



REVIEW

Lifestyles of rarity: understanding heterotrophic strategies to inform the ecology of the microbial rare biosphere

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ABSTRACT: There are patterns in the dynamics of rare taxa that lead to hypotheses about their lifestyles. For example, persistently rare taxa may be oligotrophs that are adapted for efficiency in resource-limiting environments, while conditionally rare or blooming taxa may be copiotrophs that are adapted to rapid growth when resources are available. Of course, the trophic strategies of microorganisms have direct ecological implications for their abundances, contributions to community structure, and role in nutrient turnover. We summarize general frameworks for separately considering rarity and heterotrophy, pulling examples from a variety of ecosystems. We then integrate these 2 topics to discuss the technical and conceptual challenges to understanding their precise linkages. Because much has been investigated especially in marine aquatic environments, we finally extend the discussion to lifestyles of rarity for freshwater lakes by offering case studies of Lake Michigan lineages that have rare and prevalent patterns hypothesized to be characteristic of oligotrophs and copiotrophs. To conclude, we suggest moving forward from assigning dichotomies of rarity/prevalence and oligotrophs/copiotrophs towards their more nuanced continua, which can be linked via genomic information and coupled to quantifications of microbial physiologies during cell maintenance and growth.

KEY WORDS: Microbiome · Community ecology · Community structure · Oligotroph · Copiotroph · Traits

INTRODUCTION

Environmental microbial communities are species rich and have wide phylogenetic breadth, yet most of their taxa are observed in extremely low abundance within a locality (Sogin et al. 2006, Pedrós-Alió 2007, 2012, Fuhrman 2009, Lynch & Neufeld 2015). Our modest understanding of microbial diversity, especially among these rare members, has been accentuated by our commonplace use of cultivation-independent high-throughput sequencing, which has allowed us to pull back the proverbial

sampling veil line to observe deeper into a community's standing diversity (Caporaso et al. 2011b). In parallel to this sequence-informed pursuit of understanding the microbial rare biosphere, we have accumulated much metagenome and amplicon sequencing data for environmental microbial communities. Researchers strive to make sense of these rich data, which reveal much in terms of genetic diversity and functional potential but remain limited in ecological insights. This limitation is in part because microorganisms cannot be observed discretely in their interactions and behaviors *in situ* (e.g.

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Prosser 2015). However, trait-based approaches in community ecology offer some strategies for distilling complex communities into their important ecological attributes (e.g. Litchman & Klausmeier 2008). One important distinguishing microbial trait is trophic strategy. Determining the strategies of heterotrophic bacteria has a long legacy in microbial ecology and has experienced a recent reinvigoration of interest (Giovannoni & Stingl 2007, Livermore et al. 2014, Neuenschwander et al. 2015). The conceptual delineation falls between oligotrophic and copiotrophic heterotrophs, which are generally defined by growth rate and responsiveness to local increases in resources. Understanding the balance between oligotrophic and copiotrophic microbial strategies is of particular interest in aquatic systems, where both strategies contribute to temporal and spatial dynamics of community structure and may play different roles in the transfer of nutrients in aquatic food webs (Neuenschwander et al. 2015).

The ecology of rarity and trophic strategies are intrinsically linked. A heterotrophic population's standing abundance within its community is an outcome of its strategy, and changes in the environment or biotic interactions have direct implications for the success of particular trophic strategies over time and space. There are 2 common but contrasting patterns of local rarity, and each can be framed in the context of trophic strategy. On the one hand, there are taxa that are rare but occasionally bloom to achieve a relatively larger standing population. These taxa, called conditionally rare (Shade et al. 2014, Shade & Gilbert 2015), may be hypothesized to be copiotrophs that can quickly utilize available resources or respond to changes in environmental conditions to gain temporary advantage. On the other hand, there are also taxa that are rare but relatively constant in their population size, suggestive of an oligotrophic strategy. Distinguishing the ecological mechanisms underlying patterns of rarity will likely include consideration of trophic strategy and will allow researchers to gain insights into the implications of biodiversity for community dynamics and processes.

Here, we explore the potential relationships between community structure and heterotrophic strategy and the ongoing challenges in understanding these relationships. We do not include discussion of rarity due to competitive inferiority or local transience but focus instead on persistent mechanisms of rarity that may be explained partially by trophic strategy within a locality.

FOUNDATIONAL CONCEPTS

Rarity

Rarity has long been considered in traditional ecology for communities of larger organisms and typically is delineated into either temporal or spatial aspects. A classic conceptual framework that addresses the spatial forms of rarity was suggested by Deborah Rabinowitz, and her framework has been applied widely to biogeographic studies of diversity. In the Rabinowitz framework, geographic range, habitat specificity, and local population size are used to provide a nested framework for spatial rarity (Rabinowitz et al. 1986). Also, the works of Anne Magurran and colleagues have explored the relationship between transience and standing diversity over time, inclusive of uncommon taxa (Magurran & Henderson 2003, Magurran 2007, Shimadzu et al. 2013), for example, using the fit of the species abundance distribution to partition the transient and persistent contributors to the community over time.

Like communities of larger organisms (Locey & Lennon 2016), microbial communities typically contain an extremely large number of rare taxa (Sogin et al. 2006, Pedrós-Alió 2012, Lynch & Neufeld 2015). This microbial rare biosphere was discovered because of high-throughput sequencing (Sogin et al. 2006, Reid & Buckley 2011), which has allowed researchers to observe a larger proportion of the microbial community in a given environment and thus attribute more sequences to rare taxa (Huse et al. 2010, Kunin et al. 2010, Quince et al. 2011). Thus, the dynamics of rare members are often tracked with genetic markers of microbial diversity (e.g. the 16S rRNA gene). 16S rRNA gene sequencing has revealed that some rare microbial members are transients passing through an environment (van der Gast et al. 2011, Shade et al. 2013), while others are persistent and have periodic blooms that are linked to environmental cues (Caporaso et al. 2011a, Vergin et al. 2013, Shade et al. 2014). It is hypothesized that rare microbes contribute to community stability as part of a diversity reservoir that can rapidly respond to environmental changes (Jones & Lennon 2010, Lennon & Jones 2011, Shade et al. 2012). Interest in the biology and ecology of the microbial rare biosphere is increasing, as evidenced by the recent number of opinion and review pieces (e.g. Bachy & Worden 2014, Lynch & Neufeld 2015).

Seasonal and spatial patterns of rare taxa have been observed in different aquatic systems (Galand et al. 2009, Campbell et al. 2011, Hugoni et al. 2013, Vergin

et al. 2013, Alonso-Sáez et al. 2014, 2015, Székely & Langenheder 2014), suggesting that rare taxa are constrained by environmental conditions and may share common drivers with their more prevalent counterparts. There is evidence that rare taxa are or can become active in their communities (Hunt et al. 2013, Wilhelm et al. 2014, Aanderud et al. 2015), sometimes providing key functions (Pester et al. 2010, Sauret et al. 2014). Additionally, some rare taxa are sensitive to disturbances (Sjöstedt et al. 2012, Coveley et al. 2015, Vuono et al. 2016), notably after the recent *Deepwater Horizon* oil spill (Newton et al. 2013). Rare soil taxa that were below detection not only responded to pulses of precipitation by blooming but also contributed to increased carbon dioxide and methane production, demonstrating that rare taxa collectively can contribute to ecosystem function (Aanderud et al. 2015). Similarly, typically rare but fast-growing, cultivable taxa from Lake Zurich, Switzerland, could offer substantial contributions to carbon cycling (Neuenschwander et al. 2015). A latitudinal analysis of marine bacterioplankton showed that fewer rare taxa are detectable in polar waters, possibly because these communities comprise fewer but more abundant taxa (Amend et al. 2013). There are many additional recent studies documenting the diversity or dynamics of the rare biosphere in various habitats (e.g. Elshahed et al. 2008, Youssef et al. 2010, Hugoni et al. 2013, Gies et al. 2014, Lawson et al. 2015). Thus, patterns of rare taxa are readily documented and described using commonplace cultivation-independent methods, like 16S rRNA gene amplicon sequencing. From these patterns, we can begin to hypothesize the ecological mechanisms underpinning the dynamics of rare taxa.

Heterotrophic strategies

When using a categorical definition, oligotrophs and copiotrophs are distinguished by their ability to utilize resources. Copiotrophs have been called opportunist or blooming populations that rapidly grow in response to increases in resources, while oligotrophs are typically marathoners that are adapted for efficiency and optimal scavenging under low-resource conditions. Notably, the classification of a microorganism as an oligotroph or a copiotroph is separate from the habitat in which it resides (Semenov 1991, but see Koch 2001 for an opposing definition); both oligotrophic and copiotrophic microorganisms can persist in both low- and high-productivity environments. Indeed, populations of each strategy may complement each other in their dynam-

ics in seasonally fluctuating or heterogeneous environments (Neuenschwander et al. 2015).

Numerous excellent works have delved deeper into the precise distinctions between oligotrophs and copiotrophs. Some of these works have focused on defining general traits of members exhibiting each strategy. For example, it has been hypothesized that most oligotrophs cannot produce hydrolases, can accumulate polymers, have low maximal specific growth rates, and can maintain growth in low-substrate concentrations (as summarized by Semenov 1991). Other proposed oligotrophic traits include permease activity to allow for passive nutrient transport across the cell membrane, a low surface:volume ratio, fewer copies of 16S rRNA genes (but see Blazewicz et al. 2013), and high-fidelity ribosomes (Schmidt & Konopka 2009). Responses to starvation may also be distinct between copiotrophs and oligotrophs (Lever et al. 2015): copiotrophs tend to shrink the size of their cells and decrease their DNA content, while oligotrophs do not have the same dramatic downsizing because, generally, they are already small. Copiotrophs have been suggested to have larger genomes than oligotrophs (Kirchman 2016), and extreme oligotrophy is sometimes observed to correspond with genome streamlining, attributed to increased efficiency (Giovannoni et al. 2014).

If rarity is linked to trophic strategy, considering the distinctive genomic and physiological traits of microorganisms with different trophic strategies can provide insights into the ecological importance of rare taxa. There are several physiological distinctions between oligotrophs and copiotrophs that can be quantified in the laboratory. Semenov (1991) posits that oligotrophs possess transport systems that have relatively high affinities for substrates, require low-maintenance energy for survival, and have a clear rate-limiting step, typically in respiration, that controls metabolism and promotes efficiency. Another key physiological distinction between copiotrophs and oligotrophs is the relative importance of maintenance energy, or the energy required for survival, versus growth energy, the energy required for biomass accumulation (e.g. Fierer et al. 2007, Schmidt & Konopka 2009, Lever et al. 2015). Though oligotrophs and copiotrophs both require growth and maintenance energy, maintenance energy is thought to be relatively more important for oligotrophs, while growth energy is thought to be relatively more important for copiotrophs. However, quantification of both growth and maintenance parameters remains a key unknown for many heterotrophs, as few growth- and maintenance-related measurements are per-

formed at the population level. Rather, aggregate- or community-level growth rates are easier to measure in environments (Kirchman 2016), which muddies our ability to quantitatively observe the spectrum of growth rates and delineate oligotrophs from copiotrophs based on cellular growth. Furthermore, sequence-based data suggest that common freshwater genera are made up of numerous populations with distinct habitat distributions (Jezbera et al. 2011, 2013, Newton & McLellan 2015) and substrate specificities (Kasalický et al. 2013), suggesting that within the same genus, there can be multiple trophic strategies. Therefore, measuring the growth and maintenance energies of mixed populations, even of the same lineage, may be misleading for understanding individual requirements. Furthermore, it has been noted that many heterotrophic bacteria are unlikely to be routinely achieving their maximum growth rates *in situ* (Schmidt & Konopka 2009, Kirchman 2016), suggesting that understanding the limits of growth and maintenance energy requirements may be especially difficult within an environmental context.

Physiological measurements can be complemented and potentially informed by the wealth of genomic information available from various environments. The genomic underpinnings of oligotrophy and copiotrophy were investigated in an archetypal marine oligotroph and copiotroph (Lauro et al. 2009) and then discussed later in detail and expanded by others (e.g. Lever et al. 2015, Kirchman 2016). A comparative genomics approach revealed that copiotrophs had more diverse transporters, while oligotrophs had fewer multifunctional transporters with generally higher affinities. Attributed to the higher diversity of transporters and therefore the higher diversity of entry mechanisms for phage, copiotrophs had more lytic phage infections than oligotrophs. Marine copiotrophs also had relatively more exoenzymes, which were hypothesized to be related to a predominately particle-associated lifestyle. Similarly, it has been suggested that copiotrophs are more likely to be motile (Livermore et al. 2014, Lever et al. 2015), have larger investments in signal transduction mechanisms, and have the genetic capacity to use a wider array of carbon substrates (Livermore et al. 2014). Finally, there was some evidence that oligotrophs have more secondary metabolite biosynthetic pathways (Lauro et al. 2009).

Grazing preferences can reinforce temporal patterns that link heterotrophic lifestyles to rarity, as both viruses and protists would tend to cull relatively more active (copiotroph) and more abundant popula-

tions (kill the winner) than inactive or rare populations. Therefore, grazing could manifest as the same pattern as expected for taxa that transition between rarity and prevalence. Faster-growing taxa often have wider variability in abundance because top-down pressures are relatively higher for them. Rare oligotrophs are thought to have little predation pressure, and thus the lack of predation also relates to a restriction in abundance variability over space and time. This argument was presented previously by Pedrós-Alió (2006). We additionally posit that if grazing pressures achieve equilibrium with the growth of a copiotrophic taxon, then abundance patterns may also hold steady across space and time and potentially mask dynamics. On the one hand, viral predation should impact population dynamics for abundant microbes (i.e. kill the winner). On the other hand, slow-growing microbes can be highly dispersed in aquatic systems despite their high abundance. For example, abundant oligotrophs are likely not growing fast enough, such that it is uncommon to observe dense populations of abundant oligotrophs in the open water. This greater spatial dispersion, combined with the likelihood that these microbes are small and have reduced surface molecules, may reduce viral predatory impact and lead to less variable population dynamics.

UNKNOWN AND CHALLENGES: DETERMINING TROPHIC STRATEGIES OF RARE TAXA

Studies investigating the temporal dynamics of rare taxa have provided evidence that, within a locality, some rare taxa are persistently rare, while others have appreciable variability and ranges in their relative abundance. This evidence underlies the hypothesis that persistently rare taxa are more likely to have oligotrophic strategies, while more variable or dynamic rare taxa are more likely to have copiotrophic strategies (Lynch & Neufeld 2015, Shade & Gilbert 2015). However, there are many technical challenges and conceptual unknowns that complicate directly testing this hypothesis. We discuss some of those challenges in the sections below.

Detection bias against rare taxa

Perhaps the most cumbersome challenge is that of inherent detection bias for rare taxa and, by extension, of measuring physiological or trait-based information about them. Essentially, we cannot measure

what we cannot detect. The detection of rare taxa will have relatively high variability, not necessarily because they are ecologically more variable (though they may be) but because of challenges with consistent detection and making observations near the limits of measurement (Anders & Huber 2010, Dickie 2010, Reeder & Knight 2010, Bowen et al. 2012, Lan et al. 2012, Albertsen et al. 2013, Haegeman et al. 2013, Hugoni et al. 2013, Delmont et al. 2015). For example, rare members are often only present in either the rRNA gene pool or the rRNA pool but not both (İnceoğlu et al. 2015), suggesting a combination of technical detection limitation, high variability in activity/transcripts, and a large contribution of dormant taxa to the rRNA gene pool that collectively overwhelms signals from the rare biosphere. New evidence also cautions that relic DNA (remnant signatures from dead cells) may be contributing to DNA-based observations of community structure (e.g. Lynch & Neufeld 2015, Carini et al. 2016), further confounding our ability to detect the true rare biosphere. Finally, because of detection biases for rare taxa, it is often difficult to determine if their biogeographic or temporal distributions (e.g. along environmental gradients) are driven by true environmental constraints or are a byproduct of non-exhaustive or biased sampling efforts. A study in salt marsh sediment suggested that replicated detection of the rare biosphere can be consistent, at least for some habitats (Bowen et al. 2012).

Many of the rare biosphere members observed using cultivation-independent methods are presumed to be as yet uncultivable. Though the exact link between trophic status and rarity remains unclear, it has been suggested that some copiotrophic members of the rare biosphere have been cultivated readily since the dawn of environmental microbiology (Pedrós-Alió 2006, Reid & Buckley 2011, Shade & Handelsman 2012), which can provide insights into the identity and ecology of these cultivable members. There is a renewed interest in cultivation of oligotrophic or generally slow-growing organisms (Schmidt & Konopka 2009, Carini et al. 2013, Henson et al. 2016), which, if successful, will provide precise measurements of their growth and maintenance requirements and insights into their consequences for community structure. There has also been discussion of obligate oligotrophs and copiotrophs—those that cannot survive in the presence of high or low resources, respectively (Koch 2001). Though it is difficult to ascertain whether resource conditions are truly bacteriocidal or the cultivation conditions are not optimal in

other unknown ways, Koch (2001) presents a series of interesting hypotheses as to physiological mechanisms underlying obligatory trophic conditions for both copiotrophs and oligotrophs. Relatedly, Kuznetsov et al. (1979) provide a discussion of different cultivation strategies, including media type and carbon source, that lead to historically nuanced definitions of oligotrophy.

Linking activity to growth and maintenance

Another challenge for studying microbial rarity is in linking the activity of cells to their growth rate. This remains a challenge whether the cells are rare or prevalent but especially for rare cells because of detection limitations as discussed above (see 'Detection bias against rare taxa'). There are several complications in connecting growth rate and activity levels. First, within a microbial community, some cells are metabolically inactive or dormant and therefore do not contribute to activity measurements (e.g. Jones & Lennon 2010) but can contribute to DNA-based measurements of community structure, which skews our perspective of the rare biosphere. Thus, the contribution of dormant members may make the active rare biosphere appear larger (more taxa) than it is. Also, if evaluated for activity, this may make the active rare biosphere appear to contribute less to overall activity than it does. Because oligotrophs are thought to be generally less active than copiotrophs or, instead, expend maintenance energy rather than growth energy, it is important to consider how to make a distinction between true oligotrophs that are very slow growing (or maintaining) with low activity and dormant cells that are not active.

Many studies use 16S rRNA:rDNA ratios as a proxy for relative activity of community members, which carries multiple assumptions and caveats that have been discussed in detail by others (Blazewicz et al. 2013, Lankiewicz et al. 2016). Interpretation of rRNA-based growth rates (or metabolic activities) is confounded by the fact that organisms with different ecological strategies, such as oligotrophs and copiotrophs, may have different intrinsic activity levels for maintenance and growth. There is evidence from marine systems that oligotrophs and copiotrophs have fundamentally different relationships in their rRNA:rDNA ratios both in laboratory culture and in the environment (Lankiewicz et al. 2016). It also has been shown that degradation rates of rRNA in bacteria entering starvation periods and subsequent rRNA

production rates during nutrient recovery can vary among organisms and depend on the environmental conditions leading to starvation, so changes in rRNA:rDNA may not be a consistent indication of slowing/increasing growth when comparing among organisms.

The ability to sort marine cells into high nucleic acid (HNA) and low nucleic acid content (LNA) fractions based on nucleic acid fluorescence, in combination with low-light and high-light scatter as a proxy for cell size, may provide some insights into the relationship between heterotrophic growth rate and activity (e.g. Vila-Costa et al. 2012). However, this sorting technique has some of the same limitations as the use of rRNA:rDNA ratios for a similar purpose, including ambiguity in interpretation of the relative activity of HNA and LNA fractions (e.g. Bouvier et al. 2007, Wang et al. 2009). HNA cells are either more active or have generally larger genomes, while LNA cells are either less active, are inactive, or have generally smaller genomes. Amplicon sequencing was conducted on the 16S rRNA gene of HNA/LNA and small/large cell size fractions on a seasonal dataset of coastal Mediterranean waters to assess each fraction's community structure (Vila-Costa et al. 2012). *Gammaproteobacteria* were present in multiple fractions, suggesting plasticity in their physiological state. However, most other taxa remained constant within their fractions and within a season, showing that nucleic acid content and size are 2 distinguishable and consistent traits of marine heterotrophs. Different seasons had unique 16S rRNA compositions for each fraction, suggesting that the rare and prevalent taxa were not compensatory in their relative abundances (or in their activity as well, as can be assessed by nucleic acid content and cell sizes) over time.

In contrast, work by Campbell et al. (2011) in coastal marine waters showed that many community members cycle between rare and abundant over time, which supports the hypothesis that some rare taxa exhibit a copiotrophic strategy. Those authors show that many of the cycling taxa had distinct activity to abundance relationships, as measured by rRNA:rDNA ratios. For example, some taxa had a constant activity:abundance ratio irrespective of their abundance, while other taxa seemed to increase their activity:abundance ratio when they became more rare. It could be that as a taxon blooms, its resource competition among like cells increases significantly, which drives a proportion of those cells to become less active, thereby decreasing the overall activity:abundance ratio of that population.

Implications of activity (growth or maintenance) for local abundance

If growth rates and/or maintenance activity are distinctive for oligotrophs and copiotrophs, it is important to understand the relationship between these parameters for a population's local abundance, thereby linking rates and activities to microbial contributions to community structure. For example, Hunt et al. (2013) found a general relationship between abundance and activity in a marine study of microbial communities at 2 depths in the Pacific Ocean. However, the authors noted that SAR11, a typical pelagic oligotroph, was an exception to this pattern in that it had a very large standing population size and very low activity as measured by 16S rRNA:rDNA ratio. In aquatic environments, standing population size is determined by interacting biological traits and physical factors. For example, a population's capacity to bloom will in part be underpinned by the residence time/flow rate of the water and generation time of the cell. Below is an example of how these growth properties could intersect with sequence-based technologies for detecting blooming microbial populations within microbial communities. For this example, we will use *Limnohabitans*, a common and fast-growing freshwater taxon (Fig. 1).

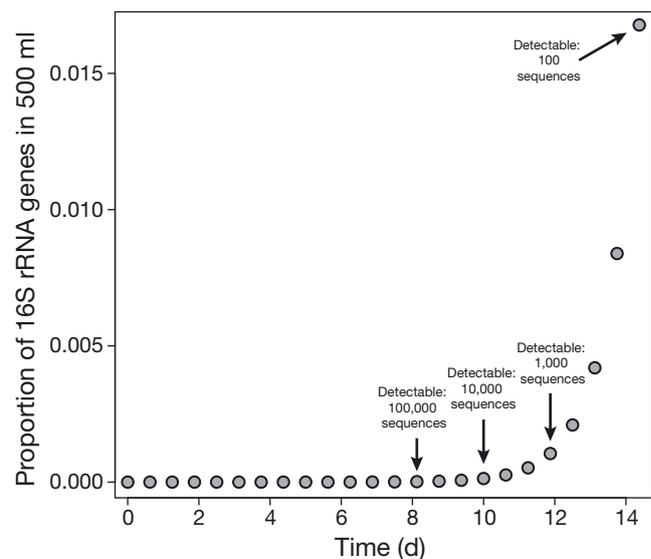


Fig. 1. Hypothetical concentration of *Limnohabitans* cells detected in 500 ml of lake water over time, assuming a standing total cell population size of 1 million cells ml⁻¹, a starting *Limnohabitans* population of 1 with one 16S rRNA gene copy, a doubling rate of 15 h, and no *Limnohabitans* cell dispersion. Common detection limits (listed as sequencing depth needed to detect at least 1 *Limnohabitans* sequence) for 16S rRNA gene surveys are indicated on the plot

Limnohabitans has an average *in situ* doubling time of 15 h (estimate in Šimek et al. 2006). We will also assume that every 1 ml of lake water has 1 million cells (here treating as equivalent to 1 million rRNA gene copies) and that growth starts from a single *Limnohabitans* cell, whose entire kin population doubles every 15 h and displaces an un-like cell from the community (i.e. *Limnohabitans* growth is balanced by an equivalent loss of non-*Limnohabitans* cells to maintain a standing stock of 1 million cells ml⁻¹). If in this hypothetical scenario we also limit the dispersal of *Limnohabitans* cells (i.e. the cells remain near each other in a lake's 3-dimensional space) and assume complete capture of the *Limnohabitans* cell population when filtering 500 ml of water, then it would take 10 d before one could detect that population at a level of 0.01% of the community and 14 d before it reached 1%. Notably, 1% relative abundance is often applied as an arbitrary cut-off for rarity, though it has no ecological rationale (Shade & Gilbert 2015). Regardless, under this scenario, it would be difficult for a localized blooming population to be detected in a reasonably large body of water, where water mixing would dilute the population signal on a time scale that may outlast the bloom. Instead, either populations with faster growth rates or a distributed rare standing stock would be needed for rare populations to be detected during blooms.

Local rarity along habitat gradients linked by regional metacommunities

A consideration that may impact our ability to understand rare lifestyles is underpinned by a meta-community framework of immigration and dispersal across interconnected local environments (Leibold et al. 2004). There is growing evidence that many microorganisms have different abundances across many connected environments and gradients (e.g. Langenheder et al. 2012, Ruiz González et al. 2015), and, of course, different habitats vary in their productivity. For example, an oligotroph in a soil habitat may, with no fundamental alteration to its physiology, fall more along the copiotrophic end of a trophic continuum than it would in a neighboring wetland habitat, though it may naturally occur in both localities. Thus, though the absolute abilities of the microorganism do not change, the environmental context may allow it to play relatively different roles along the local heterotrophic spectrum (e.g. oligotroph to copiotroph) within different communities. Of course, environmental context and other selective forces

play important roles in sorting species, but microbial dispersal allows for connectivity between disparate habitats (e.g. Peter et al. 2014), and dormancy allows for persistence within less than favorable habitats (e.g. Hubert et al. 2009). Thus, microbial mechanisms for dispersal and dormancy may allow microorganisms adapted to disparate habitats to coexist. Furthermore, habitat connectivity is important to consider in understanding the relationships between local patterns in trophic strategies and community structure.

TROPHIC STRATEGY AND RARITY IN AQUATIC ENVIRONMENTS: CASE STUDIES FROM LAKE MICHIGAN, USA

Despite the unknowns and challenges described in the previous section, there is evidence in the literature that hints at the strategies we may expect from rare taxa inhabiting aquatic environments. For example, in a study of marine microbial genomes, Yooseph et al. (2010) suggested that the most abundant and widely distributed taxa were most likely to be oligotrophs, while the less abundant taxa could be either copiotrophic or oligotrophic. In contrast, Kirchman (2016) suggested that both rare and prevalent taxa in the ocean are equally likely to be both slow and fast growers, assuming that slow and fast growth is roughly equivalent to definitions of oligotrophy and copiotrophy, respectively.

There are fewer studies in freshwater systems with parallel objectives of understanding the genomic underpinnings of trophic strategy. This is possibly because the number of available freshwater microbial genomes is considerably less than the number of marine genomes, which precludes comparative genomic assessment of trophic strategies. In one analysis by Livermore et al. (2014), the authors inferred from their genomic data 4 lifestyle strategies: (1) passive and streamlined, (2) slow augmented responders, (3) fast reduced responders, and (4) vagabonds. The passive streamlined genomes were considered oligotrophs, while the other 3 groups were considered copiotrophs because of high predicted growth rate and/or diverse carbon substrate utilization pathways.

Inland fresh waters play a critical role in the fate (burial or remineralization) of terrestrial-derived nutrients (Tranvik et al. 2009) and serve as a major source of drinking and industrial-use water to human populations (Swackhamer 2005), but these systems are in a period of rapid change resulting from increasing urbanization, nutrient deposition in ter-

restrial systems, and human consumption (Jackson et al. 2001, Carpenter et al. 2011). Given the importance of fresh waters to human well-being and the dominant role of microorganisms in the transport and fate of nutrients in these, we wanted to further extend the connections between trophic strategy and community structure to freshwater systems. We asked if, in a typical freshwater lake, there are bacteria with differing dynamics that represent rare/prevalent and hypothesized oligotrophic/copiotrophic abundance patterns. We connected abundance dynamics to trophic strategy by designating community members with low-abundance variability in space/time as oligotrophs and those with high-abundance variability in space/time as copiotrophs. We hypothesized that along a prevalence versus abundance continuum, there may be 4 generally identifiable groups of taxa: abundant oligotrophs, rare oligotrophs, blooming abundant copiotrophs, and blooming rare copiotrophs. We posited that we could first identify occurrence patterns, then use the sequence-based information to assign taxonomy, and finally ask if existing knowledge of those lineages is suggestive of either copiotrophic or oligotrophic strategies (or both). Essentially, we could ask this question: How well do dynamics hypothesized to be characteristic of certain trophic strategies correspond to the same known trophic strategies?

To identify occurrence patterns, we examined massively parallel 16S rRNA gene amplicon datasets

collected from Lake Michigan during 4 sampling transects over 3 different years ($n = 17$; National Center for Biotechnology Information Sequence Read Archive projects SRP018584, SRP059202, SRP056973). Details on the samples collected, DNA extraction, and 16S rRNA gene sequencing and processing methods can be found in Newton & McLellan (2015). After quality filtering and subsequent taxonomic assignment of sequences, we conducted oligotyping (Eren et al. 2014) on 18 common freshwater lineages (see Table 1 for lineages and Newton & McLellan 2015 for methods details). Oligotyping allows for refined sequence clustering (1 nucleotide difference between oligotypes, i.e. operational taxonomic units [OTUs]) while reducing OTU inflation from sequencing errors (Eren et al. 2014). We then used descriptive statistics (prevalence, relative abundance maximum, and coefficient of variation) on the oligotype ($n = 241$) relative abundance patterns to set boundaries for each of the 4 lifestyle categories — (1) Abundant oligotroph: oligotype with a maximum relative abundance $\geq 0.1\%$ and a coefficient of variation (CV) that is less than the lower bound of a linear model prediction interval (0.5) fit to oligotype sample occurrence versus oligotype CV (see Fig. 2 for model); (2) Rare oligotroph: oligotype with a maximum relative abundance $< 0.1\%$, a CV that is less than the lower bound of a linear model prediction interval (0.5) fit to oligotype sample occurrence versus oligotype CV, and is present in > 8 samples; (3) Abundant copiotroph:

Table 1. Lifestyles inferred from oligotype abundance patterns in Lake Michigan. See main text section 'Trophic strategy and rarity in aquatic environments: Case studies from Lake Michigan, USA' for lifestyle categorization details

Phylum	Lineage/ genus	— Oligotroph —		— Copiotroph —		Total oligotypes
		Abundant	Rare	Abundant	Rare	
<i>Actinobacteria</i>	acI-A	4	4			15
	acI-B	1	1			6
	acI-C					3
	acI-TH1					2
	acSTL					1
	<i>Aquiluna</i>			2	1	12
<i>Bacteroidetes</i>	<i>Algoriphagus</i>	1				7
	<i>Arcicella</i>	1	3		1	13
	<i>Flavobacterium</i>	2	1	4	7	39
	<i>Fluviicola</i>	1	2	6	4	31
	<i>Sediminibacterium</i>		1	1	1	24
<i>Proteobacteria</i>	LD12	1	3			12
	<i>Sphingopyxis</i>	1	1			9
	<i>Hydrogenophaga</i>		1	3	2	20
	<i>Limnohabitans</i>	3	1	4	2	18
	<i>Polynucleobacter</i>	1		2		11
	<i>Rhodobacter</i>		1	2	3	16
<i>Verrucomicrobia</i>	<i>Luteolibacter</i>		2	3	1	12

oligotype with a maximum relative abundance $\geq 0.1\%$ and a CV that is greater than the upper bound of a linear model prediction interval (0.5) fit to oligotype sample occurrence versus oligotype CV; (4) Rare copiotroph: oligotype with a maximum relative abundance $< 0.1\%$ and a CV that is greater than the upper bound of a linear model prediction interval (0.5) fit to oligotype sample occurrence versus oligotype CV (see Table 1 for results). Our strategy for assigning categories to oligotypes was chosen as appropriate for the Lake Michigan dataset, but a different and comparable strategy could be applied to any reasonably sampled spatial or temporal series. Oligotype relative abundance patterns that are characteristic of each hypothesized life strategy are depicted in Fig. 3.

The oligotype assignments to lifestyle strategies generally supported existing knowledge about the ecology of these lineages. Freshwater *Actinobacteria* lineage acI, *Alphaproteobacteria* lineage LD12, and

some members of the genus *Polynucleobacter* are considered ubiquitous, abundant members of freshwater lakes (Newton et al. 2011) with passive oligotrophic lifestyles (Hahn et al. 2012, Ghylis et al. 2014, Livermore et al. 2014). This assertion supports generally the spatial/temporal patterns we observed in Lake Michigan surface waters, where 15 oligotypes from these groups were classified as having oligotroph-like distribution patterns, including both abundant ($\geq 0.1\%$ maximum) and rare members. Although also common in fresh waters, *Flavobacterium* spp. and *Limnohabitans* spp. are generally considered more copiotrophic microorganisms (Eiler & Bertilsson 2007, Kasalický et al. 2013), capable of blooming under the right environmental circumstances. These genera also include a large diversity of metabolic capabilities (McBride et al. 2009, Zeng et al. 2012, Kasalický et al. 2013). In our analysis, *Flavobacterium* and *Limnohabitans* comprised 2 of the 3 freshwater groups that harbored oligotypes in each of the 4

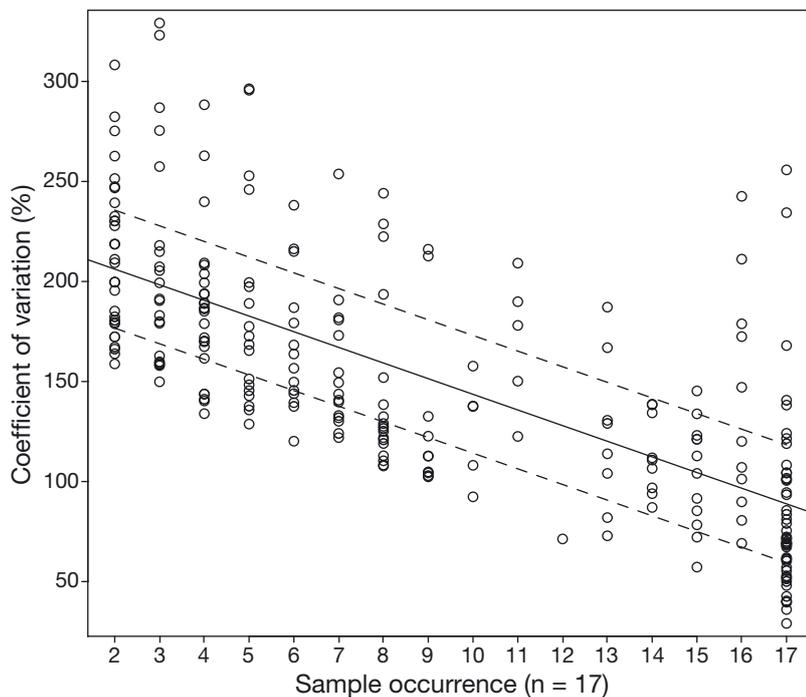


Fig. 2. Linear model fit (solid line) and prediction interval (0.5, dashed lines) for oligotype occurrence versus coefficient of variation (CV). Oligotypes with a CV above the prediction upper bound were considered copiotrophs, while those below the prediction lower bound and present in ≥ 8 of 17 samples were considered oligotrophs. For CV calculation, a relative abundance threshold of 0.0002% was applied whenever an oligotype was not detected in a sample. This threshold was set at $10\times$ below the actual detection threshold to lessen the impact of the 'no-detects' on CV calculations. A minimum presence in 8 samples requires oligotypes to be present in more than 1 sampling transect and lowers uncertainty associated with detection limits for low-prevalence, low-abundance oligotypes. Oligotypes present in only 1 sample were not used for the model fit or subsequent oligotroph/copiotroph classification

lifestyle categories, supporting the idea that these genera have large variation in traits and life strategies. For freshwater groups that are so far less studied, our data suggest *Fluviicola* spp. are diverse (31 oligotypes), often rare in communities, and present a variety of lifestyle strategies. In contrast, other taxa had more defined categorizations; 2 *Bacteroidetes*-affiliated genera, *Arcicella* and *Algoriphagus*, harbored primarily oligotroph-classified oligotypes, while *Hydrogenophaga*-, *Rhodobacter*-, and *Luteolibacter*-affiliated oligotypes were classified generally as blooming taxa or copiotrophs.

As we were most interested in the hypothesized dichotomy of rarity/abundance and oligotroph/copiotroph lifestyles, a caveat to our analysis is that our classification scheme overlooks many organisms, including those with abundance variability close to the modeled mean variability within this community and those with both low occurrence rates and low abundance. We advocate for future approaches to make use of available prevalence, genetic content, and phenotypic data to improve classification techniques for microbial traits that are indicative of microbial lifestyles in freshwater systems.

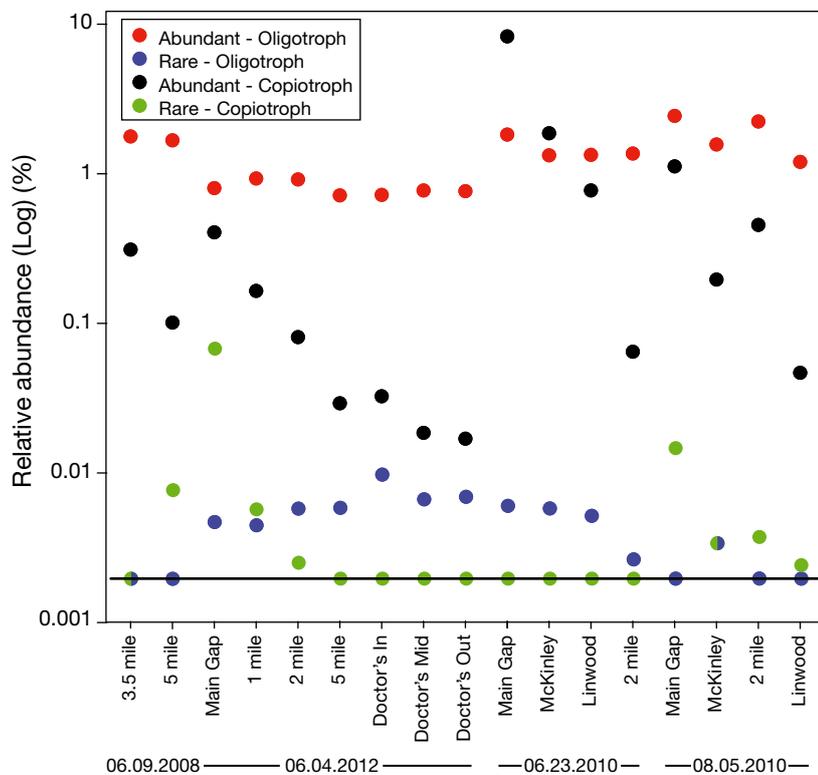


Fig. 3. Example relative abundance sample profiles for each life strategy category: (1) abundant oligotroph, (2) rare oligotroph, (3) abundant copiotroph, and (4) rare copiotroph. Sample sites and collection dates (given as mm.dd.yyyy) are included on the x-axis (for detailed locations and site characteristics, see Newton & McLellan 2015). The horizontal line indicates the relative abundance detection threshold (0.002%) used in the analyses. For plotting purposes, each abundance profile was given a relative abundance value of 0.002% when it was not detected in a sample

EXPANDING FROM DICHOTOMIES TOWARDS COUPLED CONTINUA OF ABUNDANCE AND HETEROTROPHY

For both oligotrophs and copiotrophs, a current gap in knowledge remains in distinguishing maintenance energy from true dormancy or inactivity. These measurements are difficult to observe. With renewed interest in the cultivation of oligotrophs and, potentially, an improvement in our ability to interpret nuances in rRNA:rDNA ratios or HNA/LNA measurements to make such distinctions (for instance, over time scales pertinent to observing both growth and maintenance and coupled with labeled substrate), there is promise for progress in understanding the energy requirements at the lower limits of microbial activity.

With the wealth of genomic information available for marine systems and with the upcoming availability of freshwater genomes, comparative genomic studies

will provide insights into the typical genomic features of both oligotrophs and copiotrophs. However, thinking in terms of dichotomies or extremes will only advance knowledge incrementally. Semenov ends his 1991 piece by suggesting that true oligotrophs and copiotrophs represent extremes of the spectrum and that there are few organisms that occupy these extremes, while the rest of microbial diversity falls somewhere along the continuum, with wide versatility (Semenov 1991). New comparative genomic analyses may want to consider the full spectrum of trophic strategies and begin to place common aquatic microbial genomes along a continuum instead of separated into categorical groups of copiotrophs and oligotrophs. A genomic content continuum can then be validated with information about the physiological rates—respiration rate, Michaelis constant, half-saturation constant, maintenance coefficient (m) (e.g. Semenov 1991)—that quantitatively distinguish heterotrophic strategies along the continuum.

Genomic information provides a link between community structure and trophic strategy. Thus, we can more precisely define oligotrophs and copiotrophs along a spectrum using

comparative genomics and then extract the precise 16S rRNA gene sequences from those genomes to assess the distributions of organisms (across the trophic strategy spectrum) in the environment. This approach would allow us to leverage the many existing studies of 16S rRNA amplicon sequences from many different habitats to assess the distribution of oligotrophs and copiotrophs in space and time and to ask about the consistency of their abundances across similar or connected habitats. Just as oligotrophy and copiotrophy are not true dichotomies, rarity and prevalence also exist along a continuum that can be very dynamic for some microorganisms. Variable dynamics and potentially extreme changes in relative abundance over time is a pattern expected especially for copiotrophs. Thus, relating the dynamic spectrum of member abundance to a genomic-defined (and, as possible, physiologically confirmed) spectrum of heterotrophy likely will provide new insights into lifestyles of rarity.

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