (Darriba et al. 2012).

Statistical analysis

To assess community patterns, sample clustering analyses were performed using Fast Unifrac dissimilarity matrices (Hamady et al. 2010) (unweighted Unifrac metric: the difference between samples based on sequence composition and a gene tree) and Raup-Crick dissimilarity (index based on presence/absence data) from 'phyloseq' v.1.16.2 and 'vegan' v.2.4.1 packages in R v.3.2.3 (R Core Team 2016) and principal coordinates analysis (PCoA). We removed non-ciliate sequences before statistical analysis.

RESULTS

Maine tide pool abiotic factors

The abiotic factors of salinity, pH and temperature increased in all Maine tide pools over the time the pools were isolated from the open ocean, while the abiotic measurements in the open ocean remained constant (Table 1). We observed the greatest increase in salinity, temperature and pH in the uppermost tide pools, Maine tide pool 1 (ME_TP1) and Maine tide pool 2 (ME_TP2), with salinity, temperature and pH ranging from 32 to 37 ppt, 20 to 29°C, and 7 to 9 (Table 1). The abiotic parameters in the lower tide pools, ME_TP5 and ME_TP6, remained more constant and similar to the open ocean environmental parameters, ranging from 32 ppt salinity, 21 to 27°C and 7 to 8 pH (Table 1). The intermediate tide pools, ME_TP3 and ME_TP4, had intermediate values ranging from 32 to 34 ppt, 21 to 26°C and 7 to 8, for salinity, temperature and pH, respectively (Table 1).

Table 1. Tide pools in Maine (ME) and Connecticut (CT) and associated abiotic parameters. Approximate size was estimated just after isolation, and approximate depth was estimated maximum depth. Salinity, pH and temperature ranges are reported from hourly measurements taken after isolation of pool

Location	Lat/long	Size (m)	Depth (cm)	Salinity (ppt)	pH	Temp (°C)
ME: open ocean	NA	NA	NA	32–32	7.5	20–21
ME_TP1	43° 38.785′ N 70° 13.592′ W	1.25 × 0.75	15	32–36	7–9	21–31
ME_TP2	43° 38.785′ N 70° 13.593′ W	1.25 × 0.75	15	33–37	7–9	20–29
ME_TP3	43° 38.795′ N 70° 13.353′ W	1.25 × 1.0	5	32–33	7–8	22–26
ME_TP4	43° 38.795′ N 70° 13.354′ W	1.75 × 1.25	5	32–34	7–8	21–24
ME_TP5	43° 38.785′ N 70° 13.575′ W	2.25 × 2.0	10	32–32	7–8	21–24
ME_TP6	43° 38.789′ N 70° 13.573′ W	2.0 × 2.0	10	32–32	7	22–27
CT: open ocean	NA	NA	NA	32–32	8	28–26
CT_TP1	41° 18.913′ N 72° 63.361′ W	3.5 × 1.5	8	30–39	8–9	30–31
CT_TP2	41° 18.913′ N 72° 63.861′ W	3 × 1	10	30–26	8–9	31–32

Maine tide pool ciliate diversity based on SSU-rDNA

Once isolated, the ciliate community in the Maine tide pools quickly differed from the community sampled in the open ocean in composition and abundance, in particular for the high-shore pools (ME_TP1 and ME_TP2; Fig. 2, Table 2). In total, we detected 17 OTUs at the Maine site, 13 of which were most closely related to ciliate species (Table 2). We found the presence of a tide pool specific ciliate, OTU1, abundant in all Maine tide pools across almost all sampling times, and that appeared in our first samples once pools were isolated from the open ocean (Fig. 2, Table 2). The corresponding DGGE band was not present in the open ocean samples but was present in 28 of the 32 Maine tide pool DNA samples (see Table S1 in the Supplement at www.int-res.com/articles/suppl/a080p043_supp.pdf). We excised and sequenced this band 9 times from different samples and DGGE gels, which revealed that it is most closely related to *Epiclintes auricularis* (FJ008721, 99% ID, 99% coverage; Tables 2 & S1). In a few cases our primers amplified non-target (i.e. non-Spirotrichea) taxa (e.g. OTU13, OTU23, OTU24) (Table 2).

Additionally, we found that the overall tide pool ciliate community consisted of 11 OTUs (OTUs 2 to 12) that were also abundant in the open ocean (Fig. 2, Tables 2 & S1). These shared OTUs had heterogeneous patterns of abundance across the tide pools evidenced by complex patterns of presence/absence on DGGE gels (Figs. 3 & S1, Table 2). For example, OTU2, closely related to *Strombidinopsis batos* (FJ881862, 97% ID, 98% coverage), was abundant in open ocean samples and only within a subset of tide pool samples (e.g. ME_TP1 time 14:00 h, ME_TP2 time 06:00, 08:00, 09:00 and 10:15 h; Fig. 2, Table S1).

Tide pools separated from the open ocean for the longest amount of time (ME_TP1 and ME_TP 2) had the fewest 'shared' OTUs (OTUs present both in the open ocean and in the tide pools) maintained across sampling times (Fig. 3, Tables S1 & S2). Principal coordinate analyses (PCoAs) revealed that the majority of sampling times from ME_TP1 as well as later sampling times from ME_TP2 and ME_TP3 were distant from a cluster of other Maine tide pools (Figs. 4 & S4). The distinction of the ME_TP1 samples and some of the ME_TP2 and ME_TP3 samples from the other Maine tide pools reflects a rapid reduction in OTUs over time, which was not detected in the tide pools closer to the open ocean (Tables S1 & S2). At our first sampling time, when ME_TP1 became isolated from the open ocean, ME_TP1 had lost the bulk of its diversity while the ciliate community in ME_TP2 reduced more slowly over time (Fig. 2, Tables S1 & S2). These results are in contrast to the 4 lower tide pools (ME_TP3, ME_TP4, ME_TP5 and ME_TP6) that had overall higher levels of OTU richness across time (Fig. 2, Tables S1 & S2).

Maine tide pool ciliate diversity based on SSU-rRNA

Like the abundant community members (OTUs detected in the SSU-rDNA), the active community members (OTUs detected in the SSU-rRNA) had complex presence/absence patterns across the Maine intertidal environment. Our study detected a total of 19 active OTUs at the Maine sampling site

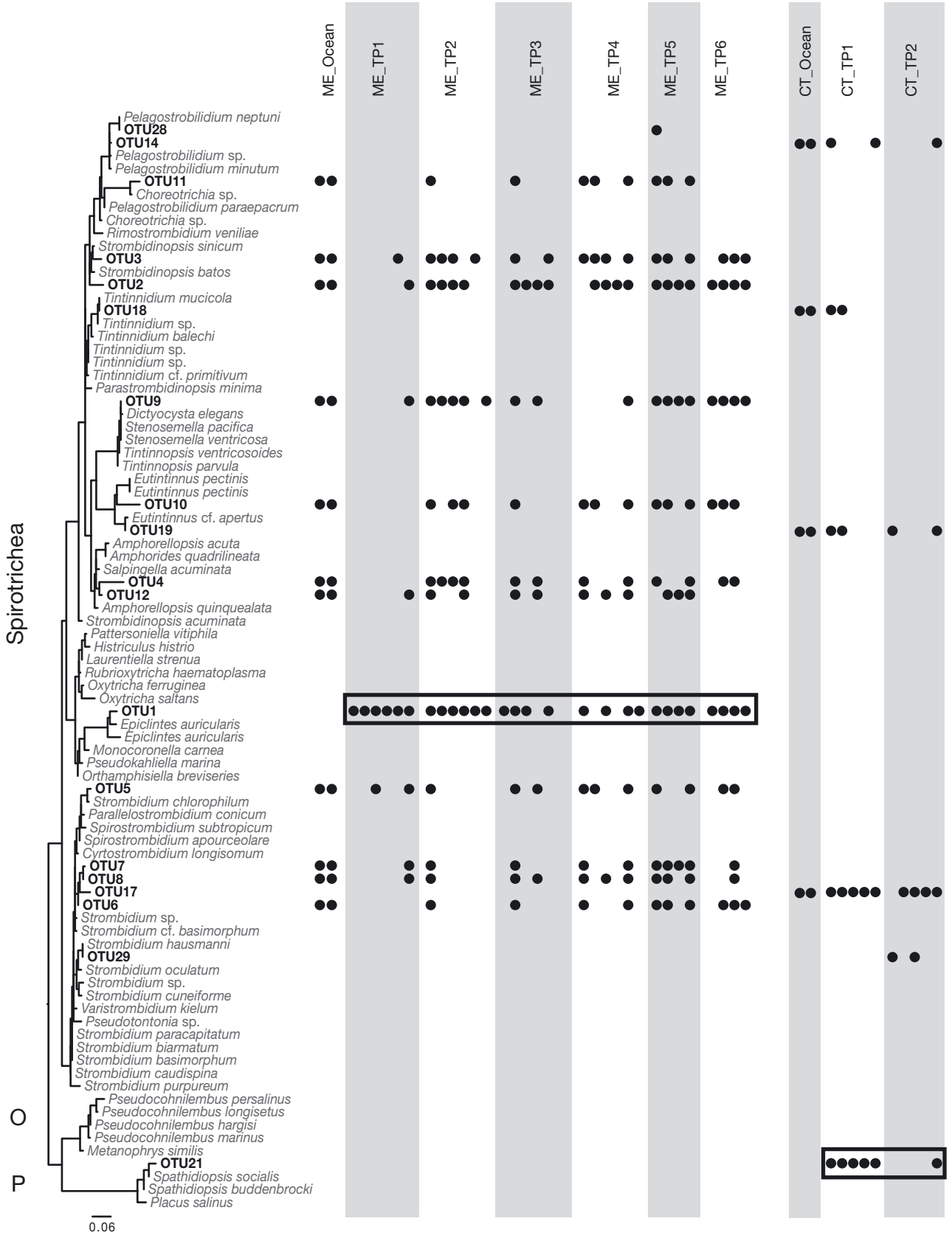

Fig. 2. Tide pool specific ciliates (boxed) appear in both Maine (OTU1) and Connecticut (OTU21), and are distinct from open ocean lineages. The gene tree was constructed from sequenced denaturing gradient gel electrophoresis (DGGE) bands and morphospecies available in GenBank. Black circles indicate presence of OTUs in specific samples. 'O' and 'P' represent the classes Oligohymenophorea and Prostomatea. The scale bar represents the number of differences per base pair

Table 2. Distribution and identification of ciliate taxa (operational taxonomic units, OTUs) sampled in Maine (ME) and Connecticut (CT) tide pools. The numbers (1 to 5) and letter (O) in columns represent tide pool and open ocean OTUs, respectively. **Bold** numbers and letters signify that the OTU was found more than once in the sample, and superscript [a] indicates that the OTU was found across all sampling times. Superscript [b] next to species name represents non-ciliate taxa detected using our primers. For further details see Tables S1 & S2 in the Supplement at www.int-res.com/articles/suppl/a080p043_supp.pdf

OTU	ME DNA	ME RNA	CT DNA	CT RNA	Best BLAST hit	ID (%)	Coverage (%)	GB No.
OTU1	**1[a],2[a],3,4,5[a],6[a]**	**1[a],2[a],3[a],4,5[a],6[a]**			*Epiclintes auricularis*	99	99	FJ008721
OTU2	**O[a]**,1,**2,3,4,5[a],6[a]**	**O[a],1,2,3[a],4,5,6**			*Strombidinopsis batos*	97	98	FJ881862
OTU3	**O[a]**,1,**2,3,4,5[a],6**	**O,1,2,3[a],4,5[a],6[a]**			*Strombidinopsis batos*	99	100	FJ881862
OTU4	**O[a],1,2,3,4,5[a],6**	**O,1,2[a],3,**4,**5,6**			*Tintinnidium* sp.	94	100	JN831803
OTU5	**O[a]**,1,2,**3,4,5,6**	1,2,3,4,**5[a],6**			*Strombidium chlorophilum*	99	100	KM084726
OTU6	**O[a]**,1,2,**3,4,5,6**	**1,**2,3,4,**5,6**			*Cyrtostrombidium longisomum*	99	100	KJ609053
OTU7	**O**,1,2,3,**4,5[a]**,6	**O[a],1,5,6**			*Strombidium caudispina*	99	100	KP260513
OTU8	**O[a]**,1,2,**3,4,5,**6	2,**3**,4,**5[a]**			*Strombidium paracapitatum*	99	100	KP260511
OTU9	**O[a]**,1,**2,3**,4,**5[a],6[a]**	**O[a],1,2,5[a],6[a]**			*Stenosemella ventricosa*	100	100	KU715764
OTU10	**O[a],2**,3,**4,5,6**	**1[a],5,6**			*Eutintinnus pectinis*	95	98	AF399170
OTU11	**O[a]**,2,3,**4,5**	5			*Choreotrichia* sp.	99	98	LN870020
OTU12	**O**,1,**2,3,4,5**	1,2,3,**4,5**,6			*Salpingella acuminata*	99	98	EU399536
OTU13	**1,2**	2			*Bysmatrum subsalsum*[b]	88	95	HQ845326
OTU14			**O[a],1**,2	**O[a],1**,2	*Pelagostrobilidium* sp.	99	99	JQ781699
OTU15			**O[a]**,1	**O[a]**,1,2	*Gymnodinium* sp.[b]	99	100	AF274260
OTU16			**O[a],1[a],2**	**O[a],1[a],2[a]**	*Lepidodinium viride*[b]	99	100	JF791033
OTU17			**O[a],1[a],2**	**O[a],1[a],2[a]**	*Spirostrombidium apourceolare*	97	100	KU525746
OTU18			**O[a],1**	**O[a],1,2[a]**	*Tintinnidium mucicola*	100	100	KU715767
OTU19			**O[a],1,2**	**O[a],1[a],2[a]**	*Eutintinnus cf. apertus*	99	100	KU715759
OTU20		O			*Oxytricha saltans*	99	99	AF370028
OTU21			**1[a]**,2	**1**	*Spathidiopsis socialis*	100	99	HM051055
OTU22		2,4,5,6			*Tintinnopsis parvula*	100	100	KU715771
OTU23	1,5				*Aureoumbra lagunensis*[b]	98	100	HQ710574
OTU24	**1**				*Dinophyceae* sp.[b]	96	100	AY251288
OTU25		1			*Pseudocohnilembus marinus*	99	100	Z22880
OTU26		1			*Astrosphaera hexagonalis*[b]	99	100	AB490706
OTU27		1			*Lepidodinium* sp.[b]	95	99	KU156670
OTU28	5				*Pelagostrobilidium neptuni*	100	100	AY541683
OTU29			**2**		*Strombidium hausmanni*	99	100	KJ609049
OTU30		**1,4**			*Pseudotontonia* sp.	99	100	JX178819

(Table 2). Of the 19 active OTUs, 15 were also abundant taxa (thus present in both the SSU-rDNA and the SSU-rRNA). In the initial open ocean sample, 11 abundant taxa were detected (OTUs 2 to 12) but only 3 of those were active (detected in SSU-rRNA) (OTUs 2, 7 and 9; Tables 2 & S1). We also observed a shift within tide pools as activity patterns changed over time (e.g. OTU 2 to 13; Table S1). The majority of

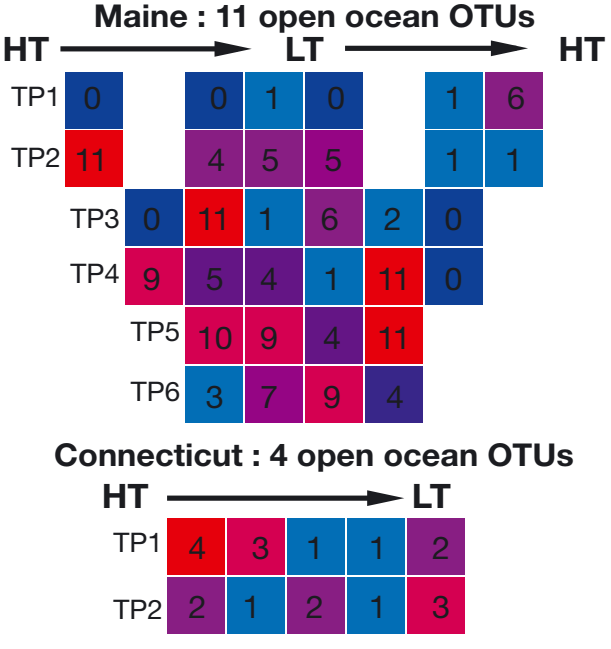

Fig. 3. Heterogeneous occurrence of open ocean operational taxonomic units (OTUs) in the tide pools during their isolation period shows the dynamism of the tide pool environment. Numbers in squares represent open ocean OTUs present in each tide pool at each sampling time. Colors correspond to number of shared OTUs: warmer colors correspond to higher numbers and cooler numbers correspond to low numbers. South Portland, Maine, high tide (HT) occurred at 03:57 h, low tide (LT) occurred at 10:01 h and high tide occurred again at 14:18 h on 21 August 2015. At Avery Point, Connecticut, HT occurred at 10:48 h and LT occurred at 17:25 h on 31 July 2015. For more details see Table 1

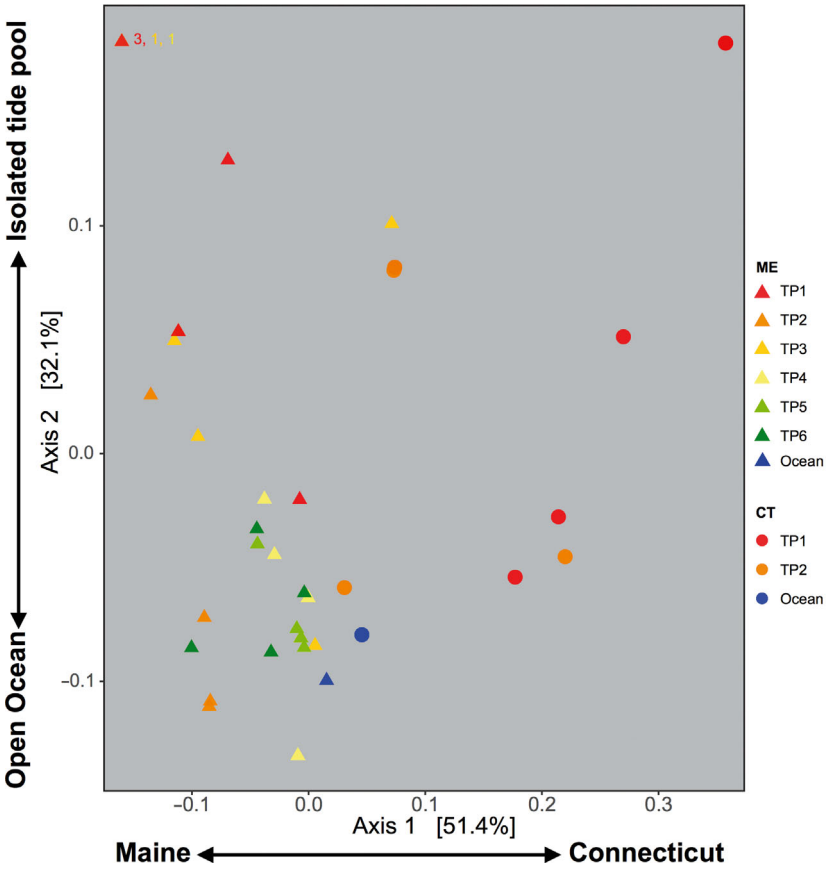

Fig. 4. Principal coordinate analyses using unweighted Unifrac metric reveal clusters (1) by locations (left: Maine; right: Connecticut), and (2) time of isolation from the open ocean (bottom: connected; top: isolated for a long duration). We labeled only the uppermost overlapping points with the numbers to the right indicating how many points this triangle represents. All other overlapping points are provided in Fig. S4 in the Supplement at www.int-res.com/articles/suppl/a080p043_supp.pdf

active community members in the Maine tide pools were also abundant (i.e. OTUs 2 to 13), although the patterns of each varied temporally (e.g. OTU2 in ME_TP2, abundant: 06:00, 08:00, 09:00 and 10:15 h; active: 06:00 08:00, 09:00, 12:45 and 14:00 h). One OTU had very similar activity and abundance patterns; OTU1, only observed in tide pools, was both abundant and active across all tide pools over multiple sampling times (Table S1). Lastly, there were a few taxa that were active for short periods in the tide pools but were not abundant (OTUs 20, 22, 25, 26, 27 and 30; Table 2).

Connecticut tide pool abiotic factors

The environmental parameters of the 2 tide pools sampled in Connecticut changed substantially over the time the pools were isolated from the ocean, as observed with the Maine tide pools. Following isolation from the ocean, salinity, temperature and pH fluctuated from 30 to 39 ppt, 30 to 31°C and 8 to 9 in CT_TP1, and from 30 to 26 ppt, 31 to 32°C and 8 to 9 in CT_TP2, while temperature, salinity and pH remained relatively constant at 32 ppt, 28 to 26°C and 8 in the open ocean (Table 1).

Connecticut tide pool ciliate diversity based on SSU-rDNA

While we sampled only 2 tide pools in Connecticut compared to 6 in Maine, the diversity patterns in the Connecticut tide pools were quite distinct from the Maine tide pools (Table 2, Fig. 2). Like in Maine, in a few cases our primers amplified non-target (i.e. non-Spirotrichea) taxa (i.e. OTUs 15 and 16) (Table 2). Of the 8 common OTUs sequenced from the Connecticut tide pools, none were shared with the Maine tide pools or oceanic samples (Table 2). However, principal coordinate analysis using Unifrac dissimilarity matrix (which accounts for phylogenetic relationships) revealed that the open ocean samples in Maine and Connecticut were composed of phylogenetically similar taxa (Figs. 4, S4 & S5).

We found the presence of a tide pool specific ciliate in Connecticut, OTU21. This tide pool specific species is closely related to the morphospecies *Spathidiopsis socialis* (HM051055, 100% ID, 99% coverage). The OTU was present in all the sampling times at CT_TP1 and present in only one of the sampling times at CT_TP2 (Fig. 2, Table S2). Our phylogenetic tree revealed that OTU21 is distant from the tide pool specific OTU1 found in Maine (Fig. 2). In fact, this OTU is an example of a non-target taxon as it belongs to the class Prostomatea rather than Spirotrichea (Figs. 2 & S5). CT_TP1 was distinct from the open ocean in the principal coordinate analysis, while CT_TP2 was only distinct at the end of the sampling period when OTU21 appeared (Fig. 4, Tables S1 & S2).

OTUs shared between the open ocean and tide pool environment were less frequent in the Connecticut tide pools than in the Maine tide pool environment (Fig. 2; OTUs 14 to 19; Fig. 3, Table S2). We found that the shared taxa (OTUs 14 to 19) were heterogeneously distributed throughout the 2 tide pool sampling times, evidenced by complex patterns of presence/absence on DGGE gels (Figs. 2 & S2, Table 2). For example, OTU14, closely related to *Pelagostrobilidium* sp. (JQ781699, 99%ID, 99% coverage) was abundant (present in the SSU-rDNA) in both open ocean samples in Connecticut and was found 3 out of the 10 times in the sampled Connecticut tide pools (e.g. CT_TP1 times 13:00 and 17:15 h, CT_TP2 time 15:00 h; Fig. 2, Table S2).

Connecticut tide pool ciliate diversity based on SSU-rRNA

All active Connecticut tide pool community members (detected in the SSU-rRNA) were also abundant community members (detected in the SSU-rDNA; OTUs 14 to 19 and 21; Table 2). A total of 7 OTUs were active and abundant in Connecticut tide pools, with an additional abundant but not active community member. However, like the Maine site, the active and abundant communities differed from each other within tide pools (e.g. OTU 14 abundant: 13:00 and 17:15 h, active 17:15 h). Active OTUs also differed in the open ocean, as only 4 (OTUs 14, 17, 18 and 19) out of 6 (OTUs 14 to 19) of the abundant taxa were also active (Table 2). The ciliate present only in the tide pools (OTU21) was active at the majority of the sampling times in CT_TP1 (Table S2; times: 13:00, 14:00, 17:15 h). In CT_TP2, only 2 open ocean taxa (OTU17 and OTU19) were active at multiple sampling times (Tables 2 & S2).

DISCUSSION

The key findings of our study of ciliate communities in the tidal environment were (1) the presence of tide pool specific species in Maine and Connecticut that were active and dominant almost immediately after tide pools became isolated from the ocean, and (2) the heterogeneous presence/absence and active/dormant patterns of taxa shared between the open ocean and tide pool suggest a complex relationship between abundance and changing abiotic and biotic factors within the pools.

Abundant tide pool specific OTUs

Most notable among the findings of this study is the rapid appearance of 2 tide pool specific species, OTU1 in Maine and OTU21 in Connecticut. The Maine taxon OTU1 is closely related to *Epiclintes auricularis*, a member of the Subclass Stichotrichia (Class Spirotrichea); this lineage quickly dominated in our high-shore tide pools even though it was not detected in our open ocean samples (Table 2, Fig. 2). In fact, this OTU has never been detected in samples along the coast of New England that were analyzed using the same primers in clone libraries (Doherty et al. 2007, 2010a,b) or more recently using high throughput sequence analyses (Santoferrara et al. 2014, 2016, Grattepanche et al. 2016a,b). The absence of this OTU in studies of near-shore water and marine sediment using the same primers suggests that OTU1 is indeed specialized for tide pool environments. The ecological role of *E. auricularis*, the closest match to OTU1 on Genbank, is unknown as previous work on this species has focused primarily on morphological characterization (Carey & Tatchell 1983, Song & Warren 1996, Wicklow & Borror 1990). The most recent of these studies reports finding specimens in the Yellow Sea, China (e.g. Hu et al. 2009a,b).

Similarly, the Connecticut tide pool specific taxon OTU21 is most closely related to the morphospecies *Spathidiopsis socialis* (Class Prostomatea; Table 2) and was also absent in previous studies using the same primers (Doherty et al. 2007, 2010a, Tamura et al. 2011, McManus et al. 2011, Grattepanche et al. 2014, 2015, 2016b, Santoferrara et al. 2014), including one that focused on benthic ciliates (Doherty et al. 2010b). The best match for this OTU, *S. socialis*, has been isolated from both brackish and freshwater benthic environments from the east coast of the United States (Lipscomb et al. 2012, Lipscomb & Riordan 2012, Azovsky & Mazei 2013). Here, both morphology-based studies of *Spathidiopsis* and our DGGE analyses suggest this taxon may be specialized for environments of low or fluctuating salinities, such as tide pool and estuarine environments, rather than the open ocean. Notably, our sampling sites in Avery Point, Connecticut where OTU21 was found are relatively isolated from rivers or freshwater input.

Resident tide pool ciliate morphospecies have been argued to persist despite daily flushing by high tide due to specific morphological and/or behavioral characteristics (Jonsson 1994, Montagnes et al. 2002, Esteban & Finlay 2007). Esteban & Finlay (2007) found that tide pool morphospecies have oblong, flattened morphologies that may be more resistant to

dislocation by waves than rounder, planktonic species. Furthermore, the movement of benthic ciliate species into the water column may be influenced by fluctuations in environmental factors such as oxygen availability (Berninger & Epstein 1995). Thus, within tide pools, benthic species may suspend themselves in the water column in order to cope with changing environmental factors. In studies of *Strombidium oculatum*, both Jonsson (1994) and Montagnes et al. (2002) described the species' ability to become dormant by attaching itself to the substrate of tide pools as cysts during the high tide. The closest hits to tide pool specific ciliates in Maine and Connecticut, *E. auricularis* and *Spathidiopsis* sp., respectively, are both oblong taxa; yet, the ability of these specific lineages to encyst must await future studies.

The rate at which the tide pool specific species OTU1 and OTU21 became abundant (i.e. observable in DGGE) in the tide pools is striking (Fig. 2) and consistent with a life strategy adapted for tide pools. Morphological studies of the tide pool species *S. oculatum* indicate a life history that allows this taxon to quickly dominate the tidal environment: before encysting, *S. oculatum* begins asexual division by doubling its genome content and swelling to twice its normal size so that it can divide and begin feeding quickly when it excysts in isolated tide pool (Montagnes et al. 2002). In our study, the rapid appearance of OTU1 and reduction in open ocean ciliate community members in Maine coincides with extreme fluctuations in abiotic conditions, suggesting plasticity may also be a key component in the life cycle of OTU1 (Tables 1, 2, & S1).

Complex patterns of presence/absence of open ocean taxa within tide pools

The majority of the OTU diversity was shared between the open ocean and tide pool environment, but the abundance and activity of these taxa varied considerably both between open ocean and tide pools, and among individual pools (Fig. 3, Tables S1 & S2). Changes in environment can cause rapid reorganization of community composition of ciliate species in the marine environment (e.g. Gertler et al. 2010, Guizien et al. 2014, Lewandowska et al. 2014). For example, a molecular study investigating the effect of the grazing capabilities of protist communities in the presence of crude oil reported a succession in common ciliate community members as environmental parameters fluctuated (Gertler et al. 2010). In our study, the communities in individual tide pools in both Maine and Connecticut had fewer present/abundant OTUs (i.e. present in the SSU-rDNA) than the initial open ocean community (Figs. 2 & 3, Table 2). In addition, these shared OTUs were heterogeneously dispersed over the duration of a tide pool's isolation (Tables 2, S1 & S2), which suggests that the ephemeral nature of the tide pool environment rapidly reorganized the community structure within each pool. Interestingly, the active community (i.e. in SSU-rRNA) in the intertidal environment varied from the active community in the open ocean (Table 2), again demonstrating that the tidal environment supports a different community than the open ocean.

The active community members (present in SSU-rRNA) detected in the tide pools in both Maine and Connecticut generally showed similar presence/absence patterns as the abundant (present in the SSU-rDNA) community members were a subset of open ocean taxa (Tables 2, S1 & S2). The Maine tide pool specific taxon (OTU1) dominated both the active and abundant community across all sampling times (Table 2). The OTUs shared between the open ocean and the tide pools had heterogeneous patterns of abundance and activity. The rapid reorganization of the active ciliate communities in the tide pools suggests that species are encysting and/or dying in response to the rapidly changing tidal environment (Kamiyama & Aizawa 1992, Kamiyama 1994, Kim & Taniguchi 1997, Montagnes et al. 2002, Hu et al. 2016, Debroas et al. 2015). In a few cases, OTUs appeared to be active (i.e. abundant in rRNA) in Maine tide pools even though we did not detect them in DNA (OTUs 25 to 27; Table 2). Perhaps these taxa represent active rare lineages that can respond rapidly to environment change (Hu et al. 2016, Debroas et al. 2015).

In Maine, where we sampled 6 tide pools at 3 heights (Table 1), we found patterns of diversity that varied with distance from low tide mark. There was a greater loss of open ocean OTUs in the upper Maine tide pools (ME_TP1 and ME_TP2) than in the lower Maine tide pools (ME_TP5 and ME_TP6) (Fig. 2, Table S1), though the tide pool specific lineage, OTU1, appeared in all tide pools (Fig. 2, Table S1). In the upper Maine tide pools, the community composition was quickly dominated by the tide pool specific taxon upon isolation from the open ocean (ME_TP1) or within 2 h of isolation (ME_TP2; Fig. 2, Table S1). In contrast, the lower pools in Maine (ME_TP5 and ME_TP6) showed greater heterogeneity in turnover patterns of open ocean taxa (Table 2). This fast turnover of community composition in the upper Maine tide pools coincided with the largest changes in

abiotic conditions (Fig. 2, Table 1). The zonation pattern observed in this study may be a result of both environmental and biotic stress. Environmental stress gradients across the intertidal zone can cause a reduction in species richness in tide pools farther away from the open ocean in planktonic and sessile species (e.g. Kooistra et al. 1989, Scrosati & Heaven 2007, Valdivia et al. 2014). However, species that are able to withstand variable environmental conditions are able to dominate the upper tidal environment. For example, the copepod *Tigriopus californicus* demonstrates unique adaptations including a carotene pigment, which allows the species to deal with the high heat and high salinity environment of the upper tide pools (Dethier 1980, Kelly et al. 2013, McAllen & Brennan 2009). This suggests that in-depth analyses of life history traits in the tide pool specific ciliates characterized here will yield evidence of similar adaptations to enable persistence in extreme environments.

CONCLUSIONS

Our study revealed evidence of resident tide pool ciliates in New England (OTU1 and OTU21; Fig. 2) that are not supplied by the resurgence of tidal water. These lineages appear and dominate immediately upon isolation of the tide pools from the open ocean (Fig. 2, Table S1), and we speculate that they encyst in tide pools before high tide. In contrast, open ocean lineages showed heterogeneous fates across tide pools, suggesting the changing environment impacts their abundance in a complex and perhaps stochastic manner. In the face of changing climates, exploring the dynamics of species that are potentially better adapted to extreme fluctuations in abiotic conditions is crucial for understanding and monitoring the impacts of a changing climate on local biodiversity and ecosystem functioning.

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LITERATURE CITED

Azovsky A, Mazei Y (2013) Do microbes have macroecology? Large-scale patterns in the diversity and distribution of marine benthic ciliates. Glob Ecol Biogeogr 22: 163–172

Berk SG, Brownlee DC, Heinle DR, Kling HJ, Colwell RR (1977) Ciliates as a food source for marine planktonic copepods. Microb Ecol 4:27–40

Berninger UG, Epstein SS (1995) Vertical distribution of benthic ciliates in response to the oxygen concentration in an intertidal North Sea sediment. Aquat Microb Ecol 9:229–236

Calbet A, Alcaraz M, Atienza D, Broglio E, Vaque D (2005) Zooplankton biomass distribution patterns along the western Antarctic Peninsula (December 2002). J Plankton Res 27:1195–1203

Carey PG, Tatchell EC (1983) A revision of the genus *Eipclites* (*Ciliphora*: *Hypotrichida*) including a redescription of *Epilcintes felis* comb. n. Bull Br Mu 45:41–52

Chen C, Qiao R, Wei R, Guo Y and others (2012) A comprehensive survey of copy number variation in 18 diverse pig populations and identification of candidate copy number variable genes associated with complex traits. BMC Genomics 13:733

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772

Debroas D, Hugoni M, Domaizon I (2015) Evidence for an active rare biosphere within freshwater protists community. Mol Ecol 24:1236–1247

Dethier MN (1980) Tidepools as refuges: predation and the limits of the harpacticoid copepod *Tigriopus californicus* (Baker). J Exp Mar Biol Ecol 42:99–111

Díez B, Pedrós-Alió C, Marsh TL, Massana R (2001) Application of denaturing gradient gel electrophoresis (DGGE) to study the diversity of marine picoeukaryotic assemblages and comparison of DGGE with other molecular techniques. Appl Environ Microbiol 67:2942–2951

Doherty M, Costas BA, McManus GB, Katz LA (2007) Culture-independent assessment of planktonic ciliate diversity in coastal northwest Atlantic waters. Aquat Microb Ecol 48:141–154

Doherty M, Tamura M, Costas BA, Ritchie ME, McManus GB, Katz LA (2010a) Ciliate diversity and distribution across an environmental and depth gradient in Long Island Sound, USA. Environ Microbiol 12:886–898

Doherty M, Tamura M, Vriezen JA, McManus GB, Katz LA (2010b) Diversity of Oligotrichia and Choreotrichia ciliates in coastal marine sediments and in overlying plankton. Appl Environ Microbiol 76:3924–3935

Esteban GF, Finlay BJ (2007) Exceptional species richness of ciliated Protozoa in pristine intertidal rock pools. Mar Ecol Prog Ser 335:133–141

Ganning B (1971) Studies on chemical, physical and biological conditions in Swedish rockpool ecosystems. Ophelia 9:51–105

Gertler C, Nather DJ, Gerdts G, Malpass MC, Golyshin PN (2010) A mesocosm study of the changes in marine flagellate and ciliate communities in a crude oil bioremediation trial. Microb Ecol 60:180–191

Grattepanche JD, Santoferrara LF, Andrade J, Oliverio AM, McManus GB, Katz LA (2014) Distribution and diversity of oligotrich and choreotrich ciliates assessed by morphology and DGGE in temperate coastal waters. Aquat Microb Ecol 71:211–221

Grattepanche JD, Santoferrara LF, McManus GB, Katz LA (2015) Distinct assemblage of planktonic ciliates dominates both photic and deep waters on the New England shelf. Mar Ecol Prog Ser 526:1–9

Grattepanche JD, Santoferrara LF, McManus GB, Katz LA (2016a) Unexpected biodiversity of ciliates in marine samples from below the photic zone. Mol Ecol 25: 3987–4000

Grattepanche JD, McManus GB, Katz LA (2016b) Patchiness of ciliate communities sampled at varying spatial scales along the New England shelf. PLOS ONE 11:e0167659

Guizien K, Dupuy C, Ory P, Montanie H, Hartmann H, Chatelain M, Karpytchev M (2014) Microorganism dynamics during a rising tide: disentangling effects of resuspension and mixing with offshore waters above an intertidal mudflat. J Mar Syst 129:178–188

Hamady M, Lozupone C, Knight R (2010) Fast UniFrac: facilitating high-throughput phylogenetic analyses of microbial communities including analysis of pyrosequencing and PhyloChip data. ISME J 4:17–27

Hu XY, Hu XZ, Al-Rasheid KAS, Song WB (2009a) Reconsideration on the phylogenetic position of *Epiclintes* (Ciliophora, Stichotrichia) based on SSrRNA gene sequence and morphogenetic data. Acta Protozool 48:203–211

Hu XZ, Fan XP, Lin XF, Gong J, Song WB (2009b) The morphology and morphogenesis of a marine ciliate, *Epiclintes auricularis rarisetus* nov sspec. (Ciliophora, Epiclintidae), from the Yellow Sea. Eur J Protistol 45: 281–291

Hu SK, Campbell V, Connell P, Gellene AG, Liu Z, Terrado R, Caron DA (2016) Protistan diversity and activity inferred from RNA and DNA at a coastal ocean site in the eastern North Pacific. FEMS Microbiol Ecol 92:fiw050

Huggett J, Griffiths CL (1986) Some relationships between elevation, physicochemical variables and biota of intertidal rock pools. Mar Ecol Prog Ser 29:189–197

Jonsson PR (1994) Tidal rhythm of cyst formation in the rock pool ciliate *Strombidium oculatum* Gruber (Ciliophora, Oligotrichida): a description of the functional biology and an analysis of the tidal synchronization of encystment. J Exp Mar Biol Ecol 175:77–103

Jousset A, Lara E, Nikolausz M, Harms H, Chatzinotas A (2010) Application of the denaturing gradient gel electrophoresis (DGGE) technique as an efficient diagnostic tool for ciliate communities in soil. Sci Total Environ 408: 1221–1225

Kamiyama T (1994) Effects of extracellular products from phytoplankton on the excystment of tintinnids from marine sediments. Mar Ecol Prog Ser 105:199–201

Kamiyama T, Aizawa Y (1992) Effects of temperature and light on tintinnid excystment from marine-sediments. Bull Jpn Soc Sci Fish 58:877–884

Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: improvements in performance and usability. Mol Biol Evol 30:772–780

Katz LA, McManus GB, Snoeyenbos-West OLO, Griffin A, Pirog K, Costas B, Foissner W (2005) Reframing the 'Everything is everywhere' debate: evidence for high gene flow and diversity in ciliate morphospecies. Aquat Microb Ecol 41:55–65

Kelly MW, Grosberg RK, Sanford E (2013) Trade-offs, geography, and limits to thermal adaptation in a tide pool copepod. Am Nat 181:846–854

Kim YO, Taniguchi A (1997) Seasonal variation of excystment pattern of the planktonic oligotrich ciliate *Strombidium conicum*. Mar Biol 128:207–212

Kooistra W, Joosten AMT, Vandenhoek C (1989) Zonation patterns in intertidal pools and their possible causes: a multivariate approach. Bot Mar 32:9–26

Lewandowska AM, Boyce DG, Hofmann M, Matthiessen B, Sommer U, Worm B (2014) Effects of sea surface warming on marine plankton. Ecol Lett 17:614–623

Lipscomb DL, Riordan GP (2012) The ultrastructure of *Placus striatus* and a revision of the Family Placidae (Ciliophora). J Eukaryot Microbiol 59:407–422

Lipscomb DL, Bowditch BM, Riordan GP (2012) A molecular and ultrastructural description of *Spathidiopsis buddenbrocki* and the phylogenetic position of the Family Placidae (Ciliophora). J Eukaryot Microbiol 59:67–79

McAllen R, Brennan E (2009) The effect of environmental variation on the reproductive development time and output of the high-shore rockpool copepod *Tigriopus brevicornis*. J Exp Mar Biol Ecol 368:75–80

McManus GB, Xu DP, Costas BA, Katz LA (2010) Genetic identities of cryptic species in the *Strombidium stylifer/apolatum/oculatum* cluster, including a description of *Strombidium rassoulzadegani* n. sp. J Eukaryot Microbiol 57:369–378

McManus GB, Katz LA, Tamura M, Grant J (2011) How many kinds of ciliates in a coastal plankton sample? Comparison of clone library and pyrosequencing estimates. J Phycol 47:S34

Montagnes DJS, Wilson D, Brooks SJ, Lowe C, Campey M (2002) Cyclical behaviour of the tide-pool ciliate *Strombidium oculatum*. Aquat Microb Ecol 28:55–68

Morris S, Taylor AC (1983) Diurnal and seasonal variation in physico-chemical conditions within intertidal rockpools. Estuar Coast Shelf Sci 17:339–355

Poulsen LK, Ballard G, Stahl DA (1993) Use of ribosomal-RNA fluorescence *in situ* hybridization for measuring the activity of single cells in young and established biofilms. Appl Environ Microbiol 59:1354–1360

R Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Santoferrara LF, Grattepanche JD, Katz LA, McManus GB (2014) Pyrosequencing for assessing diversity of eukaryotic microbes: analysis of data on marine planktonic ciliates and comparison with traditional methods. Environ Microbiol 16:2752–2763

Santoferrara LF, Grattepanche JD, Katz LA, McManus GB (2016) Patterns and processes in microbial biogeography: Do molecules and morphologies give the same answers? ISME J 10:1779–1790

Scrosati R, Heaven C (2007) Spatial trends in community richness, diversity, and evenness across rocky intertidal environmental stress gradients in eastern Canada. Mar Ecol Prog Ser 342:1–14

Song W, Warren A (1996) A redescription of the Maine ciliates *Uroleptus retractilis* (Claparede and Lachmann, 1958) comb. n. and *Epiclintes ambiguus* (Muller, 1786) Butschli, 1889 (Ciliphora, Hypotrichida). Acta Protozool 35:227–234

Tamura M, Katz LA, McManus GB (2011) Distribution and diversity of oligotrich and choreotrich ciliates across an environmental gradient in a large temperate estuary. Aquat Microb Ecol 64:51–67

Valdivia N, Díaz MJ, Holtheuer J, Garrido I, Huovinen P, Gómez I (2014) Up, down, and all around: scale-dependent spatial variation in rocky-shore communities of Fildes Peninsula, King George Island, Antarctica. PLOS ONE 9:e100714

Wicklow BJ, Borror AC (1990) Ultrastructure and morphogenesis of the marine epibenthic ciliate *Epiclintes ambiguus* (Epiclintidae, n. fam.; Ciliophora). Eur J Protistol 26:182–194

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