I. Conceptual overview: Does biodiversity regulate parasite abundance?

As Earth's ecosystems experience rapid biodiversity change, disease ecologists have turned to an urgent question: how might reductions in biodiversity affect the transmission of parasites? In other words, does biodiversity loss increase the abundance of parasites by eroding natural "checks and balances" on transmission? Or does it decrease parasite abundance by removing the free-living biodiversity on which parasites depend? Answers to these questions are urgently needed if we are to mitigate or prevent an uptick in parasite transmission for ecosystems experiencing biodiversity loss, but debate rages over whether these increases in parasite transmission should be expected at all (Keesing et al. 2010, Randolph and Dobson 2012, Lafferty and Wood 2013, Ostfeld 2013, Ostfeld and Keesing 2013, Salkeld et al. 2013, Wood and Lafferty 2013, Wood et al. 2014a, Civitello et al. 2015a, Civitello et al. 2015b, Levi et al. 2016, Wood et al. 2016, Wood et al. 2017). In part, this dissension arises because we lack the comprehensive, multi-host, multi-parasite, broad-spatial-scale dataset needed to formulate a convincing empirical test. **Our team can answer this recalcitrant question, using a dataset of unprecedented replication and taxonomic and spatial resolution, by exploiting the advantages of a marine model system**. Here, we propose a natural experiment in which we will quantify the abundance of parasites across a highly resolved gradient of biodiversity, for more than 77 parasite species and 18 replicate coral reef ecosystems. Our dataset will provide a comprehensive view of the biodiversity–parasite abundance relationship, revealing how its direction, shape, and scale-dependence vary across a diverse array of parasite taxa, and resolving questions of burning interest in the disease ecology literature.

Previous work by this team of PIs hints that biodiversity has an important role to play in determining parasite abundance. Our data demonstrate that fishing drives marine parasite abundance (Wood et al. 2013, Wood et al. 2014b, Wood et al. 2015). Parasite species with complex life cycles (i.e., those that obligately require more than one host species) decline in the presence of fishing (Wood et al. 2014b, Wood et al. 2015; **Figure 1**). On the other hand, directly transmitted parasites (i.e., those parasites transmitted among hosts of the same species) can increase dramatically with increasing fishing pressure (Wood et al. 2014b, Wood et al. 2015; **Figure 1**). Meta-analysis reveals that these outcomes hold for parasites of finfish and invertebrates around the world (Wood and Lafferty 2015). While these patterns are robust, the mechanisms that produce them have not been identified.

We hypothesize that the relationship we have observed between fishing and parasite abundance is driven by an intermediary variable: the biodiversity of free-living (i.e., non-parasitic) species. Specifically, we suspect that fishing changes fish biodiversity, and that this change in fish biodiversity influences parasite abundance. Interest in the relationship between biodiversity and parasite abundance has grown rapidly in the past decade, driven primarily by work in terrestrial and freshwater ecosystems (Johnson et al. 2015). These studies posit a number of mechanistic pathways by which shifts in biodiversity may affect parasite transmission and colonization success (Keesing et al. 2006). **The work proposed here will constitute the first comprehensive test of leading hypotheses for the relationship between biodiversity and parasite abundance in any ecosystem**, identifying the conditions under which positive, negative, and neutral biodiversity–parasite abundance relationships are to be expected.

Marine ecosystems are uniquely suited to this work, and have driven substantial progress in community ecology (Poulin et al. 2016), yet marine models are seldom used in disease ecology research. Of the 45 empirical studies into the biodiversity–parasite abundance relationship conducted to date, **only three (6.7%) are from marine ecosystems** and none have been conducted in the tropics or on coral reefs (Civitello et al. 2015b). Our proposed project will exploit the exceptional tractability of marine ecosystems (diverse parasite fauna, ease of collection and diagnosis of vertebrate hosts, strong and continuous spatial variability in host biodiversity, broad scope of spatial

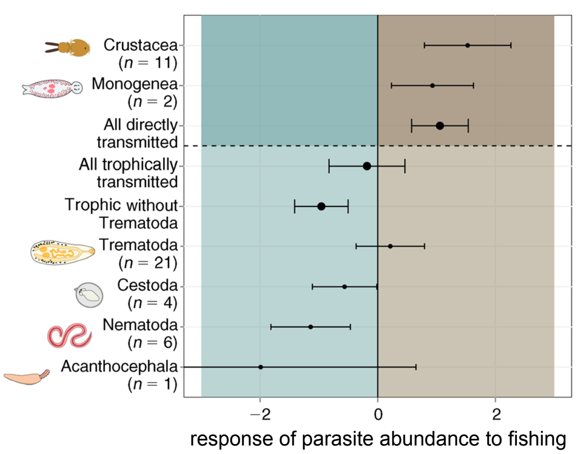

Figure 1. *Mean and 95% CI for the effect of fishing on parasite abundance for directly transmitted parasites (Crustacea, Monogenea) and complex life cycle parasites (Trematoda, Cestoda, Nematoda, Acanthocephala). Adapted from Wood* et al. *2015.*

scales, biologically realistic sampling units) to address long-standing gaps in the empirical data available to test fundamental disease ecology theory. We will accomplish this by integrating an **existing, fine-resolution dataset** on coral reef fish parasites from six equatorial Pacific islands (Wood et al. 2014b, Wood et al. 2015, Wood et al. in revisions) with **new sampling from 12 similar, nearby islands**. The focal islands vary in the amount of fish biodiversity they support. This natural experiment will allow us to explore how parasite abundance responds to variation in biodiversity at an unprecedented spatial scale, spanning 18 coral islands of three major archipelagos. Efforts will be cost-leveraged by our existing dataset and an existing, funded field project – the 100 Island Challenge – that will provide easy access to remote field sites. Our project will address three fundamental questions:

Q1. For each parasite species detected, what is the **direction and shape of the relationship between biodiversity and parasite abundance?**

Q2. What **factors (e.g., parasite traits like transmission strategy and host specificity, host traits like body size)** determine the direction and shape of the relationship between biodiversity and parasite abundance?

Q3. How does **spatial scale interact with parasite dispersal capacity** to moderate the effects of biodiversity on parasite abundance?

Intellectual merit – This work represents a new frontier for biological oceanography. It will answer long-standing questions in disease ecology through the use of a new, highly tractable marine study system, which will enrich the empirical basis for disease ecology theory, inject insights from marine ecology into a literature that is nearly bereft of data from marine systems, and deepen our understanding of marine ecosystem structure and function. The theories we propose to test here are among the most important and controversial in the rapidly growing field of disease ecology; our work represents a novel, creative approach to a recalcitrant research question – an approach that will yield transformative insights into the nature of parasite transmission in a changing world.

II. Background and significance: The biodiversity–parasite abundance relationship in disease ecology theory

We seek to exploit the unique advantages of marine systems to drive progress in an important sub-field of disease ecology – the study of biological diversity and parasite transmission. This relationship is often termed “diversity–disease”, but we will use the more specific and operational phrase, “biodiversity–parasite abundance”, to indicate that our focus is on how biological diversity might mediate the transmission and therefore the abundance of parasites (i.e., the mean number of individuals of a parasite species per host individual). The disease ecology literature is rich in hypotheses for the mechanisms that govern biodiversity–parasite abundance relationships, some of which may explain the patterns we have already observed in our coral reef study system (Wood et al. 2014b, Wood et al. 2015, Wood et al. in revision; **Figure 1**).

The dilution effect hypothesis. A dominant theory in the disease ecology literature, the “dilution effect” hypothesis was developed to explain patterns of Lyme disease (*Borrelia burgdorferi*) prevalence in forest mammals of northeastern North America (van Buskirk and Ostfeld 1995, Norman et al. 1999, Ostfeld and Keesing 2000a), but in recent years has been advanced as a general explanation for parasite transmission patterns across ecosystems (Keesing et al. 2010, Keesing and Ostfeld 2012). The theory posits that reducing biodiversity should increase the proportion of competent hosts in the population, because hosts that are competent to become infected by parasites tend to be the “fast-living”, *r*-selected species that both: (*i*) do not invest in immune defenses against infection and (*ii*) thrive in disturbed environments (Keesing et al. 2006). These host populations are regulated in high-biodiversity environments by species interactions (e.g., competition, predation) that are absent in low-biodiversity environments. Note that this is a diffuse effect: the subset of biodiversity that is predicted to affect parasite transmission contains both hosts (i.e., those species competent to transmit parasites) and non-hosts (i.e., those species that are not competent to transmit parasites, but that regulate host population density via species interactions; “diluting” hosts). Since the original studies on Lyme disease (reviewed in Ostfeld 2011), evidence has accumulated to indicate that the dilution effect operates at local scales for a variety of wildlife parasites (Clay et al. 2009a, b, Hall et al. 2009, Haas et al. 2011, Searle et al. 2011, Venesky et al. 2013, Civitello et al. 2015b). If sufficiently general, the dilution effect would produce health benefits as a side effect of biodiversity conservation – a valuable ecosystem service. **In sum, the dilution effect hypothesis predicts that biodiversity "dilutes" disease risk, producing a negative relationship between biodiversity and parasite abundance.**

The amplification effect hypothesis. Since the inception of the dilution effect hypothesis, disease ecologists have recognized the potential for an opposing response: an "amplification effect" in which increasing biodiversity amplifies parasite abundance (Ostfeld and Keesing 2000b, Ostfeld and Keesing 2000a, Keesing et al. 2006). Support for this hypothesis comes from the observation that parasites depend on primary ecosystem processes like predation to be transmitted and that they are often highly host specific or require multiple host species (i.e., high biodiversity conditions) for the completion of their life cycles. This observation has led to the suggestion that parasites might even – in direct contravention of the dilution effect – serve as bio-indicators of a healthy, functioning, high-biodiversity ecosystem (Hechinger and Lafferty 2005, Hudson et al. 2006). Amplification is expected when increasing biodiversity increases density across species, including competent host species (Mihaljevic et al. 2014) and when high-biodiversity communities are likelier than low-biodiversity communities to contain competent host species (Brooks and Zhang 2010, Joseph et al. 2013). **In sum, the amplification effect hypothesis predicts that biodiversity "amplifies" disease risk, producing a positive relationship between biodiversity and parasite abundance.**

Under what conditions do dilution and amplification hold? The dilution and amplification effect hypotheses predict diametrically opposed outcomes: negative and positive biodiversity–parasite abundance relationships, respectively. Recent empirical work has suggested that, in a single ecosystem, some parasite species may exhibit amplification while others exhibit dilution (e.g., Wood et al. 2014b, Wood et al. 2015, Young et al. 2015, Halliday et al. 2017, McLeish et al. 2017, Young et al. 2017). The next challenge for disease ecologists is to identify the conditions under which each hypothesis holds.

In order to satisfactorily address this question, we must (*i*) move beyond binary differences in parasite abundance between high- and low-biodiversity conditions, to understand the shapes that these biodiversity–parasite abundance relationships assume across a range of biodiversity values, (*ii*) identify the conditions under which each shape manifests, and (*iii*) determine how the spatial scale of observation affects these shapes. Theoretists have been active in this area, and have outlined hypotheses for the conditions under which amplification and dilution should be expected (e.g., Ogden and Tsao 2009, Brooks and Zhang 2010, Joseph et al. 2013, Miller and Huppert 2013, Mihaljevic et al. 2014, O'Regan et al. 2015, Strauss et al. 2015, Faust et al. 2017). Hypotheses have also been advanced to explain how the spatial scale of observation may influence the biodiversity–parasite abundance relationship (Cohen et al. 2016, McLeish et al. 2017, Buck and Lafferty in prep, in revision). However, testing these hypotheses in nature has proven challenging, because few systems exist in which multiple parasite species can be simultaneously measured against a backdrop of varying biodiversity. Some systems might lend themselves to such tests, including terrestrial plant parasites (e.g., Halliday et al. 2017, McLeish et al. 2017), rodent parasites (e.g., Young et al. 2015, Weinstein et al. 2017, Young et al. 2017), and parasites of coral reef fishes (Wood et al. 2014b, Wood et al. 2015, Wood et al. in review), but, to date, the critical knowledge gaps outlined above remain unexplored, even in these tractable systems.

We suspect that there is significant variability among parasites species in the direction of their response to biodiversity and, further, that within a single parasite species, there may be non-linearity and scale-dependence of the biodiversity–parasite abundance relationship. The research proposed here will assess this in a highly tractable marine model system, by (*i*) directly testing the dilution and amplification hypotheses across >77 parasite taxa from coral reef fishes, (*ii*) generating biodiversity–parasite abundance data with a highly resolved axis of coral reef fish biodiversity, which will permit exploration of the shape of the biodiversity–parasite abundance relationship and conditions under which each shape is likely, and (*iii*) exploring the influence of spatial scale on this relationship across an expansive range of scales, spanning three archipelagoes. This work will greatly improve our understanding of how parasite impacts on host populations, communities, and ecosystems may be distributed across space and time.

Why study how biological diversity regulates the abundance of parasites? Parasites are often hidden and can be easy to overlook, but they are ecologically important and affect every population of marine hosts – from zooplankton to cetaceans (Wood and Johnson 2015). Understanding the processes that control parasite abundance will allow us to predict their effects on larger, more charismatic species.

Parasites reduce host fitness – We define "parasites" as organisms that live in a spatially close and temporally durable relationship with a host, where that host suffers a fitness cost (Combes 2001). We define "disease" as pathology related to parasitic infection – that is, the loss of host fitness attributable to infection (Bush et al. 1997) – and "parasite abundance" or "burden" as the number of parasites of a particular species per host individual. Parasites are often categorized according to the kind of disease they cause. Specifically, they are binned by the intensity-dependence of their pathology (Lafferty and Kuris 2002). The offspring of **micro-parasites** go on to infect the host of their parent, and therefore, a single individual micro-parasite colonizing a host can produce significant pathology (intensity-independent pathology); this characterizes most viruses, bacteria, fungi, and protozoa (Lafferty and Kuris 2002). The offspring of **macro-parasites** disperse from the host of their parent, and therefore, pathology depends on the number of individual macro-parasites that colonize a host (intensity-dependent pathology); this characterizes parasites with complex life cycles or dispersive larval stages, including most helminth, arthropod, and other metazoan parasites (including all of the parasites that we discuss herein; Lafferty and Kuris 2002). Both micro-parasites and macro-parasites cause disease (i.e., loss of host fitness), but disease dynamics differ between the two groups. **Because their pathology is intensity-dependent, macro-parasites have minor fitness effects on hosts at low levels of parasite abundance and substantial fitness effects at high levels.** Macro-parasites can even cause host mortality at high abundances.

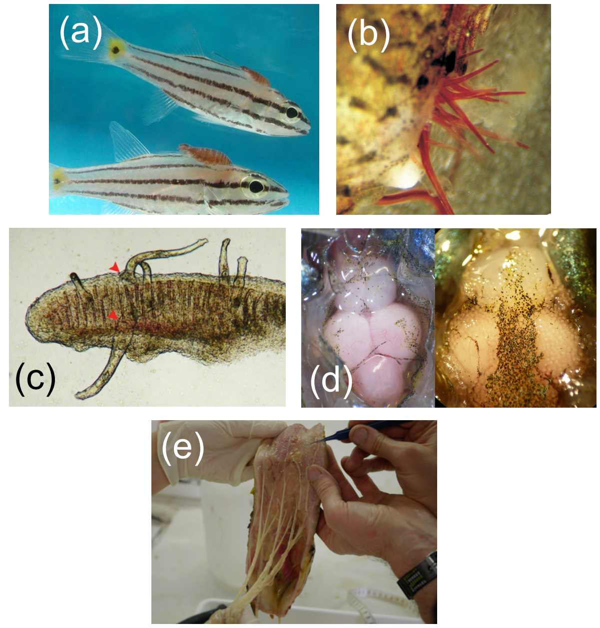

Figure 2. *Macro-parasites can impose significant fitness costs on fishes: (a) five-lined cardinalfish (*Cheilodipterus quinquelineatus*) infected with cymothoid isopods (*Anilocra apogonae*) (Fogelman and Grutter 2008); (b) intestinal nematodes protruding from the anus of a fish (Menezes et al. 2006) (c) monogenean worms attached to a fish gill filament (red arrows indicate attachment points) (Purivirojkul 2012); (d) dorsal view of the brain of an uninfected fish (left) and fish infected with larvae of the trematode* Euhaplorchis californiensis, *courtesy of K Weinersmith; (e) tapeworms clinging to the intestinal lining of an ocean sunfish, courtesy of Richard Saunders, South Australia Museum.*

There are many mechanisms by which macro-parasites can reduce host fitness and thereby cause disease. Lafferty et al. (2015) documented 67 instances in which parasites reduced the growth and survivorship of marine hosts; of these, 18% were macro-parasites. Among these examples are crustacean parasites, like ecto-parasitic isopods, which divert enough resources through blood- and mucus-feeding that they reduce host growth and survival (Fogelman and Grutter 2008, Artim et al. 2015, Triki et al. 2016; **Figure 2a**); nematodes, which can erode the intestinal lining of their hosts, reducing digestive efficiency (Menezes et al. 2006; **Figure 2b**); monogeneans, which graze on gill epithelium, disrupting the osmotic permeability of gill surfaces and thereby increasing host mortality (Lester and Adams 1974, Cone and Odense 1984, Cusack and Cone 1986; **Figure 2c**); trematodes, which can manipulate host behavior, decreasing predator-avoidance behaviors to increase the likelihood that their host is consumed by a predator (which allows the parasite to complete its life cycle; Lafferty and Morris 1996; **Figure 2d**); and tapeworms, which reduce fish growth by competing with hosts for ingested resources (Saksvik et al. 2001; **Figure 2e**). **Given these significant impacts on host fitness and the intensity-dependence of macro-parasite pathology, there is an urgent need for research that explores the drivers of parasite abundance.**

Parasites affect population, community, and ecosystem dynamics – Through their effects on individual host fitness, parasites can have cascading impacts on marine populations, communities, and ecosystems. For example, parasites (like the trematodes pictured in **Figure 2d**) commonly manipulate the behavior of hosts to facilitate transmission, often by increasing the likelihood that the host is preyed upon by the predator species that serves as the parasite's next host (Poulin 2010, Hughes et al. 2012); in this way, parasites effectively shunt energy into higher trophic levels, subsidizing predators (Wood and Johnson 2015). Parasites can control host populations through negative fitness effects, including castration (e.g., Decaestecker et al. 2005), re-shape host communities by influencing the outcome of species interactions like competition, predation, and herbivory (e.g., Wood et al. 2007), and can even affect ecosystem nutrient cycling (e.g., Mischler et al. 2016). **This substantial ecological role of parasites suggests that downstream effects of change in parasite abundance are likely to be significant for every marine population from zooplankton to cetaceans.** For example, complex life-cycle parasites that increase predation rates on their hosts (discussed above) decline substantially in the presence of fishing (**Figure 1**); we surmise that this loss of manipulative parasites might actually reduce ecosystem-level energy flows from lower to higher trophic levels. But before we can pursue a clearer understanding of how parasites control marine ecosystem processes, we must know when and where parasites are most abundant. The research proposed here addresses this unknown.

III. Coral reefs of equatorial Pacific islands: A test bed for disease ecology theory

Disease ecology theory has been developed primarily with reference to terrestrial and freshwater ecosystems, but marine systems are an equally appropriate – and in some cases, more tractable – test bed for these hypotheses (McCallum et al. 2004, Poulin et al. 2016). Our study system (the Northern Line Islands archipelago, the Southern Line Islands archipelago, and French Polynesia) contains discrete replicates (i.e., islands) that are disjunct from one another and large, thereby balancing statistical independence, biological realism, and scope for exploring the effects of spatial scale. Because most marine parasites have a pelagic larval stage, we can explicitly discriminate between long- and short-distance transmission in a way that is not possible on land or in freshwater, giving us the ability to explore the influence of dispersal capacity on infectious processes.

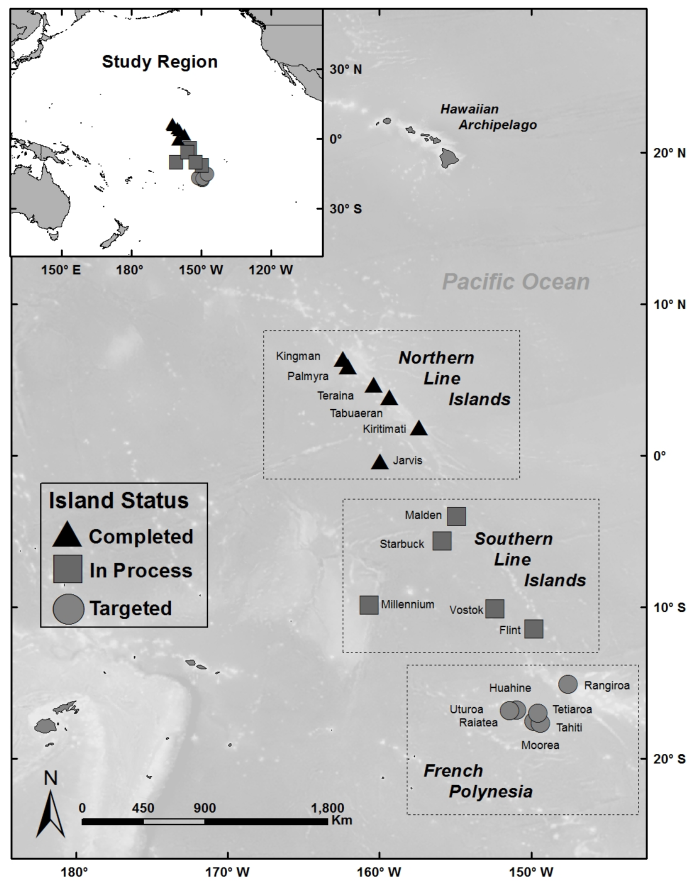

Figure 3. *Map of the study region, including the six islands in our existing dataset of fish biodiversity and parasite abundance (black triangles, Northern Line Islands), five islands where fish surveys and fish collections are complete and support is requested for parasitological dissection of these existing samples (grey squares, Southern Line Islands), and seven islands where support is requested for fish surveys, fish collections, and parasitological dissection (grey circles, French Polynesia).*

Here, we propose to build on our existing six-island dataset with 12 new islands (**Figure 3**). Over the past 10 years, our team has gained extensive experience in sampling fish from coral reefs of Pacific islands, and we have been collaborating for the past six years to characterize the parasite assemblages of these islands. Presently, our database contains records of 1,839 fish hosts of seven species from six islands (the Northern Line Islands), which yielded 988,071 individual parasites from 77 taxa, including monogeneans, trematodes, tapeworms, nematodes, acanthocephalans, and crustaceans. We currently possess an additional 875 unprocessed fish of the same seven host species collected from the five islands of the Southern Line Islands archipelago. We also currently possess fish visual survey data from the Northern and Southern Line Islands, including records of more than three million individual fish, which will allow us to make island-level estimates fish biodiversity. We propose additional collections and fish surveys at seven islands in French Polynesia (**Figure 3**). All existing data and data that we propose to collect will be made publicly available through the Biological and Chemical Oceanography Data Management Office (BCO-DMO; see *Data Management Plan*).

IV. Research questions, preliminary data, and methods

Q1: For each parasite species detected, what is the direction and shape of the relationship between biodiversity and parasite abundance?

Previous studies on the biodiversity–parasite abundance relationship have bookended the axis of biodiversity and made untested assumptions about the relationship between disturbance and biodiversity, often by grouping replicates into discrete categories: “fished” versus “unfished” (e.g., Wood et al. 2014b, Wood et al. 2015), “de-forested” versus “forested” (e.g., Bauch et al. 2015), and “large wildlife removed” versus “large wildlife retained” (e.g., Young et al. 2015). This approach has powerfully demonstrated that disturbances like fishing can drive change in parasite assemblages, but it masks the functional shape of the relationship between biodiversity change and parasitism by putting biodiversity in a “black box”. In studies contrasting infection outcomes as a function of categorical biodiversity states, it has often been assumed that a linear relationship connects the bookend categories of low and high biodiversity. But depending on the magnitude of the biodiversity value of each category, this assumption could lead to faulty interpretations – even the opposite of the actual, underlying pattern (e.g., dashed box in **Figure 4a**).

There are a few unique properties of the relationship between biodiversity and parasite abundance. First, parasites obligately require hosts. Consequently, when biodiversity equals zero, parasite abundance must also equal zero (Wood et al. 2016; **Figure 4a**). This constraint obviates the possibility of negative linear relationships, but a diversity of other relationships might result. For example, biodiversity reductions might primarily cause a decline in parasite transmission, as the dilution effect predicts (**Figure 4a[i]**). Alternately, transmission might be highest at intermediate levels of biodiversity, where competent hosts have the maximum joint likelihood of being present (i.e., increasing biodiversity increases the likelihood of any species being present) and abundant (i.e., increasing biodiversity increases the likelihood of competitors or predators being present; **Figure 4a[ii]**). Increasing biodiversity might increase parasite abundance (as the amplification effect hypothesis predicts), either linearly (**Figure 4a[iii]**), with saturation at high levels of biodiversity (**Figure 4a[iv]**), or with a peak at high levels of biodiversity, where intensifying species interactions begin to exert control on the density of any single host (i.e., with dilution effects at high levels of biodiversity; **Figure 4a[v]**). Tests of existing theory therefore depend upon a complete understanding of the trajectory of disease change – not just selective subsets (e.g., dashed box in **Figure 4a**).

To explore the shape of the biodiversity–parasite abundance relationship across a broad and well-resolved scope of biodiversity values, we will build on our existing dataset (**Figure 3**). Rather than categorizing islands as “fished” or “unfished” (as in Wood et al. 2014b, Wood et al. 2015, Wood et al. in revision), we will use two continuous metrics for characterizing fish biodiversity – one that considers fish biodiversity exclusively (fish species richness) and another that considers both fish biodiversity and

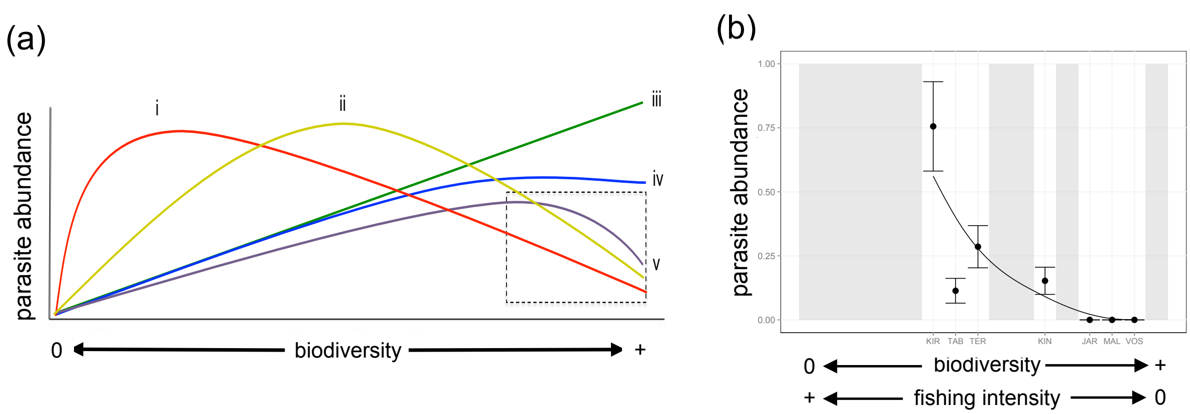

Figure 4. *(a) Hypotheses for the relationship between biodiversity and parasite abundance. (i) The dilution effect, (ii) an intermediate hypothesis, where dilution mechanisms prevail at high biodiversity and amplification mechanisms prevail at low biodiversity, (iii) amplification, (iv) amplification with saturation at high levels of biodiversity, and (v) amplification that shifts to dilution at high levels of biodiversity. The dashed box indicates how a selective frame of reference can lead to spurious conclusions. Figure adapted from (Wood et al. 2016). (b) From our existing dataset, abundance of a Cucullanidae sp. nematode in the whitecheek surgeonfish* Acanthurus nigricans, *at five islands sampled in 2010 (KIR = Kiritimati, TAB = Tabuaeran, TER = Teraina, KIN = Kingman, JAR = Jarvis) and two additional islands sampled in 2013 (MAL = Malden, VOS = Vostok). Islands are arranged along the x-axis in order of predicted biodiversity (proxied by fishing intensity). Gray areas indicate biodiversity space that will be sampled in the proposed project. Line indicates LOESS smoothing regression on means for each island, span = 2.*

evenness (Shannon diversity). Our dataset will allow us to explore a broad range of host species richness (~55–80 species) and Shannon diversity (~2.2–3.0) values. We will then examine the biodiversity–parasite abundance relationship for each of >77 parasite taxa (i.e., at least as many taxa as occur in our current, six-island dataset), providing a comprehensive portrayal of the biodiversity–parasite abundance relationship across substantial variability in fish biodiversity (Wood et al. 2014b, Wood et al. 2015).

We will synthesize data on the parasite burden of seven host species from 18 islands of the central equatorial Pacific (**Figure 3**), including the six Northern Line Islands already included in our database, five Southern Line Islands where we have already collected 875 fish but request support for performing parasitological dissections on these samples, and seven French Polynesian islands where we propose additional fish collections and surveys. Our existing database (held by PI Wood) currently reports counts of all metazoan parasites detected in seven host species from 58 geo-referenced sites at six islands (Jarvis, Kingman, Palmyra, Teraina, Tabuaeran, Kiritimati). The host species (*Cephalopholis urodeta, Acanthurus nigricans, Ctenochaetus marginatus, Paracirrhites arcatus, Stegastes aureus, Chromis margaritifer,* and *Pseudanthias bartlettorum*) were chosen to span a variety of body sizes, to represent important trophic and taxonomic groupings, and to include only broadly distributed species present at most reefs in the region (see *Power analysis and scope of inference,* below).

Fish survey data – To assess fish biodiversity, we will build on an existing fish survey dataset containing results of size- and species-specific surveys collected by members of the Sandin Lab and the National Oceanic and Atmospheric Administration's Coral Reef Ecosystem Program (part of the Pacific Islands Fisheries Science Center). The data have been collected by a limited subset of trained observers employing directly comparable methods with high taxonomic resolution (Brainard et al. 2005, DeMartini et al. 2008, Sandin et al. 2008, Williams et al. 2011, Zgliczynski and Sandin 2017). The existing data include surveys from both the Northern and Southern Line Island archipelagoes. We will perform additional surveys from seven islands in French Polynesia (see *Letter of Collaboration* from Dr. Serge Planes, CRIOBE, Moorea, French Polynesia). The final dataset will include surveys of each of the 18 islands of interest, with island-specific survey effort ranging from 7 to 50 transects located between 7 and 15 m depth on the leeward forereef of each surveyed island. All fish survey data have been and will continue to be collected using the same methodology, with methods described in detail in Friedlander et al. (2010) and Zgliczynski and Sandin (2017). The fish survey effort will be coordinated and led by the Scripps PhD student funded by this proposal, and will be overseen by co-PI Sandin. This effort will not only provide high-quality data on fish biodiversity for understanding the biodiversity–parasite abundance relationship, but will also be made freely available for other coral reef ecology studies (see *Data Management Plan*).

Fish host sampling – Lethal fish sampling will be conducted at each of the seven French Polynesian islands where we have not already conducted fish sampling (see *Letter of Collaboration* from Dr. Serge Planes, CRIOBE, Moorea, French Polynesia**; Figure 3**), will include individuals of the same seven fish species targeted in our previous efforts, and will be conducted at the same locations where fish surveys are to be carried out. Fish will be captured by scuba divers using three-pronged spears (for fish >10 cm) and hand nets (for fish <10 cm) and will be euthanized immediately upon capture by pithing (as approved by IACUC; see *Other Supplementary Documents*). At least 25 individuals will be collected per species per island, a level of replication that represents a balance between, on one hand, attaining sufficiently high replication to accurately assess the abundance of parasites (see *Power analysis,* below) and, on the other hand, minimizing the number of animals that experience discomfort, pain, and injury. Sampling will be conducted at 3–5 sites within each island, with sites chosen to match those where fish surveys are conducted. At collection, each fish will be deposited in a separate ziplock bag with a tag individually identifying the specimen. Total length, standard length, fork length, and mass will be recorded. After measurements are taken, fish will be frozen as quickly as possible and shipped on dry ice to PI Wood's facility, where they will be kept frozen until processing. The fish host sampling effort will be coordinated and led by the Scripps PhD student funded by this proposal, and will be overseen by co-PI Sandin.

Parasite sampling – A comprehensive parasitological examination will be performed for each fish, designed to detect all metazoan parasites. The following organs will be examined individually under a stereomicroscope: fins, gills, eyes, heart, liver, spleen, gonad, gills, muscle, skin, and intestines (after Wood et al. 2014b). Photographs of each parasite and its diagnostic morphological features will be linked to our database. Parasites will be identified to the lowest possible taxonomic level (Yamaguti 1963, Schultz 1969, Skryabin 1991, Khalil et al. 1994, Gibson et al. 2002, Kabata 2003, Bray et al. 2005, 2008).

We will preserve parasite vouchers in ethanol for genetic analyses (Question 3) and as an archived resource for other researchers to access and use (see *Data Management Plan*). We will also photograph each fish and archive fish tissues (see *Data Management Plan*). The parasite sampling effort will be coordinated and led by the UW graduate student and two UW laboratory technicians supported by this proposal and overseen by PI Wood.

Controlling for time of fish host collection – All fish collections will occur between June and November of year 1 of the grant (2018), to minimize the amount of time that has passed since prior collections (2010 for Northern Line Islands, 2013 for Southern Line Islands). The amount of time between sampling bouts raises the possibility that short-term change might decouple relationships between fish biodiversity and parasite abundance. Given the relative longevity of parasites in their hosts (Lafferty and Kuris 2002) and the strong inter-island variability in parasite abundance we have observed in existing data (Wood et al. 2014b, Wood et al. 2015), we view the potential for significant temporal variability to be low. However, in order to ensure that temporal variability does not influence our estimates of parasite abundance and composition, we will re-sample in 2019 two of the Northern Line Islands that were originally sampled in 2010: Palmyra and Kiritimati (see *Budget* and *Budget Justification*). We may also be able to obtain samples from the remaining Line Islands (Kingman, Tabuaeran, Teraina, Jarvis) through leveraged opportunities; a cruise to the region is currently being planned for 2019 as part of the 100 Island Challenge, and if executed it would offer a way for our team to obtain fish from Kingman, Tabuaeran, Teraina, and Jarvis at no cost. As in previous surveys, we will collect 25 individuals from each of the seven focal host species at each island. We will dissect these fishes and compare the abundance of each parasite species from each island between 2010 and 2019. In addition to explicitly assessing change over time in parasite assemblages for these re-sampled islands, we will also include time in all statistical models to account for its potential effects.

Analyses – We will analyze, for each parasite species detected and each of our two metrics of biodiversity (i.e., fish species richness and Shannon diversity), the relationship between biodiversity and abundance of parasites (number of parasites per host; e.g., **Figure 4b**) at the individual host level (after Wood et al. 2014b, Wood et al. 2015). A generalized linear mixed-effects model (GLMM) with negative binomial error structure will be used to assess whether there is a significant linear relationship between fish biodiversity and parasite abundance. Models will include fixed effects of latitude (to control for covariance in hosts and parasites not related to their direct relationship, but instead related to some third variable correlated with latitude), island fishing status (to account for the known effect of fishing on parasite abundance; Wood et al. 2014b, Wood et al. 2015), and host body size (to control for positive effects of host size on parasite burden), and random effects of island and year of collection. Those models with non-random residual structure indicating possible non-linearity in the biodiversity–parasite abundance relationship will be assessed for change points. Three techniques for change point detection will be used to ensure that change point values are robust (Aguilar et al. 2003, Gsell et al. 2016): piecewise linear regression (Muggeo 2003), the non-parametric Pettitt rank test (Pettitt 1979), and the sequential regime shift detector (SRSD) method (Rodionov 2004, 2015). For parasites that display change points, we will split the data into lower-biodiversity-than-change-point and higher-biodiversity-than-change-point sections, and compare their mean abundances with *t*-tests to derive a coefficient (standardized regression coefficient for the effect of "high" versus "low" biodiversity) that describes the direction and magnitude of the biodiversity–parasite abundance relationship for each parasite and is comparable among linear and non-linear responses to host biodiversity. Finally, we will synthesize these biodiversity–parasite abundance relationships for all >77 parasite species using meta-analytic statistical techniques (as in Wood et al. 2014b) to test whether the direction and magnitude of the relationships are different from zero when averaged across all parasite taxa. These analyses will be conducted collaboratively by the UW and Scripps graduate students and UW postdoc supported by this proposal and will be overseen by PI Wood.

Power analysis and scope of inference – The database we propose to build will be the most comprehensive ever assembled to test the biodiversity–parasite abundance relationship (for a complete list of these datasets, see Civitello et al. 2015b). That is, our dataset will offer higher replication across parasite species (>77 species) and levels of host diversity (a range of ~55–80 species) than any other dataset assembled from a single ecosystem. However, one important limitation of our study is that all the parasites to be collected will come from only seven host species (i.e., only seven host species will be targeted for lethal collection and parasitological dissection). We are limited in the number of host species we can analyze because collection and dissection are time- and resource-intensive and managers rightfully seek to limit the impact of scientific collections on the coral reefs under their stewardship.

To ensure that the level of replication we propose is sufficient to answer the questions posed above, we performed a power analysis. We calculated the correlation of the relationship between fish biodiversity and parasite abundance for each parasite taxon in our existing dataset (i.e., from the six Northern Line Islands; Wood et al. 2014b, Wood et al. 2015), using the analytic techniques described above. We then calculated power to detect a relationship of the observed magnitude, given that n = 450 (25 fish per island * 18 islands) and p = 0.05, using the pwr.r.test function in the pwr package in R. We found that, for the average effect size across parasite taxa, our power to detect a relationship between host diversity and parasite abundance is 98.3%. The lowest effect size for which we have 80% power is 0.135, meaning that our dataset will have power to detect even a very weak effect of biodiversity on parasite abundance. We are therefore confident that our dataset will provide sufficient power for detecting biodiversity–parasite abundance relationships at the proposed level of replication.

To maximize our scope of inference, we have selected focal host species that represent a cross-section of coral reef fish biodiversity. Ideally, we would sample dozens of host species across the 18 islands, both to increase statistical power (see paragraph above) and to broaden our scope of inference to include parasites of hosts that span the entire coral reef food web. Realistically, we are limited by time, costs, and logistical constraints. We selected the seven focal hosts because they are widespread (i.e., present at each of the focal islands) and common (i.e., straightforward to collect). In selecting these seven species, we made an effort to choose a representative of each major trophic group, to span a range of average body sizes, and to maximize phylogenetic diversity. We are prevented by logistical constraints (e.g., permitting, conservation, diver safety) from collecting apex predators like sharks. Given these constraints, we have selected a group of host species that is optimally representative of the coral reef food web, allowing us to obtain an optimally representative subset of parasite species.

Anticipated results – We expect that these data will support both the dilution (**Figure 4a[i]**) and amplification hypotheses (**Figure 4a[iii], 4a[iv], 4a[v]**), because we predict that there will be significant variation in the direction, shape, and strength of the biodiversity–parasite abundance relationship among parasite species. We also expect diverse change points among parasite taxa (with low change points indicating resilience to host biodiversity change and high change points indicating susceptibility). For one parasite in our existing dataset, dilution (as in **Figure 4a[i]**) is supported, but significant gaps in the axis of host diversity remain (**Figure 4b**). Our proposed sampling will fill those gaps.

Q2. What factors (e.g., parasite traits like transmission strategy and host specificity, host traits like body size) determine the direction and shape of the relationship between biodiversity and parasite abundance?

Theory developed in the past several years suggests that parasite and host traits might mediate the direction and shape of the relationship between biodiversity and parasite abundance. For example, for parasites with complex life cycles that involve multiple hosts, positive relationships between biodiversity and abundance are expected, because low-biodiversity environments are likely to lack some obligately required host, creating a "life-cycle bottleneck" (Lafferty 2012; **Figure 5a**). In contrast, directly transmitted parasites are predicted to conform to the dilution effect hypothesis, because their transmission success can be reduced if high biodiversity "wastes" parasite transmissive stages on non-competent hosts (Keesing et al. 2006). Highly host-specific parasites are predicted to conform to the predictions of the amplification effect, while low-specificity parasites are predicted to be robust to changes in biodiversity (Lafferty 2012; **Figure 5b**). Parasites of large-bodied hosts are expected to have positive relationships with biodiversity, because large-bodied hosts are likelier to be absent in low-biodiversity environments than are small-bodied hosts (Dirzo et al. 2014), while parasites of small-bodied hosts are expected to have negative relationships with biodiversity, because small-bodied hosts can sometimes benefit from the absence of large-bodied predators in low-biodiversity environments (Wood et al. 2010, Joseph et al. 2013, Dirzo et al. 2014, Mihaljevic et al. 2014, Wood et al. 2014a, Wood et al. 2014b; **Figure 5c**). To empirically test these theoretical predictions, we will assess the extent to which the direction and shape of the biodiversity–parasite abundance relationship: (**Q2a**) varies across parasite transmission strategies (**Figure 5a**), (**Q2b**) is related to host specificity (i.e., degree of parasite specialization; **Figure 5b**), and (**Q2c**) is related to host body size (**Figure 5c**).

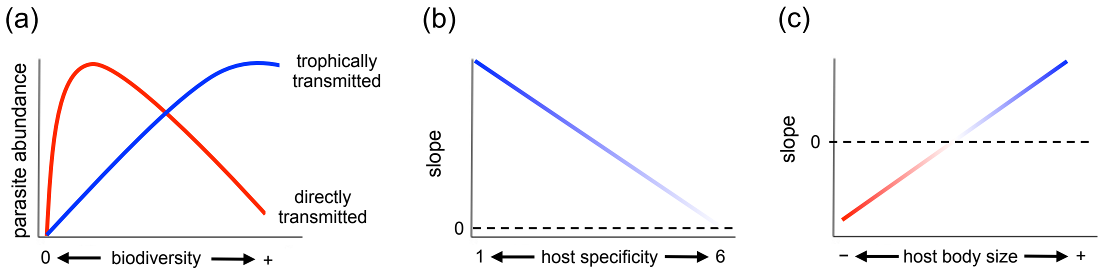

Figure 5. *We hypothesize that the relationship between fish biodiversity and parasite abundance might be moderated by (a) parasite transmission strategy, (b) host specificity, and (c) host body size. Specifically, we expect that: (a) directly transmitted parasites will conform to the predictions of the dilution effect (red), and trophically transmitted parasites will conform to the predictions of the amplification effect (blue), (b) the slope of the relationship between fish biodiversity and parasite abundance will tend to be positive (amplification effect, blue) for highly host-specific parasites (lower values on axis of host specificity) and invariant for generalist parasites (higher values), and (c) the slope of the relationship between fish biodiversity and parasite abundance will tend to be negative (dilution effect, red) for parasites of small-bodied host species and positive (amplification effect, blue) for parasites of large-bodied host species.*

Assembling data on parasite and host traits – For data on parasite life history traits (e.g., transmission strategy, host specificity), we will build on an existing database (Appendix F in Wood et al. 2014b). Information on the life cycle and natural history of each parasite will be surveyed from the literature and collated into this database. Each parasite will be classified according to its broad taxonomic group (Subphylum Crustacea, Class Monogenea, Class Trematoda, Phylum Nematoda, Class Cestoda), transmission strategy (direct versus trophic transmission, where directly transmitted parasites are transmitted among conspecific hosts, and trophically transmitted parasites are parasitic in multiple life stages among hosts of different species), and host specificity (ranked 1–6 based on Brusca 1981, Sasal et al. 1998, and Jones et al. 2007, with 1 indicating high specificity). We define "specialists" as those parasites known to use a narrow range of host species. We will surmise life history traits for each parasite based on its membership in higher-order taxonomic groups, because host specificity is known to be phylogenetically conserved within these groupings (Sasal et al. 1998, Mouillot et al. 2006). This database of parasite life history traits has already been constructed for the 77 parasite species in our current parasite dataset representing the Northern Line Islands; we will add entries for new parasites as we encounter them in fishes collected from the Southern Line Islands and French Polynesia.

For data on host life history traits, we will build on an existing database (Table 1 in Wood et al. 2014b). For each fish, we will assess the species' average body size by averaging the total lengths of all individuals of that species in our dataset.

Analysis – This analysis will assess the degree to which the direction and shape of the biodiversity–parasite abundance relationship is explained by parasite and host traits. Using the values derived in Q1 (i.e., standardized regression coefficients for the relationship between biodiversity and parasite abundance) for each of >77 parasite species, we will use meta-analytic statistical techniques (as in Wood et al. 2014b) to test whether the direction and magnitude of the relationship and the value of any change points vary systematically (**Q2a**) between complex life cycle parasites versus directly transmitted parasites, (**Q2b**) as a function of host specificity (i.e., degree of host specialization), and (**Q2c**) as a function of host body size. These analyses will be conducted collaboratively by the UW and Scripps graduate students and UW postdoc supported by this proposal and will be overseen by PI Wood.

Anticipated results – We expect that dilution will dominate among directly transmitted parasites (**Figure 5a**) and parasites of small-bodied host species (**Figure 5c**), and that amplification will dominate among trophically transmitted parasites (**Figure 5a**), those parasites with high host specificity (**Figure 5b**), and parasites of large-bodied host species (**Figure 5c**).

Q3. How does spatial scale interact with parasite dispersal capacity to moderate the effects of biodiversity on parasite abundance?

Ecological pattern tends to be governed by regional forces like climate at coarse spatial resolutions and local forces like species interactions at fine spatial resolutions (e.g., Fridley et al. 2007, Menge et al. 2015). Layered on these scale-dependent processes are cross-scale (i.e., meta-ecosystem) flows of organisms, nutrients, and energy (Loreau et al. 2003, Loreau and Holt 2004, Menge et al. 2015). But the same spatial grain is experienced in vastly different ways by different organisms; for example, disturbance to a 1-ha area might be catastrophic for a sedentary species and meaningless to a vagile species. Like many marine organisms, parasites produce pelagic larvae whose dispersal distances depend on the pelagic larval duration (PLD; i.e., the amount of time during which the planktonic parasite larva is competent). In addition to the dispersal capacity inherent in the pelagic larval phase, parasites can also disperse across space by infecting a vagile host (Blasco-Costa and Poulin 2013). As a result, parasites like monogenean flatworms, which produce weakly motile larvae with short PLDs, may disperse only tens or hundreds of meters (**Figure 6a**), while cestode flatworms, which produce larval stages with long PLDs, use zooplankton as intermediate hosts, and infect highly vagile elasmobranch final hosts, probably disperse thousands of kilometers in one turn of the life cycle (**Figure 6b**). The genetic structure of parasite populations is strongly influenced by transmission strategy among parasitic flatworms (Davies et al. 1999, Criscione and Blouin 2004, Blasco-Costa and Poulin 2013), parasitic nematodes (Blouin et al. 1999), lice (Johnson et al. 2002), and ticks (McCoy et al. 2005) and parasite populations are often more genetically subdivided than their hosts' populations (Criscione et al. 2006). This suggests that dispersal capacity has important consequences for structuring parasite populations, yet few studies have directly addressed whether a parasite's dispersal capacity mediates its response to biodiversity (but see categorical comparison in Wood et al. 2013). This problem has clear parallels in island biogeography (MacArthur and Wilson 1967), meta-community dynamics (Leibold et al. 2004), and other bodies of theory. Here, we have the opportunity to test whether these foundational ecological theories hold among parasitic organisms.

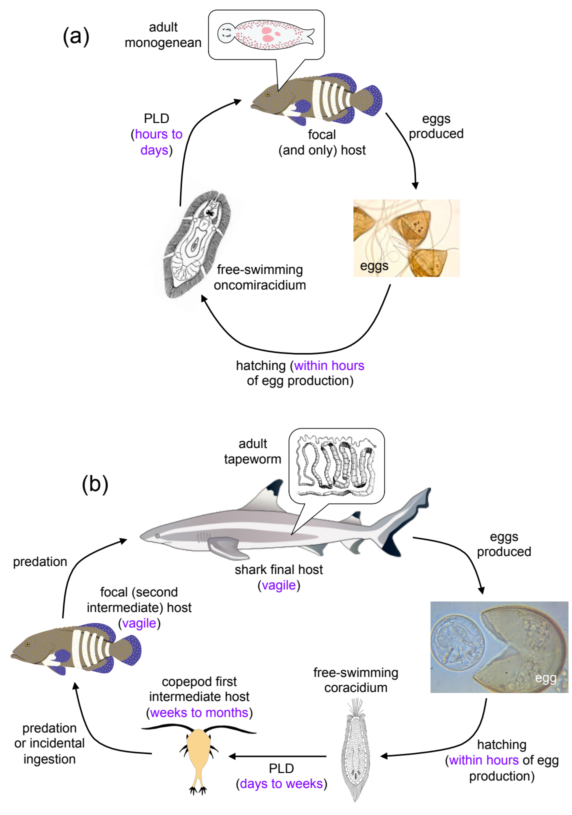

Figure 6. *Life cycles of (a) low dispersal-capacity monogenean parasites and (2) high dispersal-capacity tapeworm parasites. Factors that are likely to influence the distance traveled over the course of one life cycle are indicated in violet.*

Without considering parasite dispersal capacity, a few studies have investigated the role of scale in the biodiversity–parasite abundance relationship. In an analysis of three disease agents (amphibian chytrid fungus, Lyme disease, West Nile virus), Cohen et al. (2016) showed that the dilution effect was observable only at local scales (~1,000–10,000 km^2), while climate explained disease prevalence at larger scales (>100,000 km^2). This is consistent with the prediction from community ecology that biotic interactions should influence species distributions only at the small scales where those interactions manifest, whereas environmental conditions like climate should dominate at larger spatial scales (Huston 1999, Davies et al. 2005, Fridley et al. 2007). Studying plant viruses across an agricultural matrix habitat, McLeish et al. (2017) showed that the plant biodiversity–viral prevalence relationship was positive at large (ecosystem-level) spatial scales and undetectable at small (habitat-level) spatial scales (McLeish et al. 2017). Together, these existing studies suggest an important role for spatial scale in the biodiversity–parasite abundance relationship, but they are missing a key variable: the variation among parasites in their dispersal capabilities. In part, this gap is due to the difficulty of quantifying the dispersal capacity of parasites (Nathan 2001), which usually cannot be marked and recaptured, GPS-tagged, or induced to disperse in a meso-cosm or other laboratory apparatus. One of the main advantages of using marine organisms in disease ecology studies is the extreme inter-specific variance in their dispersal capacity (Strathmann 1990), which can range from a few meters to thousands of kilometers; this variance in dispersal capacity can be estimated with molecular techniques. Parasite transmission is a spatial process in all ecosystems, but it is exceptionally tractable to study in the oceans.

Analyses – As in Question 1, for each parasite taxon a generalized linear mixed-effects model (GLMM) with negative binomial error structure will be used to assess whether there is a significant linear relationship between biodiversity and parasite abundance. Also as in Question 1, models will include fixed effects of latitude (to control for covariance in hosts and parasites not related to their direct relationship, but instead related to some third variable correlated with latitude), island fishing status (to account for the known effect of fishing on parasite abundance; Wood et al. 2014b, Wood et al. 2015), and host body size (to control for positive effects of host size on parasite burden), and random effects of island and year of collection. This analysis will differ from that in Question 1 in that it will be repeated across spatial scales. To estimate both fish biodiversity and parasite abundance, will use a neighborhood-based approach that sequentially joins spatial units into progressively larger groups, tracking how spatial grain of observation influences the biodiversity–parasite abundance relationship. The continuously varying spatial grain will begin at a finer resolution than that used in Question 1 (collection site) and end at a coarser resolution (island groups). We will then use meta-regression (as in Wood et al. 2014b) to explore the relationship between sensitivity of the biodiversity–parasite abundance relationship to spatial scale and dispersal capacity of each parasite taxon. Analyses will be conducted collaboratively by the UW and Scripps graduate students and UW postdoc supported by this proposal and will be overseen by PI Wood.

Preliminary data and anticipated results – We predict that, at any given spatial grain, the slope of the biodiversity–parasite abundance relationship will weaken with increasing parasite dispersal capacity (**Figure 7a**). We also predict a hump-shaped relationship between slope of the biodiversity–parasite abundance relationship and spatial grain, where the peak of the hump increases with decreasing parasite dispersal capacity (**Figure 7b**). The hump-shaped relationship is expected because (*i*) fine spatial grains may reflect random heterogeneity in parasite abundance, particularly for high-dispersal parasites and (*ii*) parasite abundance at large spatial grains may be governed by large-scale forces (e.g., latitudinal productivity gradients) rather than local-scale forces, like host diversity. We expect that the peak of the hump will occur at coarser spatial grains for parasites with higher dispersal capacity, presumably peaking at a point where scale is coarse enough to reflect the large-scale dispersal process but fine enough that large-scale environmental factors (e.g., productivity) have not yet swamped the signal of dispersal. The peak will probably be lower for high-dispersal parasites, due to the swamping effect of large-scale factors (e.g., productivity) at the scales relevant for high parasite dispersal (**Figure 7b**).

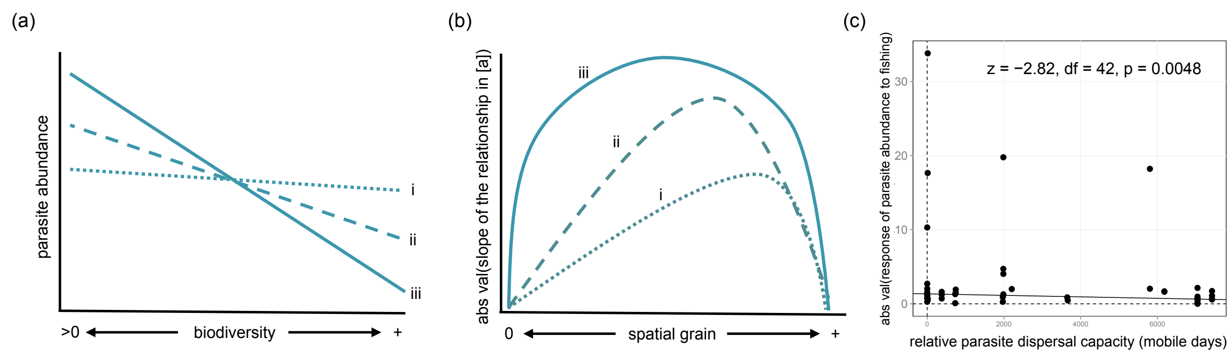

Figure 7. *We predict (a) that the slope of the relationship between host diversity and parasite abundance will shallow with increasing dispersal capacity of parasites and (b) that slope will be greatest in magnitude at intermediate spatial grains, with peaks at coarser spatial grains for higher dispersal parasites. Shown are [i] high-dispersal, [ii] medium-dispersal, and [iii] low-dispersal parasites. (c) We have already observed that the slope of the relationship between fishing and parasite abundance shallows with increasing parasite dispersal capacity.*

Preliminary data from the six islands currently in our dataset indicate a negative relationship between parasite dispersal capacity and responsiveness to fishing pressure (which we assume here is a proxy for fish biodiversity; **Figure 7c**). We used meta-regression to assess the responsiveness of parasite abundance to fishing pressure (standardized regression coefficients from Wood et al. 2014b) as a function of parasite dispersal capacity and found that – consistent with our predictions – the greater the dispersal capacity of the parasite, the less it responded to island-level fishing pressure (**Figure 7c**). The next step in this analysis is to expand the spatial scope of our dataset, so we can explore how this relationship varies across spatial scales (**Figure 7b**) and to refine our estimates of parasite dispersal capacity using molecular techniques.

Estimating parasite dispersal capacity – We will obtain information about parasite dispersal capacity in two ways: first, by collating information on the life cycle and natural history of each parasite from the literature, and second, by using molecular data to estimate dispersal for a subset of parasite taxa.

We will begin by building on an existing dataset constructed for parasites from the six islands already sampled (Appendix F in Wood et al. 2014b), by including additional information on the life cycle and natural history of each identified parasite taxon (>77 taxa). Each parasite will be classified according to its higher-order taxonomic group, transmission strategy, the identity of all known intermediate and definitive hosts, and the presence of any free-living infectious stages. We will use the literature to estimate both the average distance over which each definitive and intermediate host might disperse over its lifetime and the length of the pelagic larval duration for any free-living infectious stages. This information will be collated and parasites will be ranked by dispersal capacity. Where parasite taxa are poorly known, we will surmise life history traits based on membership in higher-order taxonomic groups. While this is a coarse approach, many life history traits are phylogenetically conserved within these higher-order taxonomic groups (Sasal et al. 1998, Mouillot et al. 2006, Wood et al. 2015).

To complement this literature-based means of estimating relative dispersal capacity for all parasite taxa detected, we will also estimate absolute dispersal capacity using molecular techniques for a subset of parasite taxa. Because assessing genetic structure across all >77 parasite taxa would create unreasonable costs, we will choose six taxa that are common across the 18 islands: two taxa that have potential for high dispersal (e.g., nematodes, cestodes), two that are expected to have low dispersal (e.g., monogeneans), and two with intermediate dispersal capacities (e.g., crustaceans, trematodes). We will use tissue samples from ~25 genetically independent individuals of each of these six parasite taxa at each island to characterize overall genetic structure of each parasite population at the mtDNA COI locus. For each of the six parasite taxa, we will begin by running samples taken from distant islands and will add islands if there is genetic heterogeneity at these initial, coarse spatial scales; given the high dispersal potential of some of these parasites, we expect to run ~2250 samples in total, a number that we can reasonably expect six undergraduate researchers and co-PI Haupt to complete in one summer of lab work (see *Broader Impacts,* below). Genomic DNA will be extracted from individual parasites using Nucleospin extraction kits (BD Biosciences, San Jose, CA) and the mtDNA COI locus will be amplified through polymerase chain reaction. The mtDNA COI locus has been used in the past for assessing genetic connectivity and estimating dispersal among marine species (Haupt et al. 2013). Primers specific to each parasite taxon at the COI locus have been identified (Hansen et al. 2003, Hu and Gasser 2006, Yazawa et al. 2008, Razo-Mendivil et al. 2010, Waeschenbach et al. 2012, Makouloutou et al. 2014). After amplification, PCR products will be sent out for sequencing in both forward and reverse directions. Sequences will be aligned and visually scored in Sequencher (Gene Codes Corporation). These sequences will then be exported in FASTA files and analyzed using Arlequin (Excoffier et al. 2007) and R. We will calculate global, group, and pairwise *F*-statistics to estimate population subdivision, measures of genetic diversity, and haplotype networks. Pairwise *F*-statistics will be used to calculate isolation by distance (IBD) to estimate dispersal potential of species (Rousset 1997). We hypothesize that parasite taxa with potential for high dispersal will have little to no genetic structure and that taxa with low dispersal will possess high levels of genetic structure throughout the sampled range. Because of this, we might not be able to calculate dispersal distance for all six parasite taxa, and for those with homogeneous genetic structure across the study region, we will make the conservative assessment that dispersal is greater than or equal to the distance between the most distant collection sites. The results from this molecular analysis will be compared to expectations based on our literature survey (see paragraph above). The population genetic analyses will be led by co-PI Haupt (California State University Monterey Bay; CSUMB) and a team of CSUMB undergraduates. The team's estimates of isolation by distance will become input for analyses designed to answer Question 3, but the data they produce will also answer other questions in the form of independent undergraduate research projects (see *Broader Impacts*). Co-PI Haupt will work closely with students from DNA extraction through data analysis to ensure quality control throughout the project.

V. Broader impacts

Integrating research and education – Our proposed project will intimately intermingle education with research by placing a group of underrepresented undergraduates in a central research role: performing the molecular analyses required to estimate parasite dispersal distance (Question 3). Co-PI Haupt, an

Assistant Professor at the minority-serving California State University, Monterey Bay (CSUMB), will lead a group of six undergraduates in a summer research experience, the Research Internship in Molecular Ecology (RIME). CSUMB is a federally classified Hispanic-Serving Institution where Latinx students comprise 36% of the student body and where a majority of students are the first in their families to go to college. The university serves a three-county region where nearly 50% of families are low-income, including many migrant farm workers.

The RIME program will be administered with the logistical support, infrastructure, and instructional resources of CSUMB's Undergraduate Research Opportunities Center (UROC; see attached *Letter of Collaboration*). UROC is a cross-campus center that trains, supports, and engages students in undergraduate research. RIME positions will be paid (see *Budget* and *Letters of Collaboration*), as financial support increases participation of students from low-income backgrounds (National Academy of Sciences 2011). The RIME program will support independent projects for six students. The five CSUMB undergraduates to be supported will be selected through a competitive application process, and we will seek out and encourage applications from students who belong to underrepresented groups (i.e., women, individuals with disabilities, URM, first-generation college students). Three students will be financially supported by NSF funds and two students will be supported by UROC funds (see *Letter of Collaboration*). We will also leverage the Monterey Bay Regional Ocean Science REU program at CSUMB to recruit an additional (non-CSUMB) student, who will be fully supported by the REU program. The three UROC-/REU-supported students will each be provided with research funds (~$1,000) and funds for conference attendance (see *Letters of Collaboration*). RIME students will spend 5 weeks during spring semester and 10 weeks during the summer performing DNA sequencing of parasite vouchers in the Molecular Ecology Lab at CSUMB's Chapman Science Center. They will receive a one-week "crash course" in molecular ecology taught by co-PI Haupt over spring break, and will begin working part-time (five hours/week) to optimize primers in the final five weeks of the spring semester, followed by ten weeks of part-time (30 hours/week) sequencing and analysis work and part-time (10 hours/week) professional development over the summer. The student supported by the REU program will only participate in the 10-week summer portion of the RIME program. Professional development activities will be administered by CSUMB's UROC/REU Summer Research Program and will include workshops on responsible conduct of research, presentation skills, writing skills, professionalism, and graduate school preparedness.

In addition to learning valuable laboratory and professional skills, students will each lead an independent research project that uses molecular data generated from parasite vouchers to address questions in parasite ecology (e.g., identifying barriers to dispersal, assessing variation in genetic structure among parasite taxa, estimating effective parasite population sizes). RIME students will receive direct one-on-one mentorship from co-PI Haupt and either PI Wood or co-PI Sandin, as appropriate to the topic of the student's chosen independent project. The end of the internship will be timed to coincide with the annual in-person meeting of the collaborators (to take place in Monterey, CA in the final year of the project), where RIME undergraduates will present and discuss their findings with postdocs, graduate students, and PIs from the Wood and Sandin Labs. All RIME students will also present a poster on their respective projects at the CSUMB Undergraduate Summer Research Poster Symposium. Students will receive authorship on all manuscripts that make use of the molecular data they produce and will be encouraged and supported to carry their research forward into undergraduate honors theses, to publish their work in peer-reviewed journals, and to present at the annual meeting of the Western Society of Naturalists in the fall semester after their internship ends. By integrating these students into the heart of our project, we will provide an unparalleled undergraduate research experience, one that allows students the opportunity to work with mentors from primarily undergraduate institutions (CSUMB) as well as large R1s (UW and Scripps/UC San Diego). Empirical data show that authentic research experiences like the one proposed here are high-impact practices for teaching (National Academy of Sciences 2011, McNair and Albertine 2012). We will work closely with UROC to assess the effectiveness of the RIME program by administering pre- and post-research experience surveys and comparing responses to a control group of CSUMB students. Outcomes to be assessed will include peer and faculty interaction, higher-order learning, academic gains, and professional development.

Our proposal will also support the training of two graduate students (one at UW and one at Scripps), one UW postdoctoral scholar, and several UW undergraduate "capstone" (i.e., honors thesis) students. All students involved in the project will participate in project meetings and receive one-on-one mentorship (see *Postdoctoral Researcher Mentoring Plan*).

Enhancing infrastructure for research and education – As our dataset continues to grow, it is imperative that we permanently archive it and make it publicly available. All of the data collected for this project – including fish data, parasite data, meta-data on sampling sites and hosts, and accession info for sequences deposited to GenBank – will be documented and made freely available through the Biological and Chemical Oceanography Data Management Office (BCO-DMO; see *Data Management Plan*). Physical specimens will be deposited with the Smithsonian Institution (see *Data Management Plan*).

Facilitating participation of women, persons with disabilities, and underrepresented minorities in science – For all of the new positions created by this proposal, we will recruit and encourage women, persons with disabilities, first-generation college students, and underrepresented minorities to apply. PI Wood is currently scouting for such candidates at the annual meetings of the Western Society of Naturalists and the Society for Advancement of Chicanos/Hispanics and Native Americans in Science (SACNAS). We will perform similar recruiting for the RIME program.

Improving biodiversity science education and increasing public engagement with biodiversity science – We will develop and disseminate a vital resource that is in extremely short supply: quality educational tools for teaching about parasite biodiversity. In collaboration with the Network of Conservation Educators and Practitioners (NCEP), a program of the American Museum of Natural History's Center for Biodiversity and Conservation, we will develop an open-access learning module (see *Letter of Collaboration*). Like all modules developed by NCEP, ours will be peer-reviewed, designed for the university and professional level (but adaptable to other audiences), and will target educational outcomes central to conservation practice. Manager of NCEP Dr. Suzanne Macey will optimize these materials for use by educators, overseen by Director of the AMNH Center for Biodiversity & Conservation, Dr. Ana Luz Porzecanski. Parasites comprise more than half of Earth's species, but are rarely addressed in conservation science education (Nichols and Gómez 2010). Our module will explore the sometimes-hidden biodiversity of marine parasites, couching marine parasitology as the study of an unseen but vibrant world that operates just below the surface of familiar seascapes. Our module will consist of a Powerpoint introduction to the system, an exercise with active learning techniques, and teacher resources, designed to foster critical thinking skills and expand student understanding of parasite ecology by posing the question: what is the shape of the relationship between biodiversity and parasite abundance? Students will learn the competing hypotheses that address this question and use real data to discriminate among those hypotheses. Our efforts will make a high-quality educational resource widely available, with the broad international reach and high volume of web traffic generated by North America's premier museum of natural history. Assessment information will be gathered by tracking downloads of the module, distributing surveys to users of the module, and including a student pre- and post-assessment in module materials, which teachers can upload to our online system.

VI. Potential for transformative insights

The research proposed herein will provide the world's first insights into the direction, magnitude, shape, and scale-dependence of the biodiversity–parasite abundance relationship across a diversity of parasite taxa, host taxa, and spatial scales, and will comprehensively identify conditions under which biodiversity is likely to be important in determining the abundance of parasites – a fundamental contribution that can be readily extrapolated from our marine focal system to freshwater and terrestrial ecosystems. **No prior study has attempted such a comprehensive investigation of how biodiversity–parasite abundance relationships play out in real ecosystems**, and this will contribute substantially to a literature that currently relies on empirical evidence from a mere handful of parasite species. For marine ecosystems in particular, our work will indicate **when and where parasitism is likely to exert important ecosystem-level effects**. In addition to its unique suitability for its scientific purpose, this project leverages existing research infrastructure – the 100 Island Challenge – to perform field collections that would otherwise necessitate a multi-million dollar investment. Our efforts will not only produce major advancements in disease ecology and biological oceanography, but will also bring authentic research experiences to undergraduates from underrepresented groups, produce and disseminate active-learning modules on parasite biodiversity, and significantly enrich fundamental ecological theory.

VIII. Results from prior NSF support

PI Wood, co-PI Sandin, and co-PI Haupt: No NSF funding in the past five years.