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Demographic consequences of disease in a habitat-forming seaweed and impacts on interactions between natural enemies

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Abstract. Diseases affecting natural ecosystems are increasing in frequency and severity, but unless obviously catastrophic, the consequences of disease outbreaks are often overlooked, relative to other ecological processes (e.g., predation, competition). Disease can have profound effects on individuals and can also strongly influence interactions between infected hosts and their natural enemies. We investigated whether a novel bleaching disease affected the survival or performance of a habitat-forming red seaweed, *Delisea pulchra*. In addition, we investigated bidirectional, multipartite interactions between this seaweed host, its pathogens, and consumers. Although we found no negative impacts of disease on survival of *D. pulchra*, bleaching had substantial, negative consequences for affected individuals, including a dramatic drop in fecundity and a significant decrease in size. In the first direct demonstration of bacterial disease-mediated herbivory of seaweeds, herbivores generally preferred to consume bleached tissue in feeding trials, and we also found higher densities of herbivores on bleached than co-occurring, healthy algae at sites where herbivores were abundant. In a conceptually reciprocal test of the effects of herbivores on infection, we showed that simulated herbivory increased susceptibility to bleaching when algae were also exposed to cultures of a bacterial pathogen. Given the high proportions of *D. pulchra* affected by bleaching during peak periods, the impacts of this disease are likely to have important implications at the population level. This work highlights complex interactions between habitat-forming organisms and their natural enemies and further emphasizes the need to consider disease in ecological research.

Key words: *Delisea pulchra*; fecundity; fitness; herbivory; host use; infection; macroalgae; performance; sublethal impacts.

INTRODUCTION

Disease is a fundamental process that affects the ecology and evolution of higher organisms. In many natural ecosystems, the frequency and/or severity of diseases appear to be increasing and this has been linked to global warming and other more localized anthropogenic impacts (Harvell et al. 2002). Consequences of diseases can be profound, often leading to mass mortalities and species declines: e.g., “chytrid fungus” (*Batrachochytrium dendrobatidis*) infections of amphibians (Pounds et al. 2006), “sudden oak disease” in woody trees (Rizzo and Garbelotto 2003), and diverse microbial diseases of corals (reviewed by Harvell et al. 2007). While these and other examples are compelling evidence for the importance of disease in natural systems, arguably, unless of catastrophic consequence, the impacts of disease receive less attention from

ecologists than other biotic interactions (e.g., herbivory, predation, and so on).

Diseases can significantly influence host population ecology without necessarily resulting in widespread mortality: Sublethal impacts of disease can directly influence hosts, because resistance or tolerance to, or recovery from infection typically incur substantial fitness or performance costs (Gemmell and Read 1998, Boots 2008). However, in ecosystems that are remote, cryptic, or otherwise difficult to observe, the direct, ecological consequences of many diseases affecting natural ecosystems may go unnoticed. Diseases can also impact hosts indirectly by altering their interactions with other organisms. For example, diseased hosts may be more or less likely to be attacked by consumers than healthy conspecifics (reviewed for terrestrial plant–herbivore systems by Stout et al. 2006), and infection may alter the competitive ability of individuals or species within a community (Park 1948, Schall 1992). The conceptual reverse of these interactions also occur, in which consumption indirectly alters host organism susceptibility to disease by providing infection sites for pathogens

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or otherwise affecting resistance (Garcia-Guzman and Dirzo 2001, Kluth et al. 2001).

Both disease-mediated changes to consumer–prey interactions and consumer-mediated changes to host–pathogen interactions have the potential to alter ecosystem function, particularly if ecosystem engineers (such as top predators or habitat-formers) are affected (Burdon et al. 2006). Despite this, such indirect, multipartite interactions between hosts, pathogens, and consumers have received little attention outside of terrestrial plant–herbivore and agricultural systems (Stout et al. 2006). A potential explanation for this may be that, traditionally, ecological experiments involving microbial pathogens were practically challenging. Recent advances in the fields of molecular and environmental microbiology have now enhanced opportunities for ecologically sensible investigations into the complex relationships between micro- and “higher” organisms.

Subtidal marine ecosystems are relatively difficult to access and observe, compared to terrestrial or intertidal habitats. Water-borne diseases can be particularly virulent (Ewald 1994), and microorganisms are ubiquitous and abundant in seawater (Reinheimer 1992). Disease may thus have important impacts in marine ecosystems, but remain unobserved. Two groups of marine organisms that have been noticeably (and often catastrophically) affected by disease are seagrasses (Orth et al. 2006) and corals (Harvell et al. 1999). As both examples are habitat-forming organisms, outbreaks of these diseases have had significant, ecosystem-scale consequences: Coral bleaching can lead to changes in associated fish (Booth and Beretta 2002) and benthic invertebrate (Przeslawski et al. 2008) assemblages and has important potential environmental and socioeconomic implications for coastal communities (Hoegh-Guldberg et al. 2007). Similarly, seagrass wasting-disease outbreaks have resulted in the collapse of fisheries, extinction of at least one associated species and declines of many more (Orth et al. 2006). Whereas the impacts of disease in these examples were obvious and involved mass mortalities, sublethal impacts of diseases may (as per other biotic interactions; e.g., herbivory [Harper 1977] and predation [reviewed by Lindsay 2010]) also have important impacts on ecosystem engineers and the communities they support, but remain unnoticed.

In temperate marine ecosystems, macroalgae are the dominant habitat-forming organisms on rocky reefs. Diseases affecting macroalgae in natural systems have been occasionally reported (Apt and Gibor 1989, Correa et al. 1993, 1994, 1997, Peters and Schaffelke 1996, Faugeton et al. 2000), but we know almost nothing about the consequences of such diseases on host populations or associated communities. There is emerging evidence that macroalgal “forests” are declining globally (Airoidi and Beck 2007, Wernberg et al. 2011), which could have widespread consequences (Steneck et

al. 2002) analogous to those resulting from declines of corals and seagrasses. Therefore, understanding how disease may affect seaweeds and their interactions with other trophic levels is crucial, particularly as the incidence of marine disease appears to be increasing (Harvell et al. 1999).

We recently described a bleaching disease in *Delisea pulchra*, a chemically defended red seaweed (Campbell et al. 2011, Case et al. 2011). Bleaching involves a whitening of normally deep-red algal tissue and may appear anywhere on a thallus, i.e., it is not restricted to the tips or any other uniform part of the alga, as would be expected were it the direct consequence of the environment (Campbell et al. 2011). This disease affects up to 80% of individuals in some populations, particularly those occurring in shallow (<4 m depths) waters during peak periods. Such outbreaks are associated with increased water temperatures, shifts in surface-associated microbial communities, and a significant reduction in tissue concentrations of halogenated furanones, *D. pulchra*'s chemical defenses (Campbell et al. 2011). Furanones have a number of functions, including inhibition of colonization and virulence of bacteria (Manefield et al. 2001, 2002). Experimentally manipulating the production of halogenated furanones in cultured *D. pulchra* increases the frequency and severity of bacterial bleaching (Campbell et al. 2011), which can be induced in “undefended” individuals via inoculation with bacterial pathogens (e.g., *Nautella* sp. R11; Case et al. 2011, Fernandes et al. 2011). Phylogenetic and functional composition of bacterial assemblages associated with *D. pulchra* appear to shift prior to visible bleaching (Campbell et al. 2011, Fernandes et al. 2012), suggesting the possibility that algal chemical defenses decrease due to environmental stress (e.g., increased water temperatures), facilitating the colonization or proliferation of pathogens that would normally be inhibited or rare.

Given the high incidence of this seaweed disease and the important role of *D. pulchra* as a habitat-former, food source, and primary producer, we assessed the direct and indirect impacts of bleaching on this macroalga. Specifically, we monitored algal populations over a six-month period to determine whether bleaching affected survivorship or performance (growth, fecundity). Additionally, we assessed whether disease affected *D. pulchra*'s interactions with locally abundant herbivores: We conducted surveys to determine whether herbivore densities were higher on bleached or healthy algae and also ran feeding assays to investigate whether consumption by herbivores was different for bleached or healthy tissue. Finally, to assess whether the grazing action of consumers affected host–pathogen interactions, we physically simulated grazing damage in *D. pulchra* individuals and recorded their susceptibility to the bleaching pathogen *Nautella* sp. R11 under field conditions. Our broad aim was to understand the consequences of bleaching disease on *D. pulchra* and

investigate potential multipartite interactions involving very different natural enemies of this abundant seaweed.

METHODS

Study sites and organisms

The red alga *Delisea pulchra* (Greville) Montagne (Bonnemaisoniales: Rhodophyta) is common in coastal, subtidal habitats in temperate Australia (Womersley 1996). Individuals of this species persist for an average of seven and a maximum of 16 months (Wright and Steinberg 2001), and detailed demographic and genetic studies of *D. pulchra* have been carried out previously (Wright et al. 2000, Wright and Steinberg 2001). Populations at two locations were studied: Bare Island in Botany Bay (33°59'32" S, 151°13'50" E) and Long Bay, Malabar (33°58'19" S, 151°14'42" E). Both locations are near Sydney, Australia, and are similarly dominated subtidally by turf-forming algae beds, kelp forests, urchin barrens, and sandstone boulders. These locations were selected for the present study because bleached and healthy *D. pulchra* individuals co-occur there during summer on reefs in "deep" (8–10 m) and shallow (1–3 m) water (Campbell et al. 2011).

Effects of bleaching on survival and performance of D. pulchra

To determine whether bleaching in *D. pulchra* influenced the survival or performance of affected algae, 50 randomly selected individuals were tagged and monitored in shallow (1–3 m) and deep (8–10 m) water reefs at Bare Island and Long Bay in September 2007. Three divers, who moved parallel to the shoreline within the specified depth range, randomly tagged each *D. pulchra* individual they encountered. Uniquely numbered, flagged plastic tags were attached to the substratum directly adjacent to *D. pulchra* individuals so that the algae were not in direct contact with the tags, but each tag corresponded with only one individual. A minimum of 60 individuals was tagged at each depth and location. At setup, the tag number (unique three-digit code), location (Bare Island or Long Bay), depth (shallow or deep), and date were all recorded and each alga was classified as either "healthy" or "bleached" (whitening of localized, nonuniform parts of the thallus, as per Campbell et al. 2011, Case et al. 2011). Approximately 35% of tagged algae at each location and depth were not observed again at any time after initial setup and these were omitted from all analyses. Algae were considered to have survived while still attached to the substratum adjacent to their unique tag and once detached, were considered to have died, since *D. pulchra* cannot reattach to the substratum.

In addition to measuring survival, algal performance was estimated by measuring algal length in situ, which was then used to estimate biomass and fecundity. We first established that length was correlated with biomass and that biomass was related to fecundity (see details in Appendix A). To determine whether total fecundity

(numbers of fertile tetrasporangia per individual), numbers of tips per branch (tetrasporangia are held on the tip of thallus branches in *D. pulchra*), or the relationship between biomass and fecundity differed between bleached and healthy individuals, 23 thalli in each condition were randomly sampled from shallow habitats at Long Bay and compared.

After the tagging study was set up and initial ($T = 0$) data had been collected, each tagged alga was revisited approximately monthly, its condition (healthy or bleached) assessed and length measured. These surveys were conducted over an austral summer from September 2007 until April 2008, so data were collected from tagged algae for up to seven months. At the end of the study, algae that had bleached at any time were classified as "bleached" and those that had not were classified as "healthy." The proportion of bleached and healthy algae that survived from one census time to another were described and compared with survivorship curves. To quantify survival duration, we calculated the number of months over which each tagged individual was observed and compared these bleached and healthy algae at each site and depth. To estimate changes in biomass and fecundity of tagged algae, the overall change in length per month was also calculated (corrected for differences in initial lengths ((final length – initial length)/initial length)/survival time), and this was compared among healthy and bleached individuals.

Effects of algal condition on herbivore abundance and feeding preferences

Surveys of algal beds.—To determine whether densities of herbivores associated with bleached or healthy algae were different, populations of *D. pulchra* growing subtidally at two locations (Bare Island and Long Bay) in 2–4 m depth were surveyed and the abundances of the urchin *Holopneustes purpurascens*, the gastropod *Phasianotrochus eximius*, and the sea hare *Aplysia parvula* (consistently the most abundant herbivores observed throughout the surveys) within each algal canopy were quantified. From each location, 30 bleached and healthy algae were randomly selected, destructively sampled, and fully enclosed in situ within sealed bags. In the laboratory, algae were rinsed thoroughly in seawater in an examination tray and the numbers of the three herbivores (>5 mm) counted. Each alga was patted dry, weighed, and the density of herbivores (numbers per gram of wet mass of algae) calculated. Because bleached *D. pulchra* tended to be smaller than healthy conspecifics, there was a risk that any condition-specific differences we observed in herbivore density could be confounded by condition-specific differences in biomass. We thus compared herbivore densities among healthy algae of varying sizes to account for this possibility, but found no effect of algal biomass on herbivore densities (see details in Appendix B).

Feeding assays.—All three herbivores sampled are found within the canopy of *D. pulchra* and have all

previously been studied with regards to their feeding preferences (Rogers et al. 2002, Williamson et al. 2004, Wright et al. 2004). To assess whether these taxa showed any preferences for consumption of either bleached or healthy algal tissues, we conducted two separate paired choice feeding experiments, in which each herbivore was offered a choice between two portions of algal tissue that were of similar biomass. In the first experiment (“bleached vs. unbleached”), 18 replicate herbivores were offered a choice between (1) bleached tissue and (2) adjacent healthy-looking tissue. In the second experiment (“healthy vs. unbleached”), 18 replicate herbivores were offered a choice between (1) healthy tissue from a completely healthy alga and (2) visibly unbleached tissue from a bleached individual, such that each replicate herbivore was offered tissue from two different individuals. In both experiments, an equal number of replicates were set up with no herbivores, to control for autogenic changes in biomass. Initial (wet) mass of algal tissue samples was measured prior to experimentation. Gentle aeration was provided in compartmentalized aquaria for 48 h at a constant temperature of 19–20°C with a 16:8 hour light:dark cycle. After 48 h, changes in algal mass in the presence of herbivores were compared to changes in mass of control algae, from which herbivores were absent.

Effects of simulated herbivory and inoculation on algal bleaching

The results of the feeding assays of bleached vs. unbleached algae indicated that disease impacted on the effects of herbivores on this alga (see *Results*). To test whether the reciprocal interaction could occur (i.e., whether herbivory could enhance infection), we assessed whether the ability of the bacterial pathogen *Nautella* R11 (hereafter referred to as “R11”) to cause bleaching in established *D. pulchra* individuals was facilitated by physical damage to the thallus (such as wounds inflicted by herbivores). We exposed experimentally “grazed” and “ungrazed” algae to R11 cultures in situ and monitored them for signs of bleaching for 48 h (see Plate 1). All replicates were inspected prior to experimentation, classified as healthy, and were all of a similar size (10–20 cm length). Eight algae were enclosed inside plastic bags in situ, with a 50-mL falcon tube containing R11 culture (for details on culturing methods and inoculum densities, see Appendix C). The tubes were opened and algae were left enclosed within the bags, exposed to the bacteria for 2 h, after which time the bags were removed. Another eight algae experienced simulated grazing (e.g., by a gastropod) via gentle scraping with a razor blade and were also inoculated with R11 as described in the previous sentence. Another set of replicates were “grazed” but not inoculated. In two procedural control treatments, eight replicate algae were bagged but not inoculated or grazed, and another eight were handled but not bagged, grazed, or inoculated. Finally, eight algae were left as unmanipulated controls.

After 48 h, each alga was revisited and the presence of any bleaching was recorded.

Statistical analyses

Survivorship data were analyzed in two ways: Firstly, cumulative Kaplan-Meier survivorship curves were fitted to the proportion of algae surviving at each time point, depth, and location and curves were compared between “bleached” and “healthy” algae using a log-rank (Mantel-Cox) test, as per Pyke and Thompson (1986). Secondly, because “bleached” algae were bleached for various lengths of time (i.e., some were always bleached, whereas others were initially healthy and then became bleached at some point during the study), the number of months for which individual tagged algae were observed alive (“survival time”) was compared among *D. pulchra* from deep and shallow waters at both locations using a two-factor analysis of covariance (ANCOVA), where the factors were location (random; Bare Island [BI], Long Bay [LB]) and depth (fixed; deep, shallow), and the proportion of the survival time each alga was classified as bleached (proportion time bleached [PTB]) was included as a covariate.

Total fecundity (numbers of tetrasporangia per gram of algae) and numbers of tips per branch were compared between bleached and healthy algae using single fixed-factor analyses of variances (ANOVAs) where the factor was condition (bleached, healthy). Change in biomass per month estimates for tagged individuals, corrected for differences in initial biomass ($[(\text{final length} - \text{initial length})/\text{initial length}]/\text{survival time}$) were compared using a two-factor ANCOVAs with PTB treated as a covariate as per the design described in the previous paragraph for survival time. Given the degree to which this data set was unbalanced among factors being compared in the model (i.e., $n = 12-72$), we also conducted a balanced ANCOVA using $n = 12$ randomly selected individuals from each group. The results of both analyses were similar, and thus, only the full (unbalanced) model including all the data is presented here.

Densities of herbivores (species analyzed separately) were compared between bleached and healthy algae (condition; fixed) at the two study locations (BI, LB; random) using analyses of variance (ANOVAs). Herbivore consumption of different tissue types was analyzed (for each species separately) by comparing the differences in mass change between the two algal tissue types in the presence and absence of herbivores using t tests as per Peterson and Renaud (1989). Binary logistic regression models with a logit link function were used to analyze the occurrence of bleaching ($\pm$) among experiments and treatments in the simulated herbivory and inoculation experiment. This analysis showed that there were no differences between experiments and no interactions between “experiment” and “treatment.” So, to compare the effects of different treatments on bleaching, we pooled the data and conducted a single-factor ANOVA on the proportions of individuals from

FIG. 1. Survivorship curves of bleached and healthy algae at (A, C) Long Bay and (B, D) Bare Island, Australia, in (A, B) deep and (C, D) shallow waters, showing the percentage of tagged algae remaining alive at each census date, with $n = 60$ –120.

each treatment, with $n = 3$ replicates (experiments). Assumptions of models were checked using Cochran's and Levene's tests and by inspecting scatterplots of residuals when appropriate and, if required, data were transformed to conform to model assumptions and these are reported alongside tables. Log-rank tests and linear regression analyses were performed in Prism Graph-Pad v5.0d (GraphPad Software 2007). ANOVAs and ANCOVAs were performed in SYSTAT v12.00.08 (Systat Software 2008) and logistic regression was done using the statistical platform R v0.97.321 (R Development Core Team 2008).

RESULTS

Condition and survival of tagged *D. pulchra*

Of the 438 algae tagged during this study, 71% were classified as “healthy” (i.e., they never had any visible signs of bleaching) and the remaining 29% as “bleached” (i.e., bleaching was observed at least once during the study). Bleaching had no consistent effects on survival of *D. pulchra* in this study (Fig. 1), with the mean ($\pm$ SE) survival times for healthy and bleached algae being 2.47 ± 0.09 and 2.46 ± 0.15 months, respectively. At Bare Island, there was no difference in survival between algae that bleached and those that did not in either deep ($\chi^2_{117} = 0.003$, $P = 0.9551$) or shallow ($\chi^2_{97} = 2.83$, $P = 0.925$) waters (Fig. 1). At Long Bay, bleaching did influence survival, but not negatively; bleached algae survived longer than healthy individuals and this difference was more pronounced in deep ($\chi^2_{162} = 10.84$, $P = 0.001$) than shallow ($\chi^2_{75} = 4.93$, $P = 0.0263$) waters (Fig. 1). Analyses of survival time between algae at the two locations and depths, including the proportion of time bleached (PTB) as a covariate, confirmed that bleaching had a positive effect on survival, but only at Long Bay and only in

deeper waters, as evidenced by a significant three-way interaction between location, depth, and PTB (Fig. 1, Table 1).

Effects of bleaching on performance measures of *D. pulchra*

In the tagging study, change in length of tagged individuals was consistently influenced by the proportion of time each alga bleached, with PTB explaining more than one-quarter of the variance in change in length ($R^2_{91} = 0.267$, $P = 0.011$). Depth also influenced whether the length of tagged individuals changed throughout the study, with shallow algae growing less (and/or losing more biomass) than deep conspecifics. These patterns were consistent at both locations (Table 2).

Fecundity was strongly and positively related to biomass in bleached ($R^2_{23} = 0.684$, $P < 0.001$) and healthy ($R^2_{23} = 0.718$, $P < 0.001$) *D. pulchra* (Fig. 2a). The slopes of these relationships were similar to each other ($F_{1,44} = 0.68$, $P = 0.414$); however, the y -intercepts

TABLE 1. ANCOVAs comparing survival time (total number of months for which each alga was observed) of tagged *Delisea pulchra* from deep and shallow waters at Bare Island and Long Bay, Australia, with the proportion of time algae were bleached (PTB) included as a covariate.

Source	df	MS	F	P
PTB	1	46.465	18.863	0.001*
Location (L)	1	20.517	8.329	0.003*
Depth (D)	1	2.865	0.488	0.514
L $\times$ D	1	5.873	2.384	0.107
L $\times$ PTB	1	0.0003	0.0004	0.953
D $\times$ PTB	1	6.967	2.828	0.102
L $\times$ D $\times$ PTB	1	28.055	11.389	0.002*
Error	430	2.463		

Note: An asterisk (*) indicates a significant effect ($\alpha < 0.05$) of the relevant factor with $n = 60$ –120.

TABLE 2. ANCOVA comparing the change in biomass of tagged *D. pulchra* in deep and shallow waters at Long Bay and Bare Island during the tagging study, including PTB (proportion of time each alga was classified as bleached) as a covariate, with $n = 12-72$.

Source	df	MS	F	P
PTB†	1	0.022	13.69	<0.01*
Location (L)	1	0.025	2.77	>0.05
Depth (D)	1	0.227	10.44	<0.01*
L × D	1	0.022	2.44	>0.05
Error	231	0.009		

Notes: Change in biomass was calculated as (final length – initial length)/initial length. An asterisk (*) indicates a significant effect ($\alpha < 0.05$) of factor on biomass change.

† Data were $\log_{(x+1)}$ -transformed to meet the assumptions of ANCOVA.

were significantly different ($F_{1,45} = 70.35$, $P < 0.001$), such that a bleached alga of a similar mass to a healthy conspecific was likely to be significantly less fecund (Fig. 2A).

When compared directly by counting the numbers of tetrasporangia per thallus and the numbers of terminal tips (where the reproductive structures are located) per branch, the effect of bleaching on fecundity was striking. Healthy algae had up to 10 times as many fertile tetrasporangia per gram of biomass than bleached conspecifics ($F_{1,46} = 18.21$, $P < 0.001$; Fig. 2B). Bleached algae also had significantly reduced (by at least an order of magnitude) numbers of terminal tips per branch ($F_{1,46} = 31.79$, $P < 0.001$; Fig. 2C) compared to healthy individuals.

Effects of algal condition on herbivore densities and feeding preferences

At sites where they were abundant, densities of each herbivore taxon surveyed were higher on bleached *D. pulchra* individuals than healthy conspecifics (Fig. 3, Table 3). At sites where herbivore species were less abundant, there were similar but nonsignificant trends for higher densities on bleached vs. healthy algae. No herbivores were found on algae collected in deep waters.

When herbivores were offered bleached vs. adjacent healthy looking (“unbleached”) tissue, *P. eximius* and *H. purpurascens* consumed more bleached tissue than adjacent unbleached tissue from the same alga (*P. eximius*, $t_{17} = 2.56$, $P = 0.055$; *H. purpurascens*, $t_{17} = 2.77$, $P = 0.013$; Fig. 4). Consumption of bleached and healthy tissue by the sea hare *A. parvula* was not significantly different, although the power of this analysis was low due to the death of many replicate sea hares during this assay (Fig. 4). In the “healthy vs. unbleached” tissue assay, where herbivores were offered a choice between visibly healthy tissue from a bleached alga and healthy tissue from a completely healthy alga, no herbivores showed a preference for either tissue type (*P. eximius*, $t_{17} = 0.77$, $P = 0.451$; *H. purpurascens*, $t_{17} = 1.34$, $P = 0.196$; *A. parvula*, $t_{17} = 1.574$, $P = 0.154$).

Effects of damage and inoculation on algal bleaching

There was a significant effect of “treatment” on whether or not algae bleached ($\chi^2_{5,136} = 43.050$, $P \ll 0.001$), with no variation among experiments ($\chi^2_2 = 141$, $P = 0.461$) and no interaction ($\chi^2_{10,126} = 6.039$, $P = 0.812$). In pairwise comparisons between treatments, algae that were both inoculated and experimentally “grazed” were significantly more likely to bleach than algae in other treatments (Tukey’s HSD $P < 0.05$ in all cases; Fig. 5). Despite some occasional bleaching in “inoculation only” and “grazing only” treatments, this was not significantly different from controls, which were all zero (Tukey’s HSD $P > 0.15$ in all cases).

FIG. 2. Comparisons of bleached (open bars and circles; dashed line) and healthy (black bars and circles, solid line) algae for (A) linear relationships between log(algal wet mass) and log(numbers of tetrasporangial sori), (B) total fecundity (numbers of tetrasporangial sori per gram of algal tissue), and (C) number of terminal tips per branch on each thalli, with $n = 30$.

FIG. 3. Densities (means $\pm$ SE) of "total" herbivores (combined densities of the gastropod *Phasianotrochus eximius*, the urchin *Holopneustes purpurascens* and the sea hare *Aplysia parvula*) on bleached and healthy *Delisea pulchra* algae at Bare Island and Long Bay, with $n = 30$. Asterisks (*) indicate a significant difference ($\alpha < 0.05$) in herbivore abundance between bleached and healthy individuals surveyed.

DISCUSSION

Bleaching disease had strong, sublethal impacts on the performance of *Delisea pulchra*. In particular, while survivorship was not consistently affected by bleaching, the loss of distal biomass meant that fecundity was reduced to near zero in bleached thalli. Bleaching disease altered interactions between *D. pulchra* and resident herbivores: Host use by abundant herbivores was higher in bleached individuals than healthy conspecifics, and we also observed greater consumption of bleached tissue. This is the first direct demonstration that a bacterial disease can alter seaweed–herbivore interactions in marine systems. Reciprocally, we presented evidence that herbivores could alter host–pathogen interactions: Simulated herbivory facilitated infection by the bleaching pathogen *Nautella* sp. R11. Together, these observations provide novel evidence for multipartite interactions in marine ecosystems and significant direct and indirect impacts of disease.

Direct effects of bleaching on survival and performance

Infection by pathogens and parasites often results in reduced growth and fecundity in animal (Myers and Kuken 1995, Villalba et al. 2004) and plant (Burdon 1987) hosts, and in a review into the influence of parasitism on invasion success, Prenter et al. (2004)

suggested that sublethal pressure from parasites and pathogens may be more important in natural communities than occasional mass mortalities. Bleaching had important sublethal impacts for *D. pulchra*, characterized by a dramatic reduction in algal fecundity (by more than an order of magnitude), suppressed growth, and in many cases, biomass loss. This disease effectively sterilized infected individuals via the loss of terminal thalli tips (which hold the tetrasporangia; Womersley 1996). The duration of this study (seven months) corresponds roughly to the mean life span of this species (Wright and Steinberg 2001), suggesting that the lifetime fitness of thalli that bleached early or at the beginning of the study was probably close to zero. To our knowledge, the only other study to investigate the effects of infection on fecundity in macroalgae, considered eukaryotic endophytic infections of a red alga, *Mazzaella laminarioides* (Faugeron et al. 2000). In contrast to our results, Faugeron et al. (2000) found no effect of the endophyte on the abundance or distribution of reproductive structures in the host, possibly suggesting differences between impacts of eukaryotic and bacterial pathogens, host identity, or environments.

The unexpectedly positive, location, and depth-specific influence of bleaching on algal survival may be due to differences in size: Bleached algae were usually smaller than healthy conspecifics, and smaller algae are

TABLE 3. ANOVAs comparing densities of herbivores (the gastropod *Phasianotrochus eximius*, the urchin *Holopneustes purpurascens*, and the sea hare *Aplysia parvula*) between bleached and healthy *D. pulchra* at Bare Island and Long Bay, with $n = 30$.

Source	<i>P. eximius</i> †				<i>H. purpurascens</i> ‡				<i>A. parvula</i> †			
	df	MS	F	P	df	MS	F	P	df	MS	F	P
Location (L)	1	0.0527	1.92	0.398	1	0.002	1.00	0.500	1	0.0052	3.49	0.313
Condition (C)	1	0.0532	1.93	0.397	1	0.0004	2.01	0.391	1	0.0019	1.29	0.459
L $\times$ C	1	0.0275	6.39	0.013*	1	0.0002	4.00	0.048*	1	0.0015	5.56	0.020*
Error	116	0.0002			116	0.0001			116	0.0003		

Note: An asterisk (*) indicates significant effect ($\alpha < 0.05$) of relevant factor on herbivore density.

† Data were $\log_{(x+1)}$ -transformed.

‡ Data were square root $_{(x+1)}$ -transformed to conform to the assumptions of ANOVA.

FIG. 4. Mass of bleached and unbleached algal tissue consumed (means + SE) by *P. eximius*, *H. purpurascens*, and *A. parvula*, and mass change in control treatments in the absence of herbivores during feeding assay 1 (bleached vs. unbleached tissue), with $n = 18$. Asterisks (*) represent significant differences ($\alpha < 0.05$) among tissue types according to post hoc pairwise comparisons.

less likely to detach due to waves and storms (Gaylord et al. 1994). This reduction in size may have thus conferred some benefit. Beneficial “pruning” by herbivores can reduce detachment risk (e.g., Black 1976) and analogous “disease pruning” may have a similarly positive influence on survivorship of bleached thalli. On the other hand, given that *D. pulchra* has nonmotile spores (Womersley 1996), becoming detached while highly fecund may be more beneficial for increased propagule release and dispersal, than remaining attached for longer, bleaching and losing reproductive biomass.

Multipartite interactions between an algal host, its consumers, and pathogens

Disease significantly affected alga–herbivore interactions in this system, with increased host use when herbivores were abundant and increased consumption by two out of three herbivores tested (the third showed a similar trend for preferring bleached tissue, but due to losses of animals during experiments, the power of this experiment was decreased). Bleached *D. pulchra* have depleted chemical defenses in affected thalli (Campbell et al. 2011). These compounds (halogenated furanones; De Nys et al. 1993) have been shown experimentally to deter several herbivores (Williamson et al. 2004, Wright et al. 2004), so any depletion is likely to alter interactions between this host and its natural enemies. It is possible that herbivores were attracted to the less chemically defended food represented by bleached thalli (although *P. eximius* and *A. parvula* are tolerant to furanones; Rogers et al. 2002, Wright et al. 2004); however, other biochemical or morphological changes could also play a role. The only other study to have considered how infection might influence herbivory in seaweeds found that the presence of microalgal endophytes within host tissues increased consumption of host algae by amphipods and isopods (Correa and McLachlan 1992). Additionally, in New Zealand during a dieback of the kelp *Ecklonia radiata*, Haggitt and Babcock (2003) observed anomalous, prolific consumption of dying plants by herbivorous amphipods. However, disease was never confirmed as the cause of this dieback (although virus-like particles, which are abundant in seawater [10^7 /mL; Reinheimer 1992], and macroalgal tissues [Van Etten et al. 1991] were isolated

from symptomatic thalli [Easton et al. 1997]). Our results are the first to demonstrate directly that bacterial disease can alter interactions between a macroalga and herbivores.

By simulating herbivory, we also demonstrated that consumers might influence host–pathogen interactions. Although this is well established in terrestrial host–pathogen systems (e.g., Garcia-Guzman and Dirzo 2001, Kluth et al. 2001), there are few examples from marine environments. An interesting exception is the fireworm *Hermodice carunculata*, which acts as a transmission vector and a winter reservoir for bacterial pathogens involved in bleaching of the coral *Oculina patagonica* in the Red Sea (Sussman et al. 2003). In general, consumers can affect host susceptibility to pathogen infection by creating infection sites through grazing damage and indeed, entire suites of plant defenses have evolved to manage wounds from

FIG. 5. Proportion (means + SE) of individuals that bleached in the three (replicate) “grazing”/inoculation experiments, including algae that were: “grazed” (damaged via scalpel scraping; G), inoculated with cultures of the bacterial pathogen *Nautella* sp. R11 (I), “grazed” and inoculated (G + I), enclosed within a bag but not inoculated (BC), handled but neither “grazed,” nor inoculated (HC), or left unmanipulated (UC), with $n = 8$ per experiment (pooled total $n = 24$). An asterisk (*) indicates a significantly different treatment effect ($\alpha < 0.05$). The inset is a picture of an individual in the “G + I” treatment highlighting the localized nature of bleaching, some of which is indicated by arrows.

PLATE 1. In situ inoculation: a *Delisea pulchra* individual being exposed to cultures of the bacterium *Nautella* sp. R11 in the field. Photo credit: A. H. Campbell.

herbivory (Ruuhola and Yang 2006, Ross et al. 2008). Although experimentally inflicted damage does not always elicit the same plant response as actual herbivory (e.g., Pavia and Toth 2000), in this case, artificial damage to *D. pulchra* thalli appeared to facilitate infection by the pathogen *Nautella* sp. R11 and lead to subsequent, localized bleaching.

As a first line of defense against attack from natural enemies and/or physical damage, many seaweeds produce an “oxidative burst,” which is a sudden and short-lived release of reactive oxygen species to impose oxidative stress on the affected cells, thereby resisting further infection (Bouarab et al. 2001, Dring 2006). An oxidative burst can occur in response to the detection of degrading compounds produced by pathogens (e.g., Potin et al. 2002) or mechanical damage (e.g., Collén et al. 1994). It is therefore possible that the bleaching we observed in the field inoculation experiments was simply a response to physical damage. However, if this were the case, bleaching should have been just as commonly observed in algae that were damaged only as in those that were damaged and inoculated, which was not the case.

Disease is an important ecological process, but remains relatively understudied in ecology. Recent advances in molecular microbiological methods, along with improved accessibility to subtidal marine environments, have made the inclusion of disease into ecological studies less problematic. Our work highlights the potential for disease to influence the ecology and evolution of affected hosts, even in the absence of catastrophic mass mortalities. Up to 80% of *D. pulchra* individuals in some populations bleach during peak periods (Campbell et al. 2011). This work suggests that disease may be a potentially important selective agent in evolutionary time, particularly with respect to the antibacterial chemical defenses produced by this alga (Wright et al. 2004). Individuals that resist bleaching for at least part of the season should perform better and have greater fitness than conspecifics that become bleached early and remain so for the rest of their lives.

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SUPPLEMENTAL MATERIAL

Appendix A

Description of investigations into relationships between algal biomass and (1) length and (2) fecundity ([Ecological Archives E095-013-A1](#)).

Appendix B

Description of investigations into relationships between algal biomass and herbivore density ([Ecological Archives E095-013-A2](#)).

Appendix C

Description of methodology for culturing bacteria used in the inoculation experiment ([Ecological Archives E095-013-A3](#)).