

Organizing principles for marine microbial consortia

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Abstract

Microbiologists commonly speak of microbial communities, but the degree to which assemblies of microbes are governed by the rules of classical community ecology is unclear. Specifically, microbes are much more easily dispersed, and have much faster growth rates, than macroscopic organisms, potentially changing the relative importance of various forces in community assembly. In the well-mixed, liquid environment of the pelagic ocean, these differences are likely to be most pronounced (and most easily studied). Here we develop a framework for understanding community assembly in marine microbial populations. We begin by constructing a simple neutral model that predicts what consortia should look like if they are randomly assembled. From there, we consider what rates determine whether consortia will persist long enough to form a “climax” community. With these possibilities representing “extremes,” we explore possible intermediate successional stages driven by lottery competition for space and/or local co-evolution toward cooperative exclusion of other species. We further discuss what empirically measurable traits we would expect to exist under these four different scenarios and suggest experiments to distinguish between these possibilities.

Section 1. Introduction

Ecological studies are traditionally defined hierarchically based on their scope, from autecology (the study of a single species and its interactions with its environment) at one extreme, ranging outward to community, landscape, and ecosystem ecology. Though they share certain overarching principles (e.g., competitive exclusion, resource limitation), these divisions are useful because the study of larger spatial scales requires different tools as environmental heterogeneity and biodiversity become increasingly important. The complex forces of ecology and evolution drive this heterogeneity, producing biogeographic patterns in species distributions. Whereas landscape and ecosystem ecology clearly transcend the domain of individual organisms and focus on biogeochemical processes integrated across entire regions, community ecology has been described as focusing on a genuine biological unit that develops and matures with distinct stages (Konopka 2009). Central to community ecology is the concept of *succession*—the development of a species assemblage from the first colonization of an uninhabited environment toward a stable *climax* community. The early stages of succession are generally dominated by species with high growth rates or dis-

persal capabilities, whereas the climax is dominated by species that maximize resource use (Odum 1969).

Whereas it remains debatable whether any community ever reaches a genuine climax, the general concept of succession—that community-level processes are functionally distinct from those of individual populations or ecosystems—is an important unifying principle in the study of terrestrial ecology (Odum 1969). Controversy exists, however, as to whether community ecology is relevant in the microbial world. The often-cited Baas-Becking maxim “everything is everywhere, but the environment selects” (De Wit and Bouvier 2006; O’Malley 2007) suggests that bacteria are ubiquitously dispersed, and therefore, the structure of extant communities is primarily a function of the niches present. An alternative to Baas-Becking might be a neutral theory of microbial biodiversity, such as the one proposed for macroscopic organisms by Hubbell (2001)—that microbial communities do not persist long enough for realistic fitness differences between niche competitors to lead to competitive exclusion, and the species abundances in consortia should be similar to that in the surrounding metacommunity. If either of these scenarios holds, microbial processes measured on the grand scale would thus also be very similar to processes measured on the small scale. This kind of thinking is implicit in the justification for much research in microbial ecology, and is particularly evident in the inference of ecological function from metagenomic data in the marine environment (e.g., Frias-Lopez et al. 2008).

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In this chapter, we seek to address this controversy from a theoretical standpoint: do microbial “consortia”—local associations, either intimate or otherwise—represent “communities” in the classical sense, with structure proceeding through predictable ecological stages involving complex interspecies interactions? Or are they merely accidental associations caused by microbes randomly searching their environment and by chance encountering each other? These questions are both interesting and largely unanswered for all microbial communities, but they are perhaps most critical for the understanding of marine microbial assemblages. Unlike organisms in most other environments, marine microbes have the capacity to freely explore their world in three dimensions, and they have a genuine and momentous evolutionary choice of whether to live as a free, planktonic cell or as a member of a community. What forces push marine microbes to choose one way or another, and how do these choices impact the community compositions we observe in nature? We here consider four hypotheses regarding the organization of marine consortia:

H0: Ecological interactions are unimportant in determining the composition of microbial consortia in the ocean. Diversity in any given microhabitat is an equilibrium based on the ability of different species to find, and persist in, that habitat.

H1: “Everything is everywhere, but the environment selects.” Microbes are ubiquitously dispersed, and habitats are composed of a finite number of niches, each of which is occupied by the single superior competitor from the surrounding planktonic metacommunity (i.e., the entire population that has any reasonable chance of joining the consortium). Habitats persist long enough that superior resource competitors dominate most niches, and stochastic primary colonization has only a minor effect on the species present.

H2: Consortia are functionally organized by “lottery.” Based on their metabolic capabilities, microbes have differential abilities to use the resources in different habitats. Whereas the species responsible may differ between consortia in nearly identical habitats due to stochastic colonization, the specific range of functions is conserved. Most habitats do not persist long enough to allow competitive exclusion to come to fruition.

H3: Consortia are strongly influenced by local co-evolution. Species share cooperative and/or antagonistic adaptations that allow them to exclude superior resource competitors, leading to stable assemblages that are very resistant to invasion. Functions beyond these specialized adaptations are largely unimportant; some niches are likely to go unfilled. Important species are consistently found together even when rare in the metacommunity.

Given that microbial consortia occupy countless habitats in the oceans, our task is to develop a testable framework for predicting and understanding their composition. To this end, we will first construct a simple mathematical description of colonization of, and competition over, uninhabited surfaces suspended in the pelagic ocean. From this, we will extract measurable parameters that will suggest experiments that can

falsify the two “extreme” hypotheses above (H0 and H1). We will then present examples of the two “intermediate” hypotheses (H2 and H3) and discuss how they can be empirically distinguished. Last, we will compare and contrast surface-associated consortia with more abstract, purely planktonic communities that may nevertheless develop and evolve in a similarly collective manner.

Section 2. The null hypothesis (H0): swimming and sticking

An increasing number of studies, using techniques ranging from straightforward microscopy to next-generation “omics” methods, have shown significant differences between planktonic and surface-associated species distributions (e.g., Crump et al. 1999; Delong et al. 1993; Sapp et al. 2007). However, we cannot simply infer from such differences that ecological forces are responsible. For instance, 42% of the ostensibly free-living cyanobacteria observed in a recent atomic force microscopy-based investigation were in tight associations with heterotrophic bacteria (Malfatti and Azam 2009). It is unknown whether these relationships are symbiotic, commensal, antagonistic, or even accidental (Malfatti and Azam 2009), yet it is vital that we address this issue because it challenges our fundamental understanding of how pelagic microbial communities are assembled.

How can we tell the difference? First, it is necessary to develop a null hypothesis for what consortia should look like in the absence of any relevant ecological differences. Assuming that all consortia are assembled on or around some type of substrate (henceforth referred to as a *habitat*, to facilitate analogy with established ecological terminology), we suggest that a proper neutral theory of consortium assembly should consider only a) the *discovery* of habitats at random by members of the metacommunity and b) the *affinity* of different species for the habitat once they associate with it. In particular, the neutral theory must disregard all forms of competition, whether over resources or space, as well as any sort of direct interspecies interactions.

We will begin by describing the simplest possible scenario: a monoculture metacommunity of randomly moving, spherical microbes M inhabiting a planktonic environment that contains a certain concentration of spherical habitats T that are targets for colonization. (For reference, variables used throughout this chapter are listed in Table 1.) M has a characteristic radius r_M and moves with speed v (representing physical forcing, intrinsic motility, or a combination of the two); each habitat also has a radius, r_T . M may further be partitioned into a planktonic (M_p) and a consortial (M_c) component, with mass balance:

$$[M] = [M_p] + [M_c] \quad (1)$$

We assume that space on T is not limiting (i.e., M_c may overgrow itself) and that there is no fitness advantage either to

Table 1.

Variable	Meaning
M	Total concentration of a microbial species in the system
M_p	Concentration of M in the plankton
M_c	Concentration of M in consortia
v	Speed
r_M	Radius of an M cell
r_T	Radius of a habitat available for colonization
S	Volume searched by microbes per unit time
T	Total concentration of habitats in the system
D	Discovery rate
k_D	Dissociation constant
k_L	Loss rate of habitats from the system
μ	Growth rate in the consortium of a microbe
N	Concentration of a superior competitor to M in a consortium
w	Fitness advantage of N over M , equivalent to the difference in their growth rates
P	Total population size of all microbes in a consortium
μ_v	Growth rate of a microbe in the vicinity of a habitat
μ_A	Growth rate of a microbe in direct attachment to a habitat
t_i	Average invasion time

the planktonic or the consortial lifestyle. Thus, there are no ecological dynamics within M that are important for colonization of T . We also assume that M and T are at steady state (production and death/loss are balanced), although T 's first-order loss rate k_L from the system (representing, for example, habitats sinking out of the mixed layer) is relevant, because a proportional loss of T also means the loss of the same proportion of M_c . Finally, M has an affinity for T (its "stickiness") that may be expressed as a first-order dissociation constant k_D .

Under these conditions, the proportion of M inhabiting consortia may be calculated. A discovery is made when an M_p intersects with, or passes tangentially to, any T , which occurs if the center of an M_p is within a distance $r_M + r_T$ of the center of a T . The volume searched by each M_p per unit time (S) may thus be described as a cylinder of radius $r_M + r_T$ and length vt :

$$S = v\pi(r_M + r_T)^2 dt \quad (2)$$

Thus, the volume searched by a cell increases linearly with its velocity, but exponentially with the sizes of the searchers and their targets (Fig. 1). S may also be viewed as the probability of any given M_p encountering any given T per unit time; as such, it functions as a second-order rate constant with units of volume \times concentration $^{-1} \times$ time $^{-1}$, and multiplication by the concentrations of T and M_p gives the *discovery rate*:

$$D = S[T][M_p] \quad (3)$$

Let us assume that all M are to some degree "sticky," such that every discovery results in at least an ephemeral associa-

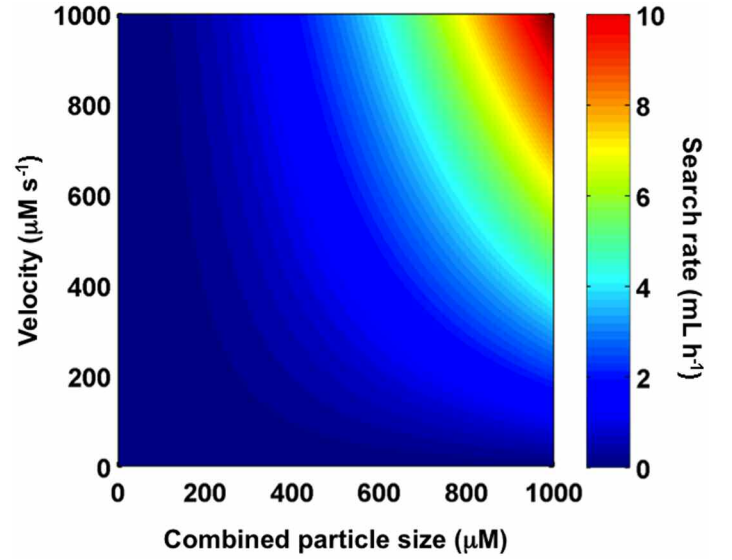


Fig. 1. Influence of velocity and size on microbial "searching." The combined size of habitat "targets" and microbial searchers exerts a stronger influence on the ability of microbes to find a particle than does their velocity.

tion, the strength of which is dictated by k_D . Then loss of M_c is a first-order process governed by k_L (and also the life span of T as described earlier). The dynamics of M_c are thus given as the sum of discoveries (Eq. 3) and losses (from dissociation and habitat loss) per unit time:

$$\frac{d}{dt}[M_c] = D - (k_D + k_L)[M_c] \quad (4)$$

By setting Eq. 4 to 0 and substituting from Eq. 1 and 3, we may calculate the steady-state proportion of M residing on T , valid for any absolute metacommunity population size:

$$\frac{[M_c]}{[M]} = \frac{S[T]}{S[T] + k_D + k_L} \quad (5)$$

Under this ecologically neutral model, the ratio from Eq. 5 can exist over many orders of magnitude with reasonable values of these basic parameters (Fig. 2), including scenarios where a species may be undetectable in one population or the other. Further, because hypothesis H0 rejects space limitation (a potential source of competition considered under hypothesis H2), it is extensible to any number of species, each of which will be represented in a given consortium as a function of a) its planktonic abundance, b) its ability to search the environment, and c) its stickiness.

Clearly, hypothesis H0 may not be trivially dismissed by the observation that a species is much more abundant in consortia than in the plankton. Rejection of this hypothesis requires knowledge of a species' abundance in both planktonic and consortial habitats as well as at least rudimentary knowledge of its motility and affinity for the habitat in question. Whereas

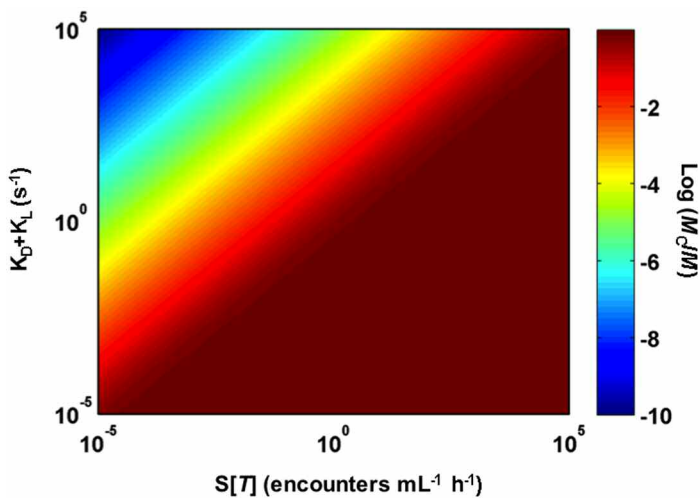


Fig. 2. Influence of searching and sticking on consortium composition. Discovery rate (plotted on the x-axis) and loss rate (plotted on the y-axis) work antagonistically to determine the proportion of the total population residing in consortia. Discovery ranges plotted span realistic values from an extreme minimum ($1 \mu\text{m}$ cells moving at $20 \mu\text{m s}^{-1}$ seeking $100 \mu\text{m}$ habitats present at $1 \text{ habitat mL}^{-1}$) to an extreme maximum ($100 \mu\text{m}$ cells moving at 1 mm s^{-1} seeking 1 mm habitats at $10,000 \text{ habitats mL}^{-1}$). Similarly, dissociation/habitat loss rates span a range from $\sim 1 \text{ loss d}^{-1}$ to $\sim 100,000 \text{ losses s}^{-1}$. Color intensity represents the log-transformed proportion of consortial cells to total cells ($\log M_C/M$).

this level of understanding is clearly unlikely for every member of the metacommunity, it is certainly achievable for specific, cultivable organisms. We suggest that an initial test of hypothesis H0 take a two-pronged approach. First, the richness and diversity of the planktonic and consortial populations surrounding the habitat of interest should be assessed using the most taxonomically informative methods available (e.g., targeted metagenomics or other culture-independent sequence-based approaches). Importantly, the taxonomies of *individual habitats* must be assessed, rather than looking at pooled samples comprised of many habitats: for example, individual marine snow particles (e.g., those collected by SCUBA) as opposed to collections from sediment traps, or individual plants from the same algal stand as opposed to plants from widely separated stands. This replication will allow statistical evaluation of the goodness of fit between Eq. 5 and the empirical community data. Second, representative cultivable organisms discovered in this dataset should be selected to include a) species more abundant in consortia, b) more abundant in the plankton, or c) roughly equal in both; ideally, both rare and common organisms would be chosen. The physical dimensions of organisms and habitats are easily measured, and motility (e.g., Deloney-Marino et al. 2003) and attachment assays (e.g., Gärdes et al. 2010) may be performed on the isolates to fill in these values. Concentrations and lifespans of habitats may be assessed micro- or macroscopically (depending on the habitat in question), and the concentrations of the chosen isolates may be assessed via FISH-tagged microscopy and/or quan-

titative PCR. These observations should allow hypothesis H0 to be supported or rejected, a necessary prerequisite to the investigation of marine microbial consortium organization.

This model is much simpler than the zero-sum ecological drift model presented by Hubbell (2001). For simplicity, we have represented species abundance with continuous variables, whereas discrete variables would be more realistic. We also allow some simple differences between species (speed and stickiness) whereas Hubbell considers all species as exactly equal in their chance of entering and leaving a community. These differences are justified by our different goals: Hubbell's focus was on a universal theory of biodiversity, whereas we are more interested in an empirically testable model. However, we note that our predictions are remarkably similar to Hubbell's—namely, that under the neutral model, the representation of a species in a given community is a relatively straightforward function of its abundance in the metacommunity.

Section 3. Competition and exclusion (H1): does the environment have time to select?

Until now we have foregone any consideration of differential fitness to an organism in or out of a consortium. However, it is certainly the case that different sorts of habitats will favor different sorts of adaptations, and some organisms will be more fit on a given surface than others. In fact, it is possible that for many marine organisms, *no growth at all* occurs in planktonic suspension. Rather, such organisms wait for an encounter with a nutrient-rich surface and grow as fast as possible until the source is exhausted—the so-called copiotrophic lifestyle (Fierer et al. 2007). Bacterial consortia surrounding detritus and marine snow are hot spots for microbial productivity in the ocean, and can represent a sizable proportion of total bacterial productivity (Simon et al. 2002). It seems likely that a copiotroph (e.g., a representative of the marine Roseobacter clade, Mayali et al. 2008) would outcompete a slow-growing planktonic oligotroph (e.g., representatives of the SAR11 clade, Giovannoni et al. 2005) were the two inoculated at their typical seawater densities into a medium containing analogues of the natural substrate under consideration (e.g., marine snow-like particles), leading to consortia dominated by the copiotroph. However, this is not necessarily the case, since competition requires time to take effect. If habitats do not persist long enough, then there may be insufficient time for fitness advantages to give copiotrophs a numerical edge on the less fit, but more numerous, oligotrophs, leading to an H0 community composition. On the other hand, given sufficient time, the Baas-Becking scenario becomes possible: every member of the metacommunity will be able to sample the new habitat, and eventually the new niche will be dominated by the single fittest species—i.e., the environment selects the superior resource competitors. In this case, the copiotroph would become numerically dominant in consortia. Thus, the realization of the Baas-Becking statement is possible, but requires a (poten-

tially calculable) period. In this section, we consider three rates—*habitat loss*, *discovery*, *invasion*—whose magnitudes will permit experimental falsification of the Baas-Becking hypothesis (H1).

Habitat loss

In order for hypothesis H1 to hold, a habitat must last long enough for early, less fit colonizers to be supplanted by latecomers with growth advantages. We have already quantified the loss rate of T in the discussion of hypothesis H0 using the first-order constant k_L ; the average life span of any given T is thus k_L^{-1} . “Life span” can mean many things, but a safe operational definition might be the time between ecological disturbances that profoundly change the parameters of habitat colonization and use. Its definition strongly depends on the type of habitat one is considering, and perhaps on the types of resources it contains. For instance, k_L for a consortium residing on a seaweed may represent the life span of the plant, although it could be much longer if the resources of the plant continue to be available to the consortium *post mortem*. For a marine snow particle, k_L may represent the time required for the particle to transit to the sediments; alternatively, it could be much shorter if we require that the attached population remain in contact with the mixed layer planktonic population from which it was seeded. Importantly, however, these questions (e.g., life histories, settling times, and so forth) have been explored for many years by microbial ecologists and oceanographers, and therefore k_L should be relatively straightforward to quantify.

Discovery

To dominate a habitat, an organism must first find it. In our discussion of H0, we established that the discovery rate D (Eq. 3) increases linearly with both an organism’s motility speed and its planktonic concentration, whereas its size, and the size of the targets, have an exponential effect (Eq. 2). In general, then, we expect larger organisms to be superior in the search for habitats, and larger habitats to be easier to find. Conversely, rare bacteria seeking sparsely distributed habitats face a daunting task. Let us assume that the superior competitor N for most habitats is rare in the plankton. (Because we know that the majority of planktonic organisms are oligotrophs such as *Pelagibacter* and *Prochlorococcus*, this is probably a reasonable assumption for many habitats.) By definition, this organism has a high growth rate μ_N once it has discovered the habitat; therefore, let us assume that $D_N \ll \mu_N$. Under these assumptions, then, only the first discovery of a habitat by N is important; subsequent discoveries are overwhelmed by the growth of the first colonizer. The average time between discoveries is simply the inverse of the discovery rate, or D_N^{-1} .

Invasion

An organism with greater fitness is able to invade a habitat, and will drive less fit organisms sharing the same niche to

extinction so long as conditions remain constant for a long enough period. Let us consider the population we described earlier, M , faced with a competitor N that is more fit by a factor $w = \mu_N - \mu_M$, where μ represents the net growth rate (inclusive of the actual rate of increase less the dissociation constant k_D) in the consortium of N or M , respectively. If we assume that each new N displaces an M (i.e., population size P is constant), then the change in the ratio of N to M over time is given by:

$$\frac{[N]}{[M]} = \frac{[N]_0}{[M]_0} e^{wt} \quad (6)$$

Let us assume that M is the organism that would dominate the habitat under H0, and that it is the only organism present when N first discovers the habitat. This is likely to be true when $D_N \ll \mu_N$ and $D_M \gg D_N$, i.e., when M is the superior seeker, and N , the superior competitor, inefficiently discovers the habitat as described in the previous section. Under these assumptions, we may construct an argument that can falsify H1 by asking if it is possible for differential fitness alone to allow N to displace M . Presuming that both M and N are cultivable, fitness w can be assessed by measuring the growth rate of pure cultures of each organism on analogues of the habitat in question (e.g., Poltak and Cooper 2011). Under our assumptions, Eq. 6 can be substituted with M_0 equal to the empirically determined mean population size P of competing individuals in a consortium (perhaps the carrying capacity of a given niche), and $N_0 = 1$. If we assume competitive exclusion occurs when $n = 0.99P$, then the average time to exclusion (t_I) is given by summing the time required for a single N to find the habitat and the time required for that colonist to displace an M monoculture:

$$t_I = D_N^{-1} + \frac{\ln(99P)}{w} \quad (7)$$

If t_I is significantly greater than k_L^{-1} (i.e., the time the habitat has experienced the current selective environment), then forces other than simple growth rate competition are at work in structuring the consortium. Such an observation would indicate that one of the organisms was more fit than axenic culture experiments would suggest, perhaps implying some sort of interspecies interaction between M and N . In such a case, hypothesis H1 is not supported.

Fig. 3 graphically depicts the influence of fitness and discovery time on the ability of superior colonizers to invade a habitat. Colors represent t_I , which clearly decreases with w and increases with D_N^{-1} . The upper portion of the figure, where $w > D_N^{-1}$, corresponds to Hypothesis H1; N rapidly fixes after its initial colonization. However, as w decreases below the rate of colonization of M , invasion takes much longer. Under such conditions, H1 is likely not supported.

One objection to this model is that it oversimplifies any real-world situation for the sake of mathematical tractability (i.e., we consider the interactions of only two species and completely disregard the concentrations of the resources for

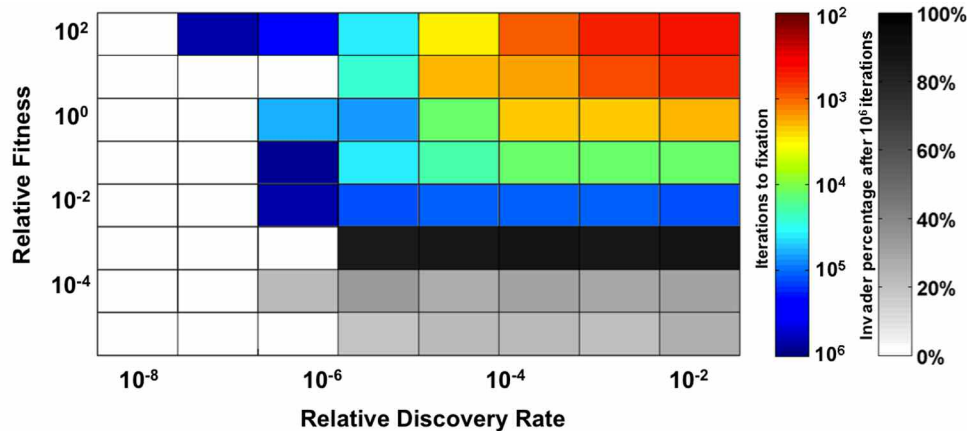


Fig. 3. Relative impact of fitness and discovery rate on the invasion time of superior competitors from the metacommunity. Colonization of a habitat was simulated in silico using parameters similar to those described under Hypotheses H1 and H2. A fixed maximum population size of 10^6 individuals was assumed, with 100% of the initial community being comprised of the superior colonizer, M . In each iteration of the model, 1% of the population was removed and replaced at random with either M or N cells. The proportion of empty spaces filled with N cells was calculated based on the growth rate advantage of N compared to M (“relative fitness,” shown on the Y-axis as a multiplier of the growth rate of M , $0.001 \text{ iteration}^{-1}$) and the relative discovery rates of the two types (shown on the X-axis as a multiplier of the discovery rate of M , $0.1 \text{ iteration}^{-1}$). Colored boxes indicate simulations where N went to fixation (99%) within 10^6 iterations; the intensity of the color represents the number of iterations required to reach this point. Grayscale boxes indicate communities that did not fix, and the darkness of the box indicates N 's proportion of the population at 10^6 iterations.

which these species are competing). This is certainly true, but we argue that almost any complication of the scenario—for instance, a distribution of fitnesses in the preexisting consortium, co-limitation by multiple resources, or spatial interactions—would tend to increase t_p , with the result that H1 would become *less* likely. Thus, Eq. 7 represents the most permissive conditions for a Baas-Becking climax community to arise, and if falsified, added complexity is unlikely to make H1 possible.

We should note that our assumption that superior competitors will not also be competent searchers of the environment is not necessarily true. For instance, consider the problem of chemotaxis toward resources. Several studies suggest that many real organisms have mechanisms for detecting desirable habitats and avoiding undesirable ones (e.g., Chet and Mitchell 1976; Kjørboe et al. 2002; Willey and Waterbury 1989; Yu et al. 1993). Such senses are obviously of great importance for habitat colonization; a bacterium that can chemotax toward a habitat has an advantage over one that must run headlong into it (Stocker et al. 2008). One may speculate that the evolution of chemotaxis would be favored in lineages that have adapted to consortial growth, i.e., are superior competitors. If this were the case, it would not be possible to disregard the discovery *rate* (as opposed to the discovery *time* incorporated in Eqs. 7 and 8) when describing recruitment of the species to a consortium. However, since we assume that consortial dynamics do not affect the metacommunity relative species abundance, discovery occurs at a fixed, linear rate (Eq. 3), whereas invasion is exponential (Eq. 6). Therefore, provided $\mu_N \gg \mu_M$, then the conclusions we draw about invasion should be *generally* sound. When μ_N and μ_M are similar,

Hypothesis H1 becomes unlikely because of the very slow resulting invasion rate (Eq. 6 and Fig. 3). We will explore this case below under hypothesis H2.

Section 4. The intermediate hypotheses (H2 and H3): lotteries and conspiracies

Hypotheses H0 and H1 represent two “extreme” views of community ecology. On one hand, H0 describes a community at the earliest stage of succession, dominated by organisms that are superior colonizers due either to their abundance or their ability to find and stick to a habitat. On the other hand, H1 communities are entirely defined by resource competition. In recent years, significant attention has been paid to identifying assembly rules or mechanisms behind microbial community assembly in a variety of environments (e.g., Jeraldo et al. 2012; Langenheder and Szekely 2011; Stegen et al. 2012), and it is becoming increasingly evident that both neutral (H0-like) and deterministic (H1-like) forces can operate simultaneously in the same habitat. Hypotheses H2 and H3 explore such intermediate cases. Unlike H0 and H1, these hypotheses are not mutually exclusive, but rather represent opposing forces (competition for space and co-evolution) whose relative magnitudes in a given consortium are likely to influence consortium succession.

H2: the lottery hypothesis

In our consideration of H1, we stated that expansion of the model to more than two organisms was unlikely to improve the odds of an H1 scenario. However, what can we expect to happen in a community subject to colonization by many organisms with the same or similar niches? In H0 and H1, we

considered communities that were initially dominated by organisms that were relatively abundant in the planktonic metacommunity, and then subsequently displaced by rarer organisms that were more fit in pairwise competitions. In both cases, we assumed that *space was not limiting*, and that organisms could essentially colonize the habitat forever, such that even the rarest organism would eventually exist in consortia at some equilibrium concentration. What happens if we lift this assumption? For instance, what if the metacommunity contains a variety of species that are each able to displace the initial colonizers of a habitat, but not each other (at least not over the life span of the habitat)? Then we might expect individual consortia to be dominated by whichever species happened to colonize it first.

Hypothesis H2 describes such a consortium and corresponds to the *lottery hypothesis*. Originally developed to describe the coexistence of similar fish on a coral reef (Sale 1977), the lottery hypothesis maintains that species within a given ecological guild (i.e., species that exploit similar ecosystem resources and thus overlap in niche requirements, Simberloff and Dayan 1991) colonize a given niche space by chance, and the first species which colonizes the niche, wins the territory. Like H0, lottery competition assumes that members of the guild do not have significant competitive advantage over one another and just as niche colonization occurs by chance, the niche is also vacated by chance (Sale 1977). Like H1, growth in the consortium is important for H2, but diversity is maintained at a high level because colonization is rare enough that colonists can usually monopolize niche space before the arrival of other guild members. In concrete terms, we expect the organisms in an H2 consortium to have very low k_D , i.e., to dissociate rarely from the community, and relatively high growth rates in the habitat. We also expect the dominant organisms to be rare in the plankton, such that colonization for any given species on any given habitat is rare (i.e., high D_N^{-1}). Like H1 consortia, we expect to see clear trophic structures and niche exploitation, as well as characteristic gene presence and expression patterns. However, we expect the diversity of organisms to be much higher than in a climax community. Further, the between-habitat variability in presence/absence of species is expected to be much higher than that in functional, niche-defining genes. In other words, certain niche-specific ecological roles or functions *will* be performed, but there is a good deal of variability as to *who* performs them.

The first application of the lottery hypothesis to marine consortia was published recently by Burke and co-authors (2011b). Bacterial epiphyte communities associated with the benthic green macroalga *Ulva australis* provide anti-fouling protection against eukaryotic larvae and fungi (Rao et al. 2007) and facilitate normal morphological development (Marshall et al. 2006). Burke et al. (2011b) found almost no crossover between the taxa present in these consortia and those in nearby planktonic populations. However, the micro-

bial community composition also varied dramatically between *U. australis* individuals, and no “core” species specific to all individuals were discovered. Similar results have been observed for microbial communities which persist within colonies of *Trichodesmium* spp. (Hmelo et al. 2012): bacterial epibionts associated with two sets of *Trichodesmium* colonies (10 colonies each set) were observed to be mutually exclusive at the ‘species’ level (97% similar operational taxonomic units based on amplification community 16S rDNA genes). Whereas no explicit test of H0 was included in these studies and these studies included no assessments of fitness in axenic culture, based on the reported levels of within-niche diversity and between-habitat variance, we may tentatively say these studies support H2. Despite high variability between individuals, the extreme discrepancy between the planktonic community and that on *U. australis* suggests that the overall epiphyte community is adapted to life on this particular host. From this observation, the authors inferred that each niche is filled by a member of a guild of ecologically redundant bacteria with diversity maintained by a strong premium on early colonization.

We propose that the lottery hypothesis also applies to consortia centered around organisms that are metabolically “incomplete.” For instance, syntrophic relationships occur when the waste products of one organism are the growth substrate for another. Perhaps the most famous example is the anaerobic oxidation of methane (Boetius et al. 2000; Knittel and Boetius 2009): in consortia of methanogenic archaea and sulfate-reducing bacteria, the bacteria make reverse methanogenesis thermodynamically viable by removing waste products generated by the archaea. Similar arrangements are found in other anaerobic methane-oxidizing communities (e.g., amongst the AMO communities that couple denitrification to AMO, McNerney et al. 2009 and references therein), although the taxonomic identities of the players change. Thus, consortium formation around a methane source requires the functions of both *types* of organism, but the specific species are not necessarily conserved, consistent with H2.

Similarly, some marine organisms depend on “helpers” to fulfill metabolic requirements that they have lost the ability to perform, perhaps due to reductive evolution (Morris et al. 2012). Often, the helper supplies an essential growth factor. For instance, many organisms require vitamin B₁₂ as an essential cofactor in methionine biosynthesis, but this compound is produced exclusively by bacteria (Giovannoni 2012). Half of the more than 300 species of eukaryotic algae studied by Croft and co-authors (2005) are vitamin B₁₂ auxotrophs, and there is some evidence of similar dependencies in cyanobacteria (Van Baalen 1961). These algae are important sources of primary production and potential food sources for bacteria; however, they cannot grow unless they are provided with B₁₂. Thus, consortium formation requires B₁₂ provision, though it appears to be irrelevant which of the many B₁₂-making bacteria perform this function. Similarly, a recent study demon-

strated that many “unculturable” marine bacteria required siderophores produced by helpers to acquire sufficient Fe for growth (D’Onofrio et al. 2010), and again the origin or type of siderophore was largely unimportant.

Helpers can also remove toxins, cross-protecting vulnerable organisms at the same time they protect themselves. This is perhaps most familiar to biologists in the appearance of “satellite colonies” on agar plates containing ampicillin. β -lactamase, the enzyme that destroys ampicillin, is secreted extracellularly. As the β -lactamase is produced by resistant colonies, it diffuses into the surrounding agar and deactivates ampicillin in the medium, thereby allowing ampicillin-sensitive cells to grow; ampicillin-resistant organisms cannot help but save susceptible organisms from being killed. In the ocean, toxin-removing helpers are emerging as an important phenomenon structuring microbial communities. For example, epibionts of the filamentous N_2 -fixing cyanobacterium *Trichodesmium* may lower the local O_2 concentration, protecting oxygen-sensitive nitrogen-fixing activities (Herbst and Overbeck 1978; Paerl and Pinckney 1996). Helpers also protect the unicellular cyanobacterium *Prochlorococcus* from solar-generated hydrogen peroxide (HOOH) (Morris et al. 2011). In this case it is the toxin, HOOH, which is freely diffusible across cell membranes, so intracellular HOOH-scavenging enzymes in the helpers ultimately lower the extracellular HOOH concentration and facilitate *Prochlorococcus*’ growth. The ability to help dilute cultures of *Prochlorococcus* survive in the laboratory was ubiquitous, with not a single non-helping species found out of dozens screened (Morris et al. 2008). As *Prochlorococcus* has evolved in the presence of the planktonic microbial community, it has lost the genes necessary to produce catalase enzymes that deactivate HOOH. By reducing the size of its genome and the burden of producing a metabolically (and trace nutrient) expensive protein, loss of catalase provides a fitness advantage in the nutrient-poor open ocean. Morris et al. (2012) speculate that the absence of HOOH-detoxifying enzymes in other marine oligotrophs (e.g., *Pelagibacter ubique*) suggest that the majority of marine bacteria are dependent on help to tolerate HOOH. Since *Prochlorococcus* probably forms the base of the food chain in the oligotrophic open ocean (Bertilsson et al. 2005), it is the focal point of a vast, planktonic consortium (a concept we will discuss in greater detail later) that requires HOOH-degrading helpers for its continued existence; however, since this function can be carried out by many different species, we expect the identity of the helpers to vary widely amongst samples, consistent with H2.

H3: Cooperative exclusion

To this point, we have not considered beneficial or antagonistic interspecies interactions between species that exploit similar ecosystem resources, although it is certain that such interactions exist and are likely to be important in some circumstances. We will consider two examples that are known to exist in marine environments and are important in the devel-

opment of consortia in other environments: interspecies quorum sensing (QS) and antibiotic production.

QS is a form of cooperative behavior, which is cell-density dependent and typically benefits individuals of high genetic relatedness (Keller and Surette 2006). QS bacteria constitutively produce low levels of small chemical signals that diffuse out of the cells into the extracellular environment. Bacteria interpret the extracellular concentration of these signals as a proxy for the density of like-cells in the immediate environment. At a given threshold concentration, the population will synchronously turn on a set of genes. One particularly well-studied QS system uses acylated homoserine lactones (AHLs) as signaling molecules. AHL-QS is used by bacteria to regulate group-beneficial metabolisms such as biofilm formation, luminescence, and antibiotic production (e.g., Miller and Bassler 2001), only turning them on when the most likely recipients of their effects are closely related organisms. All AHLs contain a homoserine lactone ring bound to a fatty-acyl or aryl side chain (Eberhard et al. 1981; Schaefer et al. 2008); importantly, QS-bacteria have evolved unique AHL-synthesis proteins, which produce structurally modified side chains that afford the signals a degree of taxonomic specificity (Miller and Bassler 2001). AHL-QS bacteria have been isolated from numerous marine environments including the surface of eukaryotic algae (Wagner-Dobler et al. 2005), cyanobacteria (Van Mooy et al. 2012), marine snow (Gram et al. 2002; Hmelo et al. 2011), corals (Tait et al. 2010), and as symbionts with marine animals (Nyholm et al. 2000). AHL-QS has been implicated in the regulation of hydrolytic enzyme activity in marine snow and within *Trichodesmium* colonies (Hmelo et al. 2011; Van Mooy et al. 2012).

Many bacterial pathogens use QS to initiate virulence upon invasion of a host (e.g., Whitehead et al. 2001 and references therein); in such cases, QS simply contributes to a single species’ fitness, and consortia strongly influenced by such intraspecies QS effects would likely resemble predictions from H1 or H2. However, there are many examples of *interspecies* recognition of QS signals. For instance, QS signals from bacteria can act as settlement cues for eukaryotic algal spores (Joint et al. 2002; Tait et al. 2005; Tait et al. 2009). There are also many examples of bacteria that can mimic or recognize the QS signals of other species whether or not they also produce the signal themselves (Dulla and Lindow 2009; Riedel et al. 2001; Subramoni and Venturi 2009 and references therein). It is possible that different species that share QS signals can co-ordinate their behaviors so as to exclude invaders that do not “speak the language.”

Cooperative behaviors, such as QS, can also be exploited to regulate competitive behaviors. For example, consider the production of antimicrobials, used both by bacteria and eukaryotes to control associations with other microbes. The production and release of antimicrobials is frequently, although not exclusively, controlled by QS (Hibbing et al. 2009). As such, bacteria associated with high cell-density environments, such

as detritus particles (Grossart et al. 2004; Long and Azam 2001; Rypien et al. 2010), are more likely to engage in antagonistic interactions than free-living bacteria (Grossart et al. 2004; Long and Azam 2001; Rypien et al. 2010). In fact, the production of antimicrobials has been demonstrated to be an important determinant in the community development of human oral biofilms (Hibbing et al. 2009; Kuramitsu et al. 2007), and we might infer it is also an important determinant in the formation of environmental biofilms.

Secreted antibiotics are strong weapons in the competition for space, and help reserve territory for the producing species and its descendants. However, the existence of antibiotic resistance complicates matters somewhat. For instance, it seems likely that organisms that are often in the presence of antibiotic-producing species (i.e., they compete for space on the same habitats) will harbor at least a minority population with antibiotic resistance genes. As an example, *Vibrio* populations closely related to antibiotic-producing strains were more likely to be resistant to those antibiotics than more distantly related genotypes (Cordero et al. 2012). A consortium composed entirely of such antibiotic-producers and -resisters could easily develop, with the single trait of antibiotic resistance producing a huge fitness advantage over any potential competitor, regardless of the relative strengths of resistant and susceptible organisms for resource competition.

Despite their obvious differences, the interactions considered under H3 share two things in common. First, their fitness effects are not *directly* related to competition for limiting resources, and therefore *only* manifest when other species are present (i.e., they would be invisible in axenic cultures). A corollary of this statement is that H3 consortia could easily leave some niches unfilled (i.e., there may be no photosynthetic organisms that can resist a certain antibiotic), a highly unlikely scenario for consortia constructed under the other hypotheses we have discussed. Second, it is at least possible for these interactions to depend on rare, heritable traits, allowing only select species to gain the benefit from the interaction. The net result of these special adaptations may be to reserve some habitats for a clique of species who “know” how to get along with each other. By mutual action, such co-evolved groupings may be able to exclude invaders who, in one-on-one competition, could exclude either of the cooperators—hence *cooperative exclusion*.

How, then, can we distinguish between H2 and H3 communities? Clearly, the broad array of manifestations of these

two concepts precludes the clean models with which we sought to address the “extreme” hypotheses. Rather, we think that metagenomics might hold the answer. First, we suggest that habitats should be sequenced *individually*, sacrificing sequencing depth for replication if need be. Second, both targeted sequencing (for species abundance, by 16S) and shotgun sequencing (for functional genes) should be performed. (As an alternative, we note that both of these goals may also be achieved using PhyloChip [Brodie et al. 2006] and GeoChip [Wang et al. 2009] microarray methods.) The unifying characteristic of H2 and H3 consortia is that their metagenomic signatures should be in disagreement with the predictions of the two extreme hypotheses, H0 and H1. Each hypothesis makes certain testable predictions, summarized in Table 2. H0 consortia should be phylogenetically consistent from one habitat to the next, should consist of organisms that are significant members of the surrounding plankton, and probably will not have any obvious niche partitioning in terms of functional genes. H1 consortia should also have low variance between habitats but, in contrast to H0 consortia, should be dominated by organisms that are rare or undetectable in the plankton, and should show clear signs of trophic structure based on functional genes. The predictions of H2 are similar to H1, but separate habitats should produce significantly different 16S libraries, i.e., there should be a high level of between-habitat variability (e.g., Burke et al. 2011b). Last, in H3 consortia certain species and/or unusual functional genes (i.e., antimicrobials or quorum sensing genes) should be consistently found together. These functional genes may or may not be directly related to resources present in the habitat, but should involve specific cooperative or antagonistic interspecies interactions that serve to exclude naïve species from the consortium. Because of these specific interactions, the dominant players of H3 consortia are unlikely to be common in the plankton.

Let us consider two recent studies that illustrate communities consistent with the predictions of hypotheses H2 and H3. First, Burke and co-workers (2011a) used a metagenomic approach to test the role of a competitive lottery model in the assembly of microbial communities associated with the alga, *U. australis*. They determined that individuals shared only 15% species similarity among their epibiotic bacterial population, although within those associated populations, functional genes (characterized using COG and SEED annotations) were 70% similar. Their data strongly support the lottery hypothesis (H2), although it remains to be seen whether these

Table 2.

Hypothesis	16S	Functional genes	k_b	Dominant organisms are
H0	Similar between habitats	No signal	High?	Common in the plankton
H1	Similar between habitats	Clear trophic structure	Low	Rare in the plankton
H2	Different between habitats	Clear trophic structure	Very low	Rare in the plankton
H3	Consistent groupings	Can go either way	Low	Rare in the plankton

functionally defined guilds are specific to *U. australis* or more broadly distributed amongst similar algal species.

In complementary work, Fan and co-workers (2012) analyzed phylogenetic and functional profiles of sponge microbiomes. In contrast to *U. australis* communities, they found the phylogenetic profile of sponge-associated bacteria varied little between individuals of the same host species. In contrast, closely related sponge species sampled from the same geographical area harbored quite different microbial flora, although the representation of functional genes was similar. These findings are suggestive of co-evolution, either between the sponge and its microbiome, between individual taxa of the microbiome, or both. Thus, the sponge microbiome appears to be best described by H3.

Section 5. Planktonic “consortia”

In the foregoing sections, we have mostly discussed traditional, surface-associated consortia. It has been stated that the majority of the world’s microbes reside in biofilms (Stoodley et al. 2002); however, in the ocean, massive populations of nominally free-living, planktonic organisms thrive, sometimes reaching densities higher than 10^6 cells mL^{-1} (Kirchman 2008). There is a temptation to think of these organisms as existing in a vacuum, only indirectly affecting each other through their manipulation of the chemical properties of the seawater. However, we think there are several ways where even some planktonic organisms may be thought of as consortia.

Let us now allow a habitat to have a variable size not limited to its physical volume—we will call this its “effective radius.” For instance, imagine a large unicellular alga that exudes some form of polysaccharide into its environment. To use this resource, a heterotroph could attach itself to the alga directly. However, we expect the sugar to be present at a decreasing concentration to a certain distance away from the cell as it diffuses into the medium (the so-called “phycosphere,” Bell and Mitchell 1972). An organism could gain *some* of the benefit of consortial existence simply by spending a good deal of its time *near* the alga, without ever actually committing to life in an attached milieu. Microscopic observation of plankton suggests that many marine bacteria may behave this way, staying close to algae without actually attaching (Azam et al. 1983). The modeling of the fitness effects of this choice are complicated by the effects of diffusion and use on the resource gradient extending from the alga (or other similar habitat, e.g., marine snow, Azam and Malfatti 2007), but we can broadly state that there are conditions where it would clearly be more advantageous to trade lower access to resources (or for that matter, any other fitness-enhancing effects, such as toxin removal) in favor of the greater flexibility of the planktonic lifestyle. Specifically, there is no advantage to direct attachment if

$$\mu_V > \mu_A - k_D - k_L \quad (8)$$

where μ_V and μ_A are the net growth rates of the organism in the vicinity of the habitat and in direct attachment, respectively. One clear way this inequality could be made true is by decreasing the life span of the target; an organism that doesn’t attach would not be at risk of disappearing with its substrate. Despite the apparent differences between these lifestyles, the dynamics of such a distributed “consortium” can generally be made to fit the models described in the previous sections, simply substituting an effective radius (i.e., the distance at which μ_V is equal to the planktonic growth rate) for r_T in Eq. 2.

The ocean is also not completely chemically homogenous, and micro- and mesoscale resource patchiness could lead to consortium-like dynamics amongst planktonic microbes. Hydrothermal vent plumes, cold-water eddies, oil spills, and algal blooms all create spatially defined regions that have greatly different resource characteristics from the surrounding waters (Edwards et al. 2011; Karrasch et al. 1996; Morris et al. 2006; Riemann et al. 2000; Sunamura et al. 2004). Colonization of these regions should proceed in much the same way as colonization of any other new habitat, and succession should proceed along the lines that we have described without any requirement for direct association.

In contrast to surface-attached populations, it is difficult to imagine hypothesis H3 holding for distributed consortia, particularly of this latter kind that applies to potentially large regions of the ocean. First, the large size of such habitats, and their general openness to discovery by any member of the metacommunity, suggests that they should be ripe targets for invasion by superior competitors. Second, co-evolution implies the reliable presence of two species in the same habitat. Whereas this *can* occur in the plankton, and even might often occur, it is difficult to guarantee it. Since diversity in the plankton probably provides a great deal of functional insurance against perturbation (e.g., the *Prochlorococcus*/helper interaction, Morris et al. 2011), individual species could easily come and go with little effect on the overall fitness environment. As the fitness benefits of co-evolved consortia depend on the continued presence of the co-evolved species in the absence of potential invaders, it stands to reason that, should such interactions develop in the plankton, they would tend toward closer and closer association until direct attachment became commonplace. In other words, if an H3-like association arose in a planktonic environment, it is highly likely that it would evolve toward a surface-attached lifestyle. There is increasing evidence, however, that this is commonplace amongst “planktonic” organisms, and that ecologically interacting species have more direct connections than are commonly supposed (Malfatti and Azam 2009).

Section 6. Where to start?

We hope that the hypotheses that we have developed here will be useful for researchers looking for new tactics in the study of marine consortia. However, the sheer scope of possible systems, and possible types of consortia, make it a daunting task to

settle down on one system that is most likely to yield useful results. We would like to suggest two well-studied consortia that we think would be ideal places to begin to test our ideas: *Trichodesmium*-associated consortia and the coral “holobiont.”

As mentioned briefly earlier, *Trichodesmium* spp. are globally important nitrogen-fixing cyanobacteria (Capone et al. 1997). Like *Prochlorococcus* (although with the notable exception of *T. erythraeum* IMS-101), they cannot be cultured axenically using traditional cultivation methods (Waterbury 1991). In nature and in culture, *Trichodesmium* form macroscopic aggregates invariably colonized by diverse ‘epibiotic’ bacteria and zooplankton (Hmelo et al. 2012; Sheridan et al. 2002). Members of these consortia appear to be *Trichodesmium*-specific: they are rarely, if ever, detected in surrounding planktonic communities and are not found in other marine biofilm communities (Hmelo et al. 2012). Similar observations have been made for consortia surrounding other diazotrophic cyanobacteria (e.g., *Nodularia spumigena* [Tuomainen et al. 2006] and *Anabaena* [Stevenson and Waterbury 2006]). Whereas these interactions are not yet well defined, it seems reasonable that the interactions are in some way mutualistic, with the heterotrophs benefiting from the fixed C and N produced by their cyanobacterial host and the hosts benefiting from as yet unknown helper effects (see above). Still, current work on these systems is not sufficient to decide which hypothesis best describes these consortia. Are they organized by functional lottery? Are epibionts to some degree vertically transmitted, leading to specific semi-hereditary symbiotic H3 associations (as in *Vibrio fischeri*, Nyholm et al. 2000)? Or are these aggregating bacteria simply large targets that are colonized more or less at random? We suggest that these communities are prime targets for empirically testing the concepts laid out in this work. Aggregating diazotrophs are relatively easy to find and measure, and they present discreet habitats that may be collected from a common site and examined separately, allowing true replication and robust statistical exploration of metagenomic data. Moreover, *Trichodesmium* aggregates may be sustained at least temporarily in cultures with their epibionts relatively intact, potentially allowing direct measurements of attachment affinities for different members of the community, even without axenic cultivation of *Trichodesmium*.

Another fascinating consortium with great import to conservation efforts is the bacterial community associated with corals. Recent work addressing the role and composition of coral microbial communities has led researchers to propose the idea that the coral along with its essential microorganisms constitute a so-called ‘holobiont’ in which all the constituent members exist in a consortial arrangement (e.g., Rohwer et al. 2002). The holobiont may be more resistant to environmental fluctuations than the axenic host because the microbial population can evolve and adapt more rapidly than can the host genome (Yachi and Loreau 1999; Zilber-Rosenberg and Rosenberg 2008). It has been suggested that the genetic information

contained within the holobiont (the “hologenome”) should be considered a unit of selection in evolution. This idea has gained favor with many microbiologists for other microbial assemblages associated with multicellular eukaryotes; for instance, it is often stated that only 10% of the cells in a human being are of human origin (Savage 1977), and therefore much of our functional genetic repertoire is encoded by our associated microbes. Whereas it is recognized that nearly all animals and plants form symbiotic relationships with microorganisms (Zilber-Rosenberg and Rosenberg 2008), the corals remain the single marine system studied as such. There are clear connections between shifts in the coral microbiota and the onset of disease (Bourne et al. 2009; Reshef et al. 2006) and bleaching events (Bourne et al. 2008), and therefore it is of pressing importance to understand how the holobiont develops as well as how it is maintained. Last, the accessibility and stability of corals, as well as generations of research surrounding specific reefs, will allow replication of community analyses not only spatially but also temporally.

Section 7. Conclusion

It appeals to human intellect to envision the natural world partitioning its productivity in a manner analogous to our own systems of economics. We have tended to look at communities of organisms with an eye pre-adapted to discovering patterns like those driving our own world: supply and demand, competition for resources and space, cooperation in the construction of public goods. However, it is also possible that none of these forces truly acts as an important factor in the structuring of natural communities, particularly microbial ones. The ocean, in particular, seems a likely testing ground for neutral theories of community ecology, because it has no rigid boundaries separating one habitat from the next, nor for the most part any strong temporal barriers to the global dominance of superior niche competitors. Thus, it is equally possible a priori that the ocean’s population could be a randomly mixed hodgepodge of species, or else an extremely non-diverse pool of nearly perfectly adapted species. In this work, we have attempted to define a falsifiable neutral theory of marine microbial consortium assembly, and also a test of the classic “everything is everywhere” hypothesis. We have also described two theoretical intermediates, as well as how we expect them to manifest in real-world communities if they are true. We feel that much fieldwork looking at marine microbial communities has been driven by interest in particular systems rather than the idea of consortium dynamics in general. It is our hope that this work will inspire future researchers in microbial community ecology to address these questions from a more fundamental perspective.

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