QUANTIFYING THE EFFECTS OF WHITE POX DISEASE AND BLEACHING IN ELKHORN CORAL IN THE FLORIDA KEYS FROM 1994-2014

by

BRETT STEPHEN BERRY

(Under the Direction of Andrew Park and James Porter)

ABSTRACT

Bleaching events and disease outbreaks have contributed to major losses of coral worldwide in recent decades. We analyze a long-term data set from 1994-2014 in the Florida Keys to determine risk factors for elkhorn coral (*Acropora palmata*) colony mortality. Historical (1994-2004) and contemporary (2008-2014) surveys, each tracking the status and fate of individual colonies through time, were compared and contrasted. Whole colony mortality, disease prevalence and severity were high in historical, and low in contemporary, outbreaks of white pox disease. Although counter intuitive, our results clearly show that corals surviving the initial bleaching or disease event, may exhibit delayed whole-colony mortality. The statistical significance of the pattern and its repeated expression at multiple sites and times on reefs throughout the Florida Keys, suggests that future studies of disease and bleaching should also investigate mechanisms that might temporarily prolong life in the face of these life-threatening stressors.

INDEX WORDS: *Acropora palmata*, coral disease, white pox, bleaching, coral colony size, survival analysis

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CHAPTER 1

Introduction

In recent decades, coral reef systems worldwide have experienced severe declines. It is predicted that these systems will continue to decline at an increasing rate (Precht & Miller 2007). While the long-term consequences of coral declines for reef and ocean ecosystems are largely unknown, the loss of coral reefs could be destabilizing for tropical marine systems (Hughes et al. 2003; G. P. Jones et al. 2004). The loss of reefs worldwide would come at a significant cost. Coral reefs provide services such as food (invertebrates and fish), construction materials (nio-mining), and pharmaceutical and cosmetic compounds (bio-prospecting) (Sakai 1998; Moberg and Folke 1999). Reefs provide regulating services such as land addition, protecting shorelines from erosion from waves and currents and play an important role providing carbon sequestration (Principe et al. 2012). Coral reef population reductions can be followed by prompt losses of reef biodiversity (G. P. Jones et al. 2004).

In the last three decades, an estimated 19% of reefs worldwide have been lost, 15% more are threatened with loss by 2030, with an additional 20% loss by 2050 (Wilkinson 2008). Declines in coral populations have been attributed to many factors including climate change influences such as global warming and ocean acidification, disease, increased nutrient flows, predation, presence of human waste and hurricanes (Hughes et al. 2003; Precht & Miller 2007). At a global level, elevated atmospheric CO_2 is increasing seawater acidification, which slows calcification processes necessary for coral growth and restoration. On a local scale, coral

colonies are constantly affected by improper fishing and land use practices that can release toxic contaminants, sediments, and potentially dangerous microbes into the reef ecosystem (Principe et al. 2012). Under this broad range of stressors the demographic and physiological composition of coral reef systems change, often creating conditions that promote disease outbreaks and bleaching events. This is especially true in the Florida Keys (Patterson et al. 2002; Precht & Miller 2007; Yee et al. 2011), in which this study is focused.

Observational Studies of Coral Reef Diseases:

The first studies of coral disease began in the 1970s and 1980s (Woodley et al. 2016). Early studies were very specific to particular systems, describing gross lesions and the affected hosts. In limited cases, only coral colony populations were monitored (Gladfelter 1982). By the mid-1990s reporting of new coral diseases increased, as did efforts to identify causal agents of the pathogens (Sutherland et al. 2004; Ward & Lafferty 2004). In the early 2000s pathogens were described for four coral diseases through the satisfaction of Koch's postulates (Kushmaro et al. 2001; Patterson et al. 2002; Ward & Lafferty 2004), a set of criteria aimed at establishing causative agents of disease.

Currently, approximately 20 coral diseases have been identified and 15 pathogens have been associated with approximately nine diseases (Sutherland et al. 2004; Woodley et al. 2016). Efforts to determine specific etiologies are limited due to difficulties in pathogen identification (Gil-Agudelo et al. 2006; Ritchie 2006; Cervino et al. 2008). Determining the specific source for a given coral disease is complex, in part because causal agents may indicate that a single pathogen causes several diseases, or conversely that multiple pathogens cause similar disease

signs. Clemens *et al*. (2015) performed a laboratory study showing that transmission mechanisms may also determine the severity of an infection (Clemens & Brandt 2015).

Although coral reef diseases pose a major threat to worldwide reef ecosystems, collectively little is known about most of them. These include causative agents, transmission characteristics, existence of reservoirs (relatively tolerant species that act as pathogen sources), and preventative measures to either reduce or control their influence on reef ecosystems. Advances are hindered by insufficient diagnostic tools and difficulties in determination of the disease causing pathogen (sole or otherwise) (Work et al. 2008).

Acropora palmata:

Stony corals (Phylum: Cnidaria; Order: Scleractinia), which provide calcareous reef infrastructure, are particularly sensitive to stressors such as elevated sea surface temperatures, bleaching and disease (Aaronson & Precht 2001). Their population numbers have severely declined in recent history. Elkhorn coral (*Acropora palmata*) and staghorn coral (*Acropora cervicornis*), exemplify this severe decline. In the late 1970s white band disease was first observed in these coral species, causing widespread mortality and changing the shallow reef ecosystem (Aaronson & Precht 2001). Since the 1980s there has been an 80-98% reduction of elkhorn corals in the Caribbean, in particular attributed to the effects of disease, but also to other climate change factors and anthropogenic influence (Patterson et al. 2002; Ruzicka et al. 2010) . The current now occurs at very low abundance, but appears to be stable (Ruzicka et al. 2010) However, in some areas the population continues to decrease while other locations are exhibiting localized recoveries (Aronson *et al*. 2008). The massive decline of elkhorn coral in the Caribbean

since the 1980s led to the species being classified as “threatened” in 2006 under the United States Endangered Species Act (Hogarth 2006).

In wave-exposed and high surge reef areas in shallow waters (<10m), elkhorn coral reefs were historically dominant in terms of abundance and growth rate. This was due to the combination of a fast growth rate, asexual reproduction through fragmentation, and resilience to lesions, which enabled elkhorn coral to thrive within the reef system (Rodríguez-Martínez et al. 2014). Fragmentation occurs when broken branches or pieces re-attach to the substrate. Elkhorn corals exhibit a higher rate of sexual recruitment than other *Acropora* species such as staghorn corals (Aaronson & Precht 2001). Further, its ability to recover quickly following hurricanes and other stressors allowed it to be a very successful and important component of the Caribbean reef system (Rodríguez-Martínez et al. 2014).

Diminishing populations of elkhorn coral have dire consequences for the overall functioning and structure of reefs in the Caribbean. Elkhorn is the only species able to provide a highly complex branching structure and high calcification rates (Young et al. 2012). Carbonate production and reef growth is drastically reduced with the loss of these corals, which affects reef growth and leads to erosion (Kennedy et al. 2013). This erosion of the remnant coral skeletons significantly reduces the three-dimensional structure of Caribbean reefs causing a reduction in biodiversity, fisheries productivity and reduces inshore land protection (Alvarez-Filip et al. 2009).

Microbial Communities:

Coral colonies house an immense array of bacterial communities, some of which are thought to be species specific (Brown & Bythell 2005; Rohwer et al. 2002). One potential source of colony mortality includes these invasive microbes, which invade the host across the surface mucus. Pathogenic microbes often thrive under conditions of increased coral stress. In a study by Ritchie (2006), mucus from healthy elkhorn colonies prohibited growth of potentially invasive microbes by up to 10-fold (Ritchie 2006). From bacteria cultured from mucus layers, 20% demonstrated antibiotic activity against one or more laboratory reference strains, including the white pox disease pathogen *Serratia marcescens*. A novel mucus-mediated selection for coral symbionts revealed a minute subset of bacteria and selected for isolates that produce antibiotics (Ritchie 2006). This result suggests that coral mucus play an important role in disease suppression by promoting the growth of bacteria with antibiotic properties. Mucus also plays a valuable role in colony survival by providing protection from UV light, sedimentation and desiccation (Brown & Bythell 2005).

Stressors: Temperature, Bleaching and Disease

Over one third of all reef building corals worldwide are threatened with extinction due to severe population declines in response to bleaching and disease outbreaks driven by increasing sea surface temperatures (Bruckner et al. 2008). Corals in the Scleractinian family Acroporidae are particularly vulnerable in the Caribbean; Adults are confined to shallow waters with high sunlight intensity and are incapable of migrating into cooler temperature waters since they are sessile. This also increases their susceptibility to localized pollution and damage from hurricanes. All of these stresses can be amplified by climate change (Bruckner et al. 2008).

Elkhorn coral are sensitive to small increases in temperature (Barron et al. 2010). Since reef building corals live near their upper thermal tolerance limit, they are at risk for either naturally or anthropogenically induced elevated temperatures (above 1.0-1.5 C° above maximal seasonal means, Baker, Glynn, and Riegl 2008). Tissue losses from bleaching or disease have been shown to occur at rapid rates in shallow water in Caribbean elkhorn corals. For diseases, loss rates averaging 2.5 cm^2 day^{-1} have been measured. These loss rates increase during periods of elevated temperatures (Patterson et al. 2002).

Increasing temperatures have the potential to increase the prevalence and severity of coral disease outbreaks due to factors such as increased pathogen growth and host susceptibility (Sokolow 2009). The period from 1998 to 2005 included the warmest five years on record in Caribbean near shore reefs up to that time; the expectation is that this trend of increasing temperatures will continue (Trenberth et al. 2007) This has the potential to lead to higher frequency and severity of outbreaks in the future. The concern is that these increasingly chronic stresses may exceed the ability of coral to resist or recover from them (Hughes et al. 2003).

Bleaching:

Elevated temperatures cause bleaching, with Elkhorn coral being particularly susceptible (Bruno et al. 2007; Precht & Miller 2007; Yakob & Mumby 2011). The process of bleaching in coral colonies subject to high temperatures occurs from the loss of photosynthetic algae endosymbionts on the surface layer. This loss of the yellow-brown pigmented zooxanthellae causes the coral to become transparent, exposing the white calcareous skeleton under the host tissue (Banin et al. 2001; Baker et al. 2008). In elkhorn coral the population of zooxanthellae has to go below 0.5×10^6 before appearing bleached (Fitt et al. 2000). Increased mortality, reduced

fecundity, and lower growth rates have all been observed as results of bleaching events (Banin et al. 2001; Patterson et al. 2002; Hughes et al. 2003; Precht & Miller 2007).

Manzello *et al* (2007) performed a long term *in situ* study to determine whether differences in short-term temperature stress, cumulative temperature stress, or variability in temperature was the largest determinant of coral reef bleaching in the Florida Keys. They found that maximum monthly sea surface temperature values and the number of days above 30.5 C° were the most significant factors contributing to bleaching occurrences in coral reef colonies (Manzello et al. 2007). These conditions have occurred in the region including the Florida Keys and are likely to continue to reoccur (Kuffner et al. 2015).

Studies have shown that bleaching events caused by high temperatures in the Caribbean and Pacific are linked to an increase of disease outbreaks (R. J. Jones et al. 2004). Bleaching outbreaks have also been shown to reduce the effectiveness of the innate immune system of elkhorn colonies (Ritchie 2006; Reed et al. 2010). Bleaching events in turn can potentially amplify the likelihood of disease outbreaks by increasing the abundance or virulence of a pathogen or by increasing host susceptibility. In 2005, both bleached and unbleached corals showed a positive correlation with disease prevalence and increased water temperatures (Muller et al. 2008). However, the amount of partial mortality caused by disease was greatest only in colonies that were bleached. This suggests that host susceptibility, not just temperature increases, can also influence *A. palmata* disease severity (Muller et al. 2008) .

White Pox Disease:

White pox disease (WPX), in coral reef systems in the Florida Keys fits the pattern of seasonal outbreaks correlated with elevated sea temperatures (Patterson et al. 2002; Yee et al.

2011). White pox is a species-specific disease, only affecting elkhorn coral (Patterson et al. 2002). WPX is distinctively recognized by irregular shaped white lesions where tissue is discolored on the skeleton. Lesions are capable of developing on any part of the surface of the coral colony. Once infected, the recently bared calcium carbonate skeleton is quickly colonized by numerous species of turf algae (Patterson et al. 2002).

The first officially documented case occurred in 1996, in the Florida Keys, at Eastern Dry Rocks Reef (Holden 1996), though following further investigation it was determined to be present in 1994, and may have occurred in the Caribbean even earlier than this (Rogers et al. 2005). WPX spread rapidly throughout the Florida Keys between 1996 and 2000. During this period the Florida Keys experienced a 38% loss of overall living coral cover. At the species level, elkhorn colonies experienced the most devastating losses in terms of percent loss and abundance (Porter et al. 2008). The rapid colonization by turf algae following infection prevents recovery of elkhorn coral tissue (Patterson et al. 2002). If these infected elkhorn colonies are incapable of recovering from stressors, such as disease, bleaching, and subsequent macro algae invasion, an increase in the relative abundance of other competing coral species will likely occur (Aaronson & Precht 2001).

Colony Size:

The effect of colony size on the outcome of whole colony survival and reef persistence is an especially important factor to consider when studying corals, such as elkhorn in the Caribbean, that exhibit a relatively low sexual recruitment rate compared to other Acroporid species in the Pacific. Previous studies have shown a link between small colony size and an increased susceptibility to whole colony mortality (Sakai 1998). Larger colonies are much more

successful at producing gametes and therefore the re-establishment of new colonies. Therefore, if mortality differentially affects large, reproductively active colonies, then this size-dependent mortality is especially deleterious to population recovery (Sakai 1998). High recruitment rates are necessary for recovery of elkhorn coral colonies following stress, and elevated temperatures can play a large role in reducing larval outputs of these colonies. Since elkhorn corals reproduce during summer months, the possibility of elevated temperatures negatively influencing recruitment success is plausible (Randall & Szmant 2009).

Muller and van Woesik (2014) have shown that colony size has an influence on WPX susceptibility. They state that large colonies are more likely to be susceptible and show sign of WPX. They also reported that the number of times a colony has been previously infected significantly affected the likelihood that a colony would be infected in the next given time point (Muller & van Woesik 2014).

Overview:

Long-term demographic studies of coral populations allow for the most accurate prediction of coral colony survival and can help identify mitigating strategies in cases of slow declines. Although various forms of political protection for the Florida Keys have been implemented beginning in the early 1960s, most are *ad hoc*, and with few programs to monitor their efficacy (Precht & Miller 2007), Ruzicka et *al*. 2014). Repeatedly sampling individuals or populations helps control for error and temporal variability (Rothman & Greenland 1998). This is also a powerful means of epidemiological analysis, but has rarely been applied in marine ecosystems. These analyses are necessary, however, to further progress our understanding of

disease outbreaks in the ocean. Although desirable, this kind of long-term study is extremely difficult to undertake at large spatial scales (Bruno et al. 2015).

We combine two long-term datasets: the Eastern Dry Rocks historical data set (EDR 1994-2004) and the Ecology and Evolution of Infectious Disease data set (EEID 2008-2014). The EDR data represents annual surveillance (biannual surveys in 1997 and 1998) at one reef that underwent dramatic decline. The EEID data involved more frequent sampling (typically three times per year) of seven reefs differently impacted during the study period. All data were accompanied by detailed field notes on white pox and bleaching status for each colony in every survey. The EDR study started prior to the initial discovery of white pox disease, and therefore allows us to document the devastating loss of coral from this disease in a matter of a few years. The more recent survey allows a direct comparison to the historical survey, for the same coral species, disease and region.

We use the data to study and quantify the fates of both historical and contemporary elkhorn populations in the Florida Keys, preceding and following white pox disease outbreaks and bleaching events. From this extensive field data spanning 20 years, we explore hypotheses that may explain differences in the fates of the same coral species undergoing similar conditions but with drastically different outcomes.

METHODS AND MATERIALS

Study Areas and Experimental Designs:

Long-term photographic data from two different studies were used to determine WPX prevalence, severity, and lethality for elkhorn corals within EDR and EEID surveys. The first study was conducted between 1994 and 2004 in the EDR (24°27.617’ N; 81° 50.583’ W. EDR is

located southwest of Key West, in the Florida Keys, contained within both the borders of the Florida Keys National Marine Sanctuary (FKNMS), the Key West National Wildlife Refuge, and the EDR Sanctuary Preservation Area (NOAA-SPA).

The EDR study area consisted of a 13.5 m^2 grid of 36 continuous frames. Eight stainless-steel survey stakes were drilled and cemented into the reef substrate to demarcate the corner boundaries of the grid. The stakes formed one large rectangle, partitioned into three areas, each 2m by 2.25m. Plastic tubing was used to attach the PVC grid to the tops of the stakes, 0.25m above the coral colonies using removable bungie cords. Each of the three areas was then divided into 12 sections that were 0.5m by 0.75m, and these were considered the framework classification for image designation (frames 1-36). A Nikos UW camera with a 0.5m x 0.75m PVC frame was then lowered onto the large 2m x 2.25m PVC grid. The 0.5m x 0.75m frame on the camera was marked with alternating black and white stripes. These "tigertail" stripes provided scale for the corals in view. This camera frames system was moved across the large grid. Photographs were taken across the entire large grid system. Velcro backed numbers (1-36) were changed for each sequential image. This grid network provided a means of accurately and consistently measuring elkhorn colonies in EDR over long periods of time.

EDR colonies within the grid were photographed annually from 1994-2004, and biannually in 1997 and 1998. Survey seasons ranged from spring (June 1997, May 1998), summer (July 1994, 1995, 1999, 2001-2004 and September 1997-1998), and winter (October 1996, December 2000). Photographic slides were scanned digitally at 600 dpi for image analysis. Due to an overlap the field of view encompassed by the fish-eye camera lens, colonies on the border of one frame were frequently visible in adjacent frames. Correction for this overlap was achieved by concatenating colonies visible in more than one frame into individual colonies

comprised of all non-repetitive parts from all adjacent frames. For accuracy and consistency, each colony within view of each frame was initially traced, measured and saved within the frame as a separate numbered colony. Therefore, for large colonies visible in more than one frame, or smaller colonies seen twice by the fish-eye lens shooting adjacent frames, a single colony number was assigned to each individual colony (Figure 1). Over the course of these surveys, many colonies underwent fragmentation. The newly formed distinct colonies were renumbered and treated as stand-alone colonies.

Seven reef sites spanning from the upper, middle and lower FKNMS (White & Porter 1985) were added in 2008 (the EEID data set) to expand our repeated long-term observational study. Each site was visited between one and five times per year from 2008 to 2014. Survey periods were binned according to seasonality: spring (March-June), summer (July-October) and winter (November-February) (Table 1). Colonies were photographed digitally with a scale bar (for calibration) in view during each survey trip. A single survey stake at each reef was used as a reference point to relocate individual colonies by knowing the distance and bearing from the stake. Colonies were assessed for signs of WPX, bleaching and predation, and were recorded on underwater datasheets *in situ*.

Image Data and Analysis:

Analysis of the digital images from both the historical EDR (1994-2004) and contemporary EEID (2008-2014) surveys were conducted with the software package ImageJ (Rasband 2016). Each whole coral colony and WPX lesions larger than $1cm^2$ were traced to measure projected surface area (projected surface area). Prevalence was determined by the percent of colonies which were WPX positive for each reef at each survey. Severity was calculated in two ways: the average number of WPX lesions and the average size of WPX

lesions both scaled relative to the measured living *A. palmata* tissue area of each colony. Determining severity in this way was necessary to maintain consistency between the EDR and EEID surveys since these metrics could be determined in both surveys. Image analysis in this manner allowed for direct comparisons of the health and survival of 92 elkhorn colonies in the EDR survey and 126 elkhorn colonies within the seven reefs in the EEID survey.

All data analyses were performed within the R environment (R Core Team 2015). Difference in disease severity of historical and contemporary surveys was established by first logit transforming severity metrics to meet assumptions of normality then applying analysis of variance to a mixed effects model (*lme* in package 'nlme') (Pinheiro et al. 2016) with colony ID as a random effect and survey (historical/contemporary) as a fixed effect.

When analyzing the photographic images, many coral colonies were observed to have spots or patches of living tissue that were discolored, often resembling diseased or bleached tissue, but were less severe. Each of these blemishes were individually measured for projected surface area, and initially labeled as "discoloration". The underwater field notes taken *in situ* were then used to assign disease lesion presence or absence to these discolored areas. Only when field notes confirmed the presence of WPX were these discolored areas labeled as WPX. Observed spots can be caused by predation (primarily snail), damselfish gardens, fish feces, or other external sources, such as bleaching. These blemishes or tissue loss areas were not included within this study.

Mortality in colonial animals such as corals takes two forms: partial (only a portion of the living tissue dies) or whole (all the living tissue dies) mortality. In this survey, three causes of death were determined for each mortality event. First, DOA (dead skeleton in place and visible during survey), second, TKO (colony physically removed and knocked out of the frame) and

finally FUS (two separate and distinct colonies fused together to form one colony). Time of death is assumed to be the last time when the colony was observed during a particular survey. Since there was no surveillance between survey periods this is important to consider in when conducting survivorship curves. Colonies observed alive in the final survey period were considered to be alive until at least this point, and were treated as right-censored in survival analysis because they were alive when the survey ended, thus we do not know their time of death.

Survival Analysis:

Tracking the fate of a set of colonies, as well as identifying risk factors correlated with mortality, can usefully be done using survival analysis. The basic function of survival analysis allows us to predict the expected duration of time before an event occurs (here, colony death). We used Cox proportional hazards regression analysis (Therneau & Grambsch 2000), a method that is well-suited to dealing with multivariate predictor variables, including mixtures of binary variables (e.g., diseased vs. never diseased) and continuous variables (e.g., colony size).

Survival analyses were performed to determine whether the presence of bleaching, white pox, or colony size, had an effect on the survival probability per time of the colonies in the survey. First, date data were converted into a monthly value, corresponding to the month that the survey was conducted within each year. Colony death date was ascertained using simple computer code to extract the date of the death status associated with each colony (Figures 2-3).

Analysis was conducted using the ‘coxph’ function in the R package ‘survival’ (Therneau & Grambsch 2000) to jointly assess how survivorship depended on colony size, presence or absence of white pox disease, and presence or absence of bleaching. While colony size was

treated as a continuous variable, for data visualization it was additionally binned into two categories, small and large, which differed for each study based on the distribution of sizes. The median colony size for each survey was used as a cut-off for small and large and this was 33.2cm^2 for EDR and 710.5cm^2 for EEID.

To determine bleached and disease status for each colony in the survival analysis, presence or absence of each was denoted by either a 1 (or 0), 1 meaning that at any time during the coral's lifetime it experienced that given stressor (disease, bleaching or both). The key output from the survival analysis is the proportional hazard, which indicates the strength and direction of an effect (e.g., diseased coral colonies exhibit a probability of dying per unit time that is X times that of non-diseased coral colonies) and its statistical significance.

RESULTS

Colony Survival through Time:

Individual colony survival was tracked over the course of 20 years in the Florida Keys for this study, allowing us to compare a historical study (1994-2004) to a contemporary one (2008-2014) (Figure 1, Table 1). In the Eastern Dry Rocks, where the historical survey began in 1994, observation started with 92 distinct elkhorn colonies contained in our photo-station. In six years, this number declined to six living colonies, and by 2004 only one colony remained (Figure 2). This rapid loss yielded a 97.8% decline in living colonies at the Eastern Dry Rocks in the space of 10 years (Table 1). In our contemporary EEID study beginning in 2008, the first initial survey

started with 126 colonies across seven reefs. By the summer of 2014, our last survey, this number had declined to 70, a 44.4% decline of living colonies in the space of six years.

WPX Severity:

WPX was observed at a minimum of three of the seven monitored reefs in the FKNMS-wide survey every year between 2009 and 2014, and reefs affected varied from year to year (Figure 2, Table 4). The WPX outbreak was most pronounced in 2014 in our survey, affecting five of the seven reefs at that time (Table 2). WPX was not observed at any reef in 2008 (Figure 4, Table 2). Western Sambo Reef was the only reef where WPX was not observed in this survey.

The vast majority of EDR losses were whole colony mortality (DOA), preceded by WPX (66 colonies). The remainders were DOA, attributed to factors other than disease, (15 colonies) and TKO (10 colonies). In contrast, partial but rarely whole colony mortality was observed throughout the duration of the contemporary FKNMS-wide survey. The loss of 56 elkhorn colonies that occurred between 2008 and 2014 was attributed to DOA (14 colonies), TKO (40 colonies) and FUS (2 colonies). Of the DOA colonies, cause of death was preceded by WPX for only one colony (Table 3).

In the summer of 2009, repeated surveys were taken at Looe Key (LK) reef. These surveys revealed partial, but not whole colony, mortality. We followed progression of the outbreak at LK with monthly surveys in June, July, August, September and November 2009 (Table 2). No whole colony mortality (DOA) occurred at LK for colonies identified as WPX positive in the previous survey time-point. Throughout the 2009 outbreak WPX caused only partial mortality. Further tissue lost during this outbreak was beginning to regrow over affected areas of *A. palmata* colonies by November.

WPX severity was calculated as average size and as average number of WPX lesions per cm^2 of living coral tissue. Average number of lesions per projected cm^2 of living coral tissue were 0.0336 per cm^2 and 0.0010 per cm^2 (Figures 5-9), in historical and contemporary surveys, respectively. The projected surface area of *A. palmata* affected by lesions averaged 8.9% in the historical survey and just 1.1% in the contemporary survey (Figures 5-9). WPX severity was significantly greater for the historical survey at EDR than for the contemporary FKNMS-wide survey (Figure 9; ANOVA applied to a fitted mixed effect model using each severity metric, $P<0.0001$).

WPX Prevalence:

WPX prevalence at EDR ranged from 0% to 71.4%. Prevalence was 30.4% (n=92 colonies) in 1994 and increased steadily thereafter, even as colony number declined, to 50.7% (n=69, 1995), 57.7% (n=52, 1996), and 60.9% (n=46, 1997) (Figure 10). In May 1998, prevalence was 62.1% (n=29). The number of *Acropora palmata* colonies at EDR declined to 9 by 1999 and to three by 2000 (Table 1, Figure 4), and WPX prevalence in these years was 44.4% and 33.3%, respectively. In both 2002 and 2004 when only two colonies occupied the EDR photo-station, WPX prevalence was 50%. No WPX signs were observed in 2001 (n=3) and 2003 (n=1) (Figure 4). The one *A. palmata* colony added to EDR between 2003 and 2004 was not a sexual recruit, but a colony outside the photo-station that had become visible by growing into the frame.

WPX prevalence in the FKNMS-wide survey ranged from 0% to 60.0%. WPX prevalence was greater in summer (July-September) than in winter (October-February) or spring (April-June) (Figures 1112). Peak prevalence in each year of the FKNMS-wide survey occurred

in warmer months. In 2009, this peak occurred in spring (June) with 52.3% of *Acropora palmata* colonies (n=117) affected. From 2010-2014, peak prevalence occurred in summer: August 2010 (60.0% of *A. palmata* affected, n=94), July 2011 (27.8%, n=87), August 2012 (23.5%, n=82), September 2013 (31.2%, n=77), and August 2014 (42.4%, n=70). Prevalence returned to zero percent of colonies affected during winter in 2011 (February, December) and during winter and spring in 2012, 2013 (February and May) and 2014 (January, February, April). No WPX was observed in August 2011, but signs were present in July and September of that year (Figures 11-12).

Monthly surveys at LK in summer 2009 demonstrate progression of WPX (Figure 12a). We first observed WPX affecting 56.7% of the *Acropora palmata* colonies (n=30) at this reef during our June 2009 routine survey. Large active WPX lesions affected the colonies in June and by July and August the disease progressed from lesion margins (Figure 12b). WPX prevalence during this outbreak peaked in July, with 61.1% of *A. palmata* colonies (n=36) affected. By November 2009 just 2.8% of colonies (n=36 colonies) exhibited WPX.

WPX was observed at Palmata Patch Reef in the Dry Tortugas National Park (DTNP) annually between 2011 and 2014 (Table 2). The 2011 DTNP outbreak was documented in July (27.7% of *Acropora palmata* colonies affected, n=18) with a bleaching event following in September. In September, 100% of *A. palmata* colonies at Palmata Patch Reef (n=18) were bleached and WPX lesions could not be distinguished or quantified on bleached colonies. By December 2011, no WPX was observed at Palmata Patch and all *A. palmata* colonies had recovered their pigmentation.

WPX and Bleach Co-Occurrence:

In both the EDR and EEID surveys, co-occurrence of WPX and bleaching outbreaks were observed during the same survey time-point (Tables 4-5, Figures 13-16). However, EDR experienced a much higher number of these, especially in the years 1997 and 1998 (Table 4). From 1994-1996, some of the highest numbers of WPX infected colonies were observed, with 28, 35 and 30 colonies infected, respectively. These numbers accounted for 30% of all colonies being infected in 1994, 51% in 1995 and 58% in 1996, showing an increase in overall prevalence over time (Table 4). Before 1997, bleaching had not been observed in EDR in this study. In 1997 and 1998, the number of colonies co-experiencing disease and bleaching was 27 and 17, respectively, accounting for 59% of all living colonies. The two years following these bleaching events and disease outbreaks showed colony loss numbers of 20 and 17, some of the highest of the survey. Figures 13 and 14 highlight the loss of colonies following co-occurring bleaching events and disease outbreaks.

Survival Analysis:

Survival analyses of EDR and EEID coral colonies revealed consistent patterns. In each case, having been bleached or diseased during the survey period significantly delayed mortality (Tables 6-8, Figures 17-18). The analysis provides the hazard ratios, which estimate that bleached EDR colonies have a mortality rate between 4-18 times lower than unbleached EDR colonies (Tables 6-7), and that diseased EDR colonies have a mortality rate between 3-6 times lower than their non-diseased counterparts (Tables 6-7). A similar pattern occurs among the EEID colonies, with bleached colonies exhibiting a seven-fold reduction in mortality and diseased colonies, a five-fold reduction (Table 8). Surprisingly, and in contrast to previous

studies, colony size was not a significant variable in any analysis (hazard ratio ~1), although the trend was for smaller colonies to be at a somewhat increased risk of mortality (EDR: p=0.28-0.87, EEID: p=0.88). For EDR, which exhibited considerable co-occurrence of WPX and bleaching at the individual colony level (Table 4, Figures 13-14), a more detailed statistical model was developed that compared non-disease, non-bleached colony survivorship to each other colony type (bleached only, disease only, both; Table 7). This model demonstrated that survival times for colonies with both stressors were similar to those for colonies with only a single stressor and significantly longer than colonies with neither stressor. In terms of model performance, this more complex model is slightly favored over the original model for EDR colony survivorship (with an AIC difference of ~6.5). A similar analysis could not be performed on the EEID data because of the small number of colonies exhibiting both WPX and bleaching, and due to those few colonies exhibiting a near-identical time of death. Collectively, the three variables, disease (0/1), bleaching (0/1) and size explain 56-60% and 39% of the variation in mortality rates at EDR and EEID, respectively (Cox proportional hazards pseudo-R^2 , Venables and Ripley 2002).

While the main survival analysis concentrates on distinguishing colonies ever infected from colonies never infected, we additionally consider two metrics of WPX manifestation: the number of times a colony was observed infected, and the maximum number of lesions observed on a colony during a single observation (Figures 19-22). In all cases, the mean time of death was dependent on these categorical groupings (all ANOVA p-values < 0.001), with increasing values (i.e., increasing number of infection observations and number of lesions) corresponding to increasing lengths of time to death (i.e., long survivorship).

DISCUSSION

Coral reef populations are in severe decline on a global scale and coral colonies are subject to progressively unfavorable conditions, such as disease outbreaks, rising sea surface temperatures and increased pollution and eutrophication, which are capable of amplifying coral mortality (Rodríguez-Martínez et al. 2014). While elkhorn coral colony losses have been estimated between 80%-98% within the last three decades in the Caribbean, there are relatively few published studies of quantitative changes in distribution and abundance (density and cover) of this species through time are lacking (Rodríguez-Martínez et al. 2014; Ruzicka et al. 2010).

This study examined two separate data sets with coral reef surveillance spanning twenty years in the Florida Keys. The EDR dataset is unusual in that it is a long-term study, complete for all frames in all years, which is accompanied by detailed field notes on white pox and bleaching status for each colony in every survey. This study began before the initial appearance of white pox disease and documents the devastating loss of coral over only a few years. With the inclusion of the EEID survey, we were able to quantify the fates of individual elkhorn coral colonies at greater temporal and spatial scales, and EEID surveys further highlight the influence of seasonality on WPX outbreaks.

Unlike the EDR survey (1994-2004) whose WPX outbreaks were more severe and resulted in high whole colony mortality, less severe EEID (2008-2014) outbreaks caused only partial colony mortality (Figure 9). The number of colonies at EDR declined from 92 in 1994 to just two in 2004 (98% whole colony mortality). This study was coincident with WPX signs occurring in eight years of this decade-long survey (Figure 4). The seven-year contemporary FKNMS-wide survey, however, showed lower (44.4%) whole colony mortality (from 126 to 70

colonies) than the EDR survey. Although WPX was detected every year from 2009 to 2014, only partial but not whole colony mortality of WPX-affected *A. palmata* was observed (Figures 11-12).

During historical EDR and contemporary FKNMS-wide surveys, WPX prevalence ranged from 0% to approximately 70%. This prevalence is comparable to the 0% to 53% reported for the United States Virgin Islands between 2004 and 2010 (Muller et al. 2014). WPX prevalence during the EEID survey showed a seasonal pattern and increased during warmer summer months and declined in winter and spring. Peak prevalence in each year of the FKNMS-wide survey ranged from 23.5-60.0% of colonies affected (Figure 11). WPX prevalence is known to increase seasonally (Williams & Miller 2012; Rodríguez-Martínez et al. 2001), including at EDR (Patterson et al. 2002). WPX prevalence has also been observed in association with bleaching (Rogers & Muller 2012). During our historical survey at EDR, simultaneous signs of WPX and bleaching occurred in September 1997 and September 1998. The only *Acropora palmata* bleaching observed during our FKNMS-wide survey occurred in summer 2011 at Rock Key Reef, Molasses Reef (August), and Palmata Patch Reef (September).

Through survivorship analyses we found a consistent and intriguing pattern between datasets that both WPX and/or bleaching, observed in at least one survey, delay mortality and temporarily increase survivorship. The mortality rate of EDR bleached colonies was 4-18 times less than that of unbleached colonies. Likewise, diseased colonies exhibited a mortality rate that was 3-6 times lower than of corals without disease signs. EEID bleached colonies showed a seven-fold lower mortality rate, and EEID diseased colonies exhibited a mortality rate five times lower than EEID corals free of disease signs.

The ultimate explanation behind these patterns lies in data beyond the reach of this study, but we can lay out the candidate explanations, which may guide future research efforts. One simple explanation might be that the intervals between our photographic surveys were too infrequent. Under this hypothesis, between surveillance, corals did develop white pox or bleaching and died before the presence of the stress could be photographed. Since the last photograph did not show disease signs on the colony, its subsequent loss from the population would not be attributable to either bleaching or disease. The stressor came and went undetected, but resulted in mortality never-the-less. One argument against this possibility is that the positive relationship between disease and delayed mortality occurred in both the annual and the tri-annual surveys (EDR and EEID, respectively). Differences in tolerance and resistance between individuals can lead to variation in survivorship following infection or bleaching events. For instance, there may be strong selection favoring individuals who can maintain high levels of fitness even while infected. Alternatively, others may have invested in resistance to prevent infection. These individuals might never catch the disease and therefore never exhibit signs of illness. Resistance to pathogens often comes at a life-history cost, such as longevity (Råberg 2014). Consequently, a costly resistance-strategy could reconcile the patterns of short life-span being associated with absence of disease. Confirming such a hypothesis would require detailed life history analysis as well as knowledge of genetically-controlled traits of resistance and tolerance. Currently not much is known about disease resistance in elkhorn corals, however, closely related staghorn coral genotypes have been observed to be resistant to white band disease (WBD). Vollmer and Kline (2008) found that roughly six percent of staghorn genotypes (3 of 49) from four reefs were resistant to WBD through *in situ* transmission experiments and field surveys in Boca Raton, Panama (Vollmer & Kline 2008). This natural resistance in threatened

staghorn populations is the first evidence of disease resistant reef-building corals, and could explain why pockets of staghorn were able to withstand and survive the Caribbean-wide WBD epidemic of the last thirty years (Vollmer & Kline 2008).

Differences in population abundances of naturally resistant colonies with advantageous genotypes between reefs may explain why some populations have been able to persist more effectively. Colonies surveyed at EDR may have genetically been more susceptible to WPX when the outbreak first started in 1994, as compared to colonies within the 2009 EEID survey, which may have survived after a period of strong natural selection that occurred as this species declined rapidly between 1994 and 2009. EEID colonies may also have naturally been more resistant to WPX, or those colonies that were not may have had their immune system competence stimulated by the presence of the disease. Without doubt, however, several EEID colonies that seemed to acquire WPX annually seem to exhibit tolerance to the disease by being able to recover rapidly from the disease, and by experiencing only partial mortality (Figure 12).

Aside from coral resistance from the individual host, individual coral colony microbial communities and their conferred immune function can affect survival in the presence of a pathogen (Ritchie 2006). Corals are thought to maintain a close relationship with their associated bacterial communities (Ritchie 2006). If so, this might allow them to eliminate potentially harmful microbes through the use of a multitude of microbial and lower invertebrate immune defense mechanisms (Van De Water et al. 2015). Since coral mucus layers play an important role in disease suppression (Ritchie 2006), individual differences could be attributed to a combination of susceptibility and resistance. It is possible that those colonies surviving to the end of the EDR survey and those in the EEID survey may have had microbial communities promoting the growth of bacteria with antibiotic properties.

In both surveys, colonies with the highest longevity also were ones capable of clearing the disease often (Figures 19 and 21). This could have a simple explanation, that the clearance rate for all individuals identical across the board, thus those living longer would inevitably clear disease more often. This pattern may also be explained by seasonality, with WPX outbreaks occurring most frequently in summer months and with colonies returning to a healthy status during winter months. However, there may be variation in individual colony effectiveness at clearing disease, with some being able to clear the disease more quickly and efficiently, and others not as well.

Colony size also exhibited a trend that larger colonies tended to live longer than their smaller counterparts on a population level. Coral colony vigor, colony size, and tissue regeneration are linked traits that may contribute to increased longevity of individual colonies, even when faced with higher disease prevalence. The larger the surface area of living tissue of corals compared to that of smaller colonies makes them more susceptible to disturbance, damage, and encounters with disease propagules. However, the ability of an injured coral to regenerate is positively correlated with an increase in body size. Henry and Hart (2005) found size-dependent survivorship of wave-generated or experimentally-derived fragments of scleractinian corals, which suggests a link between increases in regenerative capacity and relatively large colony size (Henry & Hart 2005). This could explain the trend in this study that smaller colonies were at an increased risk of mortality in comparison to larger colonies.

While bleaching and disease are positively associated with survivorship in both studies, an important difference between these two studies is reflected in the amount of partial mortality expressed during these two periods. The early survey had high rates of partial colony mortality; the more recent survey had much lower rates of partial mortality. In the early survey, disease

killed almost all colonies (98%) (Amortized to an average loss over the 10 years of this survey of 9.8% absolute loss per year); in the more recent survey, disease killed only (44%?) Of the colonies, which amortizes over the 6 years of the survey to 7.3% absolute loss per year [brett, i am not sure these numbers are right, but what i am fishing for here is a way to compare the lethality of pox in the first survey and the last survey. If we normalize these by dividing by the number of years of the survey, i think we can do this. But it is key that we only include in this analysis coral colony deaths where pox=y in the previous survey. Although the historical survey only included EDR, and only two colonies survived to the last survey point (2004), we can speculate that the two surviving colonies may have survived this WPX zoonotic due to individual host heritable resistance characteristics that may also have been properties of coral colonies surveyed at the beginning of the contemporary study (which started in 2008). Although speculative, the disease-related mortality well documented in the first study would have, imposed an exceedingly strong natural selection favoring disease-resistance in surviving colonies. Surviving colonies may have been subject to canalizing natural selection by a virulent form of the pathogen. Hosts exposed to such a pathogen may have been unable to recover from, and instead succumbed to mortality, whereas those more resistant were able to survive and reproduce either sexually or asexually. There are many individual host factors that could contribute to survival differences, such as general vigor, colony size, tissue regeneration capability, colony immunity, and differences in microbial communities.

Additionally, environmental factors such as temperature may have changed subtly between the two time periods of the survey, thereby contributing to differential colony survival and persistence in the EDR and EEID studies. Even slight increases in sea surface temperature might have many effects on individual colonies, thereby influencing microbial community

composition, regeneration capability, or increased bleaching. Elevated temperatures can negatively affect the survivorship of coral larvae, which reproduce in the summer months (Randall & Szmant 2009). Temperature increases may also cause changes in the in both the susceptibility of the host and the abundance and virulence of pathogens. While bleaching of elkhorn colonies was not widespread in our surveys, WPX was most prevalent during warmer months. These are consistent with the hypothesis that WPX can express in the absence of bleaching, and that WPX may be completely decoupled from bleaching. Our data are also consistent with the assertion that slightly elevated late-temperatures promote WPX infection, even at sub-lethal values that are insufficient to produce signs of bleaching on *A. palmata* host colonies.

The specific pathogen(s) which cause WPX disease and their mechanisms are still being studied (Sutherland et al. 2016). Although the enteric bacterium *Serratia marcescens* was identified as the causal agent in the early study on eastern dry rocks (Patterson et al. 2002), this pathogen has not been seen in abundance in the second survey (Joyner et al. 2015) This leads to several possibilities, that: (1) over time, *S. marcescens* has lost its extraordinary virulence, (2) over time, coral colonies have been naturally selected for resistance to this pathogen, or (3) over time, a new pathogen is at work producing disease signs that are virtually indistinguishable from those produced by the old pathogen.

Over time pathogen virulence may also evolve to better persist within a host community (Alizon et al. 2009). Allowing hosts to live longer with the pathogen (lower virulence) can lead to increased transmission opportunities for the pathogen. If the pathogen is highly virulent, the host may die before it can spread within a population. It is possible that the strain of the pathogen which exhibited WPX in the EDR survey had a higher virulence than the pathogen we are seeing

in the subsequent EEID survey. There may also be a tradeoff between environmental durability and virulence (Cressler et al. 2015), meaning that over time virulence may decline as the physiology and molecular biology of this non-marine microbe adapts to survive in salt water. Either declining pathogen virulence or increasing host resistance could explain the high mortality rates seen in the historical dataset in comparison to lower mortality rates seen in our contemporary one. Moreover, it has been difficult to establish an etiological agent which produces the new WPX disease signs (Sutherland et al. 2016), and it is possible that the pathogen responsible for WPX in our EEID data is different from the one causing the disease observed in the EDR survey.

Much of this research suggests that patterns on a population level can partly be explained by inter-individual variation. In order to fully understand the dynamics of this system it is important to characterize this variation. Accurate diagnosis of coral disease etiology requires well-informed analyses of not only gross disease signs and host coral affected, but long-term monitoring of affected host populations that includes assessment of environmental parameters including temperature and water quality. Expanding surveillance measures can provide a more comprehensive interpretation of the processes at work. Increased surveillance in future studies could include more frequent monitoring, genetic analysis of the microbial community of every lesion, genetic analysis of the host, and closer examination of regeneration rates. This study was capable of linking patterns to candidate processes, but future work could further refine and/or exclude these hypotheses. Especially, by expanding surveys both temporally and spatially, use of static underwater cameras, sampling for microbial communities on the surface of colonies, accurate measurements of local temperature reading during each survey, and the use of flow meters to monitor changes in water currents promises to fill knowledge gaps and mechanistically

understand patterns of disease and population declines, to further progress the field of coral disease epidemiology.

Coral reefs are one of the most diverse and important ecosystems on the Earth, providing habitats for immense amounts of marine biota, regulating services such as protecting coastlines from erosion by waves and currents, and playing an important role in global carbon sequestration. Within the last three decades nearly 20% of reefs worldwide have been lost and these losses come with a significant cost in reductions in reef biodiversity. This biodiversity loss may prove to be destabilizing for tropical marine environments in the future. Although coral diseases are a major threat to global reef systems, little is known about them collectively. Disease outbreaks and bleaching events are occurring more frequently and show no signs of slowing down, therefore it is imperative that we expand our understanding of these systems in order to aid strategies of coral reef conservation efforts in the future.

This study quantified the impact of WPX disease and bleaching on elkhorn coral colonies within the Florida Keys over the course of 20 years. We were able to compare a historical dataset with a more contemporary one, finding differences in colony mortality and disease prevalence and severity and similarities in characteristics of survival probabilities in colonies experiencing WPX and bleaching presence. This study identifies, for the first time the potentially counterintuitive fact that in a direct comparison of survivorship, corals with either bleaching, disease, or both bleaching and disease, had delayed mortality relative to colonies that did not exhibit either bleaching or disease signs. These observations suggest that low-level stress from either non-lethal disease infection or non-lethal bleaching may somehow retard (but ultimately not prevent) whole colony mortality. The physiological mechanisms covering delayed mortality are, at this moment, completely unknown. This study aims to be a valuable asset for future

comparisons of partial and whole mortality patterns of elkhorn coral systems and other corals in the Florida Keys and beyond, especially as these important species continue to decline.

TABLES AND FIGURES

Table 1: EHD and EEID Colony Survival Numbers

Survey Time	Year EEID	Year EDR	EEID Colony Number	WPX Y/N	EDR Colony Number	WPX Y/N
1	2008	1994	119	N	92	N
2	2009	1995	110	Y	69	Y
3	2010	1996	95	Y	52	Y
4	2011	1997	94	Y	42	Y
5	2012	1998	83	Y	21	Y
6	2013	1999	79	Y	9	Y
7	2014	2000	75	Y	3	Y
8		2001			3	N
9		2002			2	Y
10		2003			1	N
11		2004			2	Y

Table 2: EEID Seasonal WPX Presence

		2008			2009			2010			2011			2012			2013			2014		
Reef	**Coords.**	**Wi**	**Sp**	**Su**	**Wi**	**Sp**	**Su**	**Wi**	**Sp**	**Su**	**Wi**	**Sp**	**Su**	**Wi**	**Sp**	**Su**	**Wi**	**Sp**	**Su**	**Wi**	**Sp**	**Su**
Carysfort	25°13.248' N 80°12.594' W			1			1 w			1 w		1 w	1	1	1	1	1	1	1	1	1	1 w
Molasses	25°00.528' N 80°22.590' W		1				1			1 w		1 w	1	1	1	1 w	1	1	1 w	1	1	1
Sombrero	24°37.518' N 81°06.696' W		1			1 w			1	1		1 w	1	1	1	1 s	1	1	1 w	1	1	1 w
Looe Key	24°32.700' N 81°24.400' W		1		1 w	1 w	3 w		1 w	1 w		1	1	1	1	1 s	1	1	1 s	1	1	1 w
Western Sambo	24°28.680' N 81°43.026' W			1			1			2		1	1	1	1	1	1	1	1		1	1
Rock Key	24°27.270' N 81°51.534' W			1			1			1			1	1	1	1	1	1	1		1	1 w
Palmata Patch	24°37.243' N 82° 52.042' W	1				1			1		1		1 w		1 w	1 w		1	1 w		1 w	

Table 3: EDR and EEID Causes of Colony Mortality
DOA = Dead on Arrival, TKO = Technically Knocked (Out of the Frame) and FUS = Fusion

	DOA	DOA preceded by WPX	TKO	FUS
EDR	81	66	10	0
EEID	14	1	40	2

Table 4: EDR Bleaching and WPX Co-Occurrence and Colony Survival

Year	WPX and BLCH	WPX	BLCH	Healthy	Total	Colony Loss	% Colony Loss	WPX Y	%_Inf
1994	0	28	0	64	92	0	0	28	0.30
1995	0	35	0	34	69	23	0.25	35	0.51
1996	0	30	0	22	52	17	0.25	30	0.58
1997	27	8	5	6	46	8	0.15	35	0.76
1998	17	5	3	4	29	17	0.37	22	0.76
1999	1	3	1	4	9	20	0.69	4	0.44
2000	0	1	0	2	3	6	0.67	1	0.33
2001	0	0	0	3	3	0	0.00	0	0.00
2002	0	1	1	0	2	1	0.33	1	0.50
2003	0	0	0	1	1	1	0.50	0	0.00
2004	0	1	0	1	2		0.00	1	0.50

Table 5: EEID Bleaching and WPX Co-Occurrence and Colony Survival

Year	WPX and BLCH	WPX	BLCH	Healthy	Total	Colony Loss	% Colony Loss	% Inf
2008	0	0	0	109	109	0	0.00	0.00
2009	0	44	1	73	118	0	0.00	0.37
2010	0	10	0	86	96	22	0.23	0.10
2011	5	14	22	57	98	0	0.00	0.19
2012	0	19	0	70	89	9	0.10	0.21
2013	0	15	0	66	81	8	0.10	0.19
2014	1	26	0	48	75	6	0.08	0.36

Table 6: EDR Colonies (n=92) Survival Analysis with Independent Factors

Time of death is predicted by independent factors WPX, bleaching and colony size. For Factor (X:Y) with hazard ratio H, individuals with state X have a probability of dying per unit time that is H times that of individuals with state Y.

Contrasted Factor (ratio)	Hazard ratio (95% confidence interval)	P-value
WPX (0:1)	3.64 (2.18-6.08)	<0.001
Bleaching (0:1)	4.78 (2.71-8.40)	<0.001
Size (small:large)	1.0 (0.99-1.0)	0.87

Table 7: EDR Colonies (n=92) Survival Analysis with Size, and with Colony Type compared with the Non-Diseased, Non-Bleached Type.

Time of death is predicted by independent factors WPX, bleaching and colony size and the interaction between WPX status and bleaching status (B). For Factor (X:Y) with hazard ratio H, individuals with state X have a probability of dying per unit time that is H times that of individuals with state Y.

Contrasted Factor (ratio)	Hazard ratio (95% confidence interval)	P-value
X=WPX0,B0, Y= WPX0,B1	18.46 (5.17-65.96)	<0.001
X=WPX0,B0, Y= WPX1,B0	6.01 (3.16-11.43)	<0.001
X=WPX0,B0, Y= WPX1,B1	17.33 (8.10-37.06)	<0.001
Size (small:large)	1.0 (0.99-1.0)	0.28

Table 8: EEID Colonies (n=138) Survival Analysis with Independent Factors

Time of death is predicted by independent factors WPX, bleaching and colony size. For Factor (X:Y) with hazard ratio H, individuals with state X have a probability of dying per unit time that is H times that of individuals with state Y.

Contrasted Factor (ratio)	Hazard ratio (95% confidence interval)	P-value
WPX (0:1)	5.07 (2.74-9.39)	<0.001
Bleaching (0:1)	7.06 (2.54-19.58)	<0.001
Size (small:large)	1.0 (0.99-1.0)	0.08

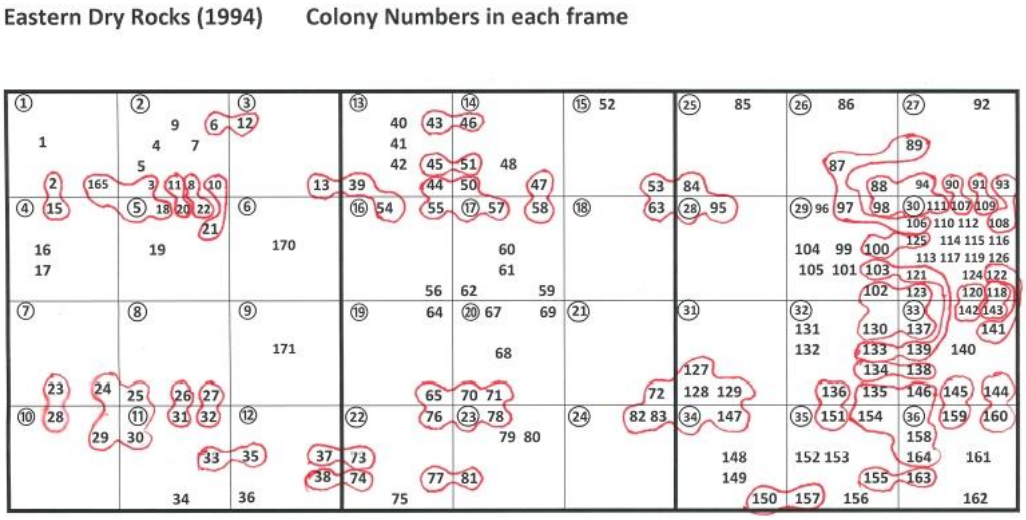

Figure 1: Eastern Dry Rocks Colony Concatenation Diagram

Colony numbers circled in red were merged together to be one distinct colony number for data analysis.

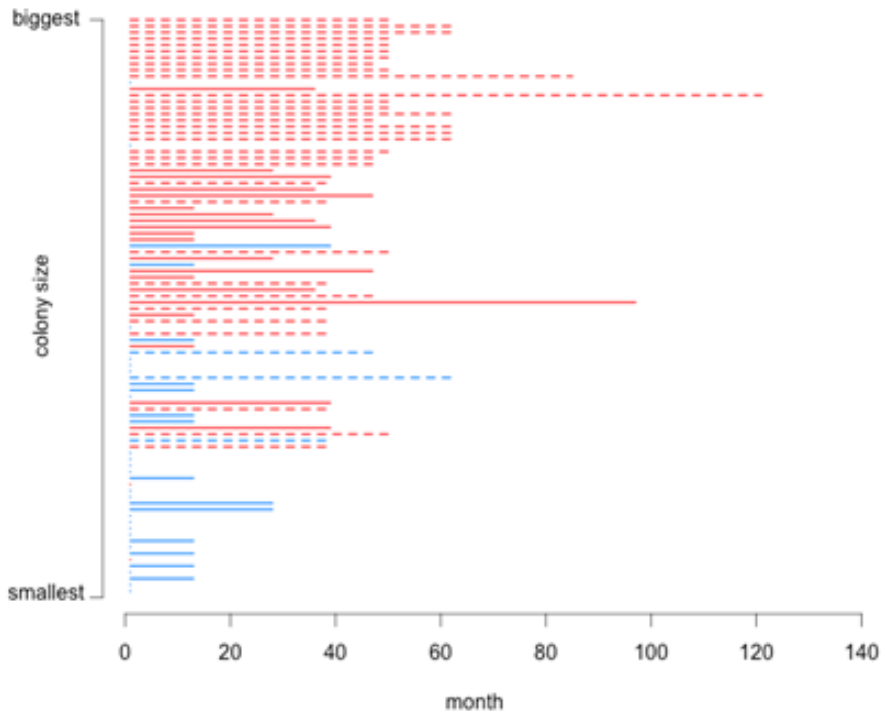

Figure 2: Longevity of Eastern Dry Rocks colonies arranged in size order

Line color distinguishes colonies that were ever WPX positive (red) from those not (blue). Line style distinguishes colonies which were ever bleached (dashed) from those that were not (solid).

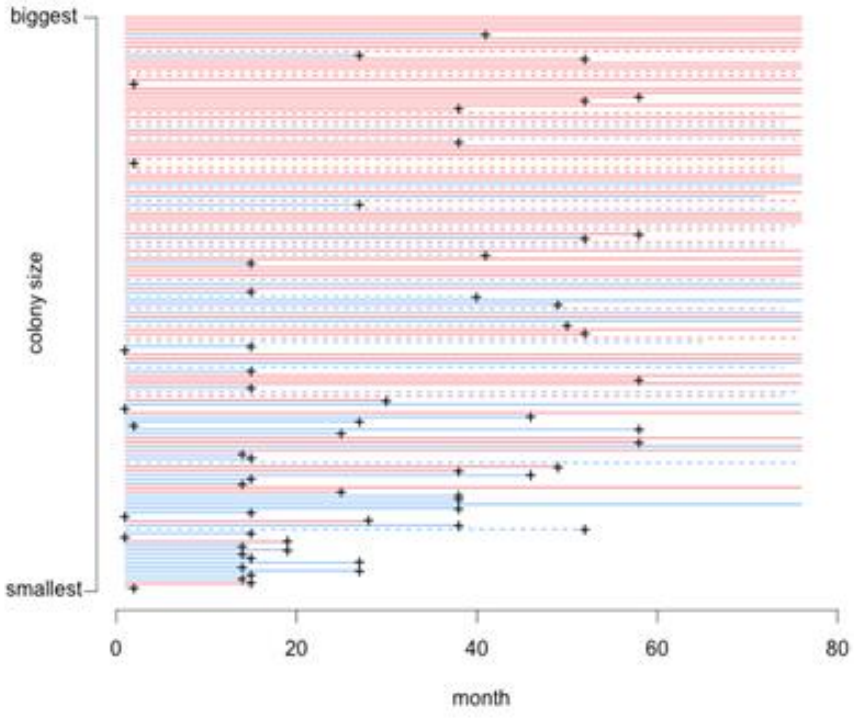

Figure 3: Longevity of EEID colonies arranged in size order

Line color distinguishes colonies that were ever WPX positive (red) from those not (blue). Line style distinguishes colonies that were ever bleached (dashed) from those that were not (solid). Plus (+) symbol indicates time of death, with some colonies (fully extended lines with no '+') still alive at the end of the survey period (i.e., right-censored).

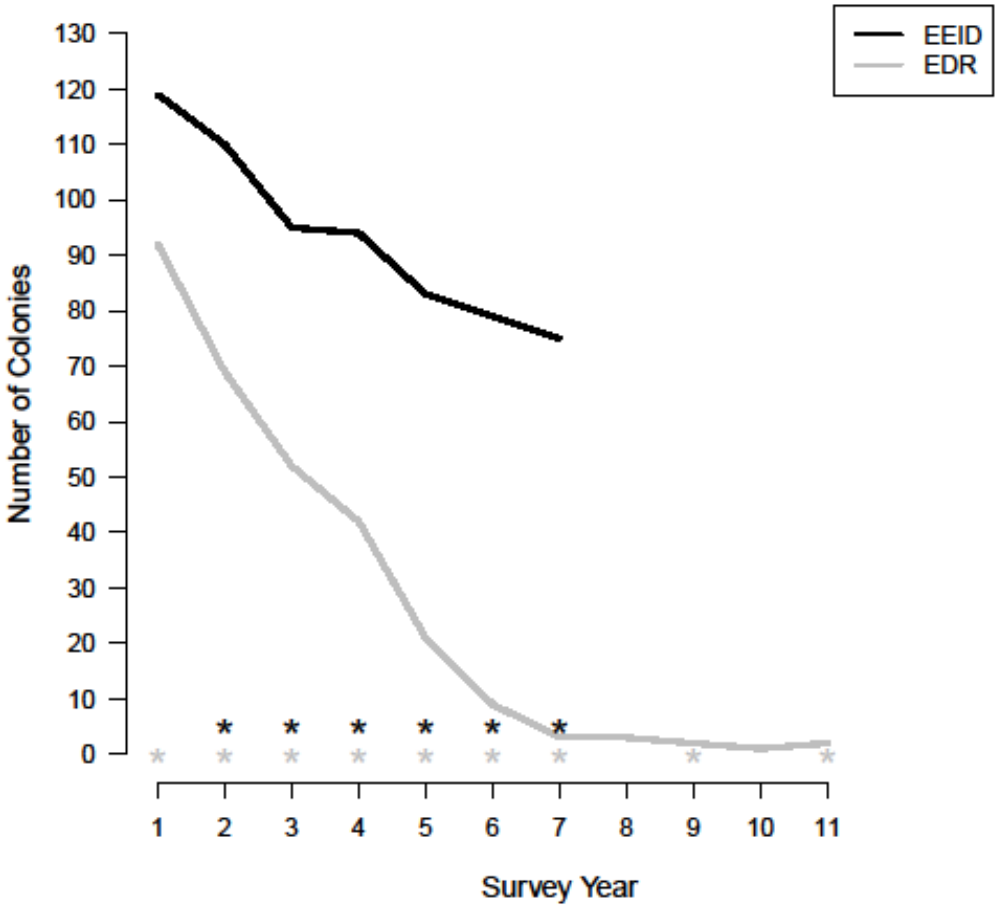

Figure 4: Colony Survival

Stars denote when WPX was present at that year for each survey.

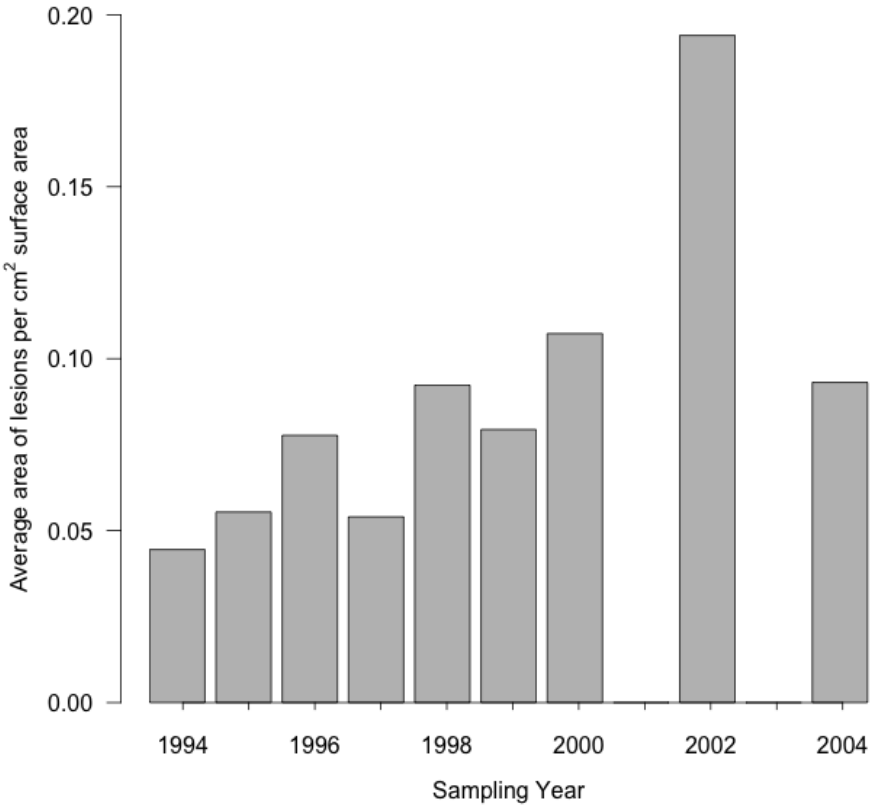

Figure 5: EDR Severity (Percent Infected Tissue per Projected Surface Area per Year)

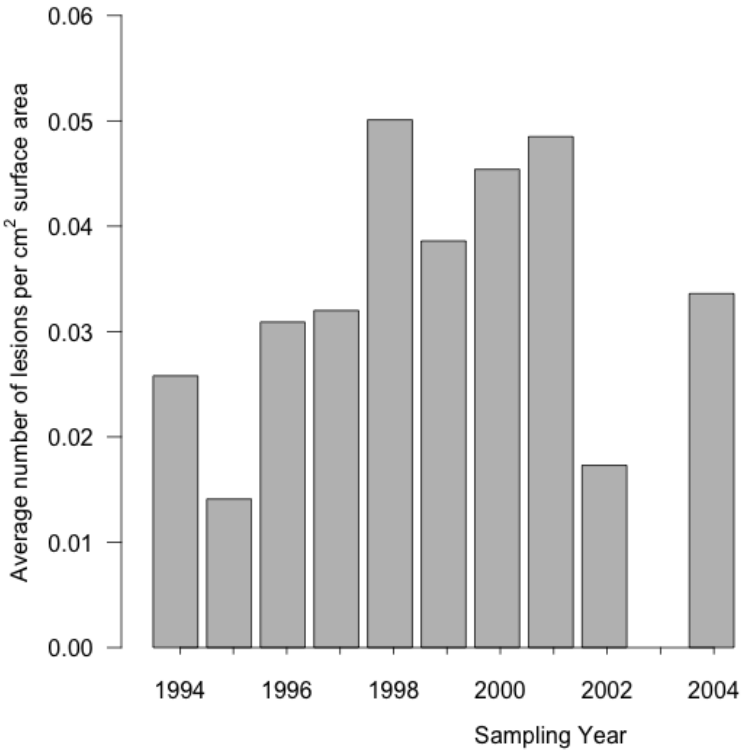

Figure 6: EDR Severity (Mean Number of Lesions per Projected Surface Area per Year)

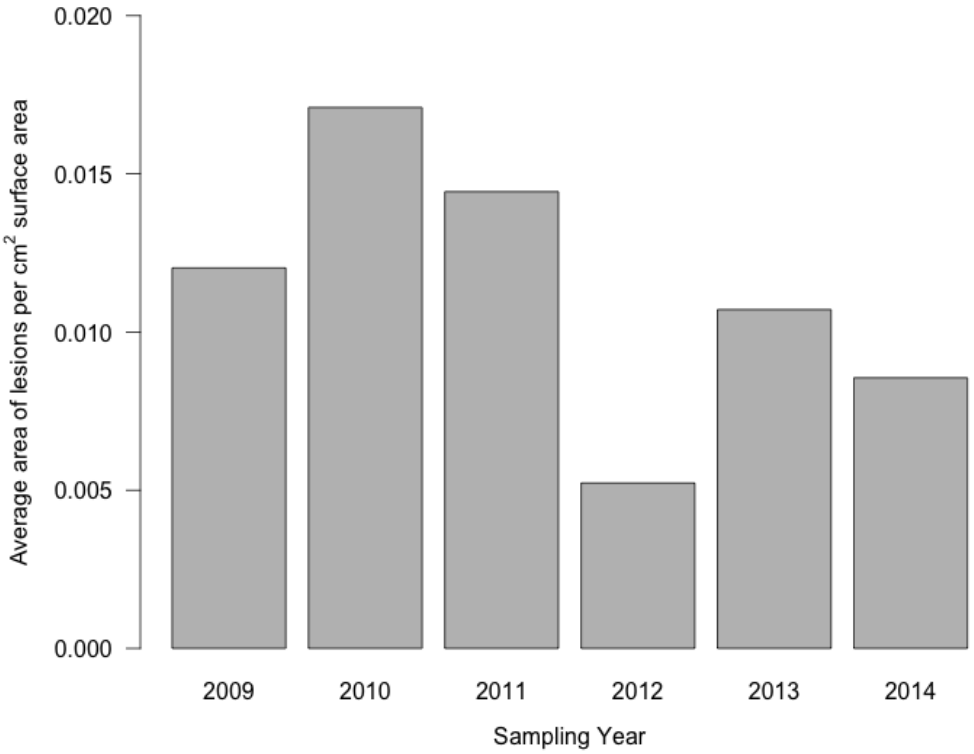

Figure 7: EEID Severity (Percent Infected Tissue per Projected Surface Area per Year)

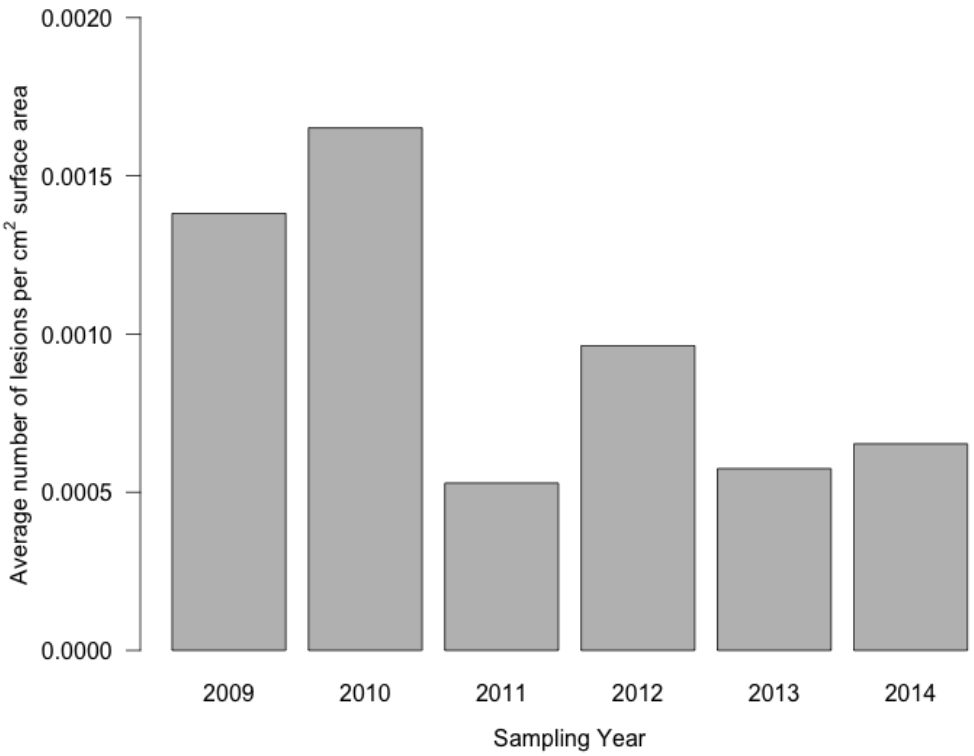

Figure 8: EEID Severity (Mean Number of Lesions per Projected Surface Area per Year)

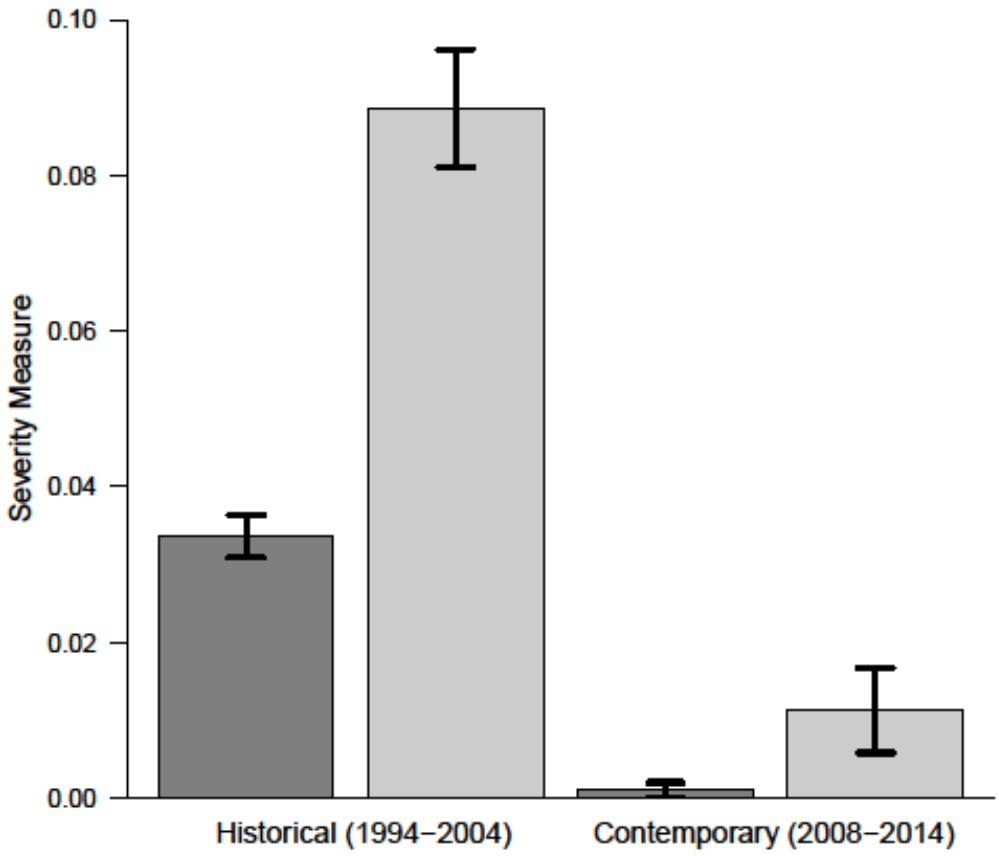

Figure 9: EDR and EEID Severity Averages

Darker shading denotes area of lesions per cm^2 of live tissue area and lighter shading denotes the number of lesions per cm^2 of live tissue area.

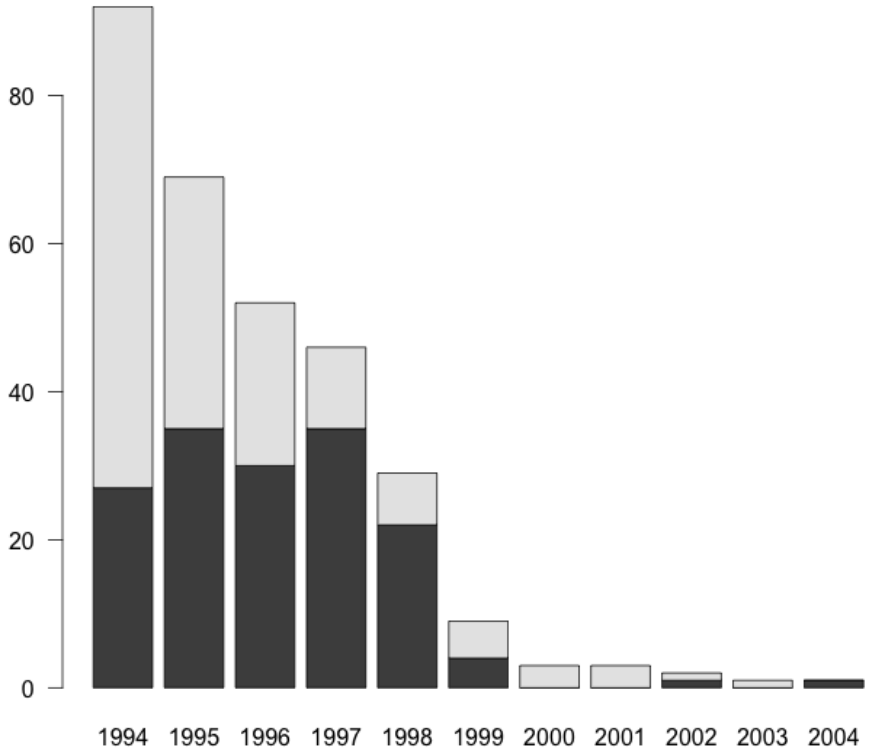

Figure 10: EDR Prevalence per Year

Darker shading denotes the number of colonies infected with WPX each year and lighter shading denotes the total number of colonies alive each year.

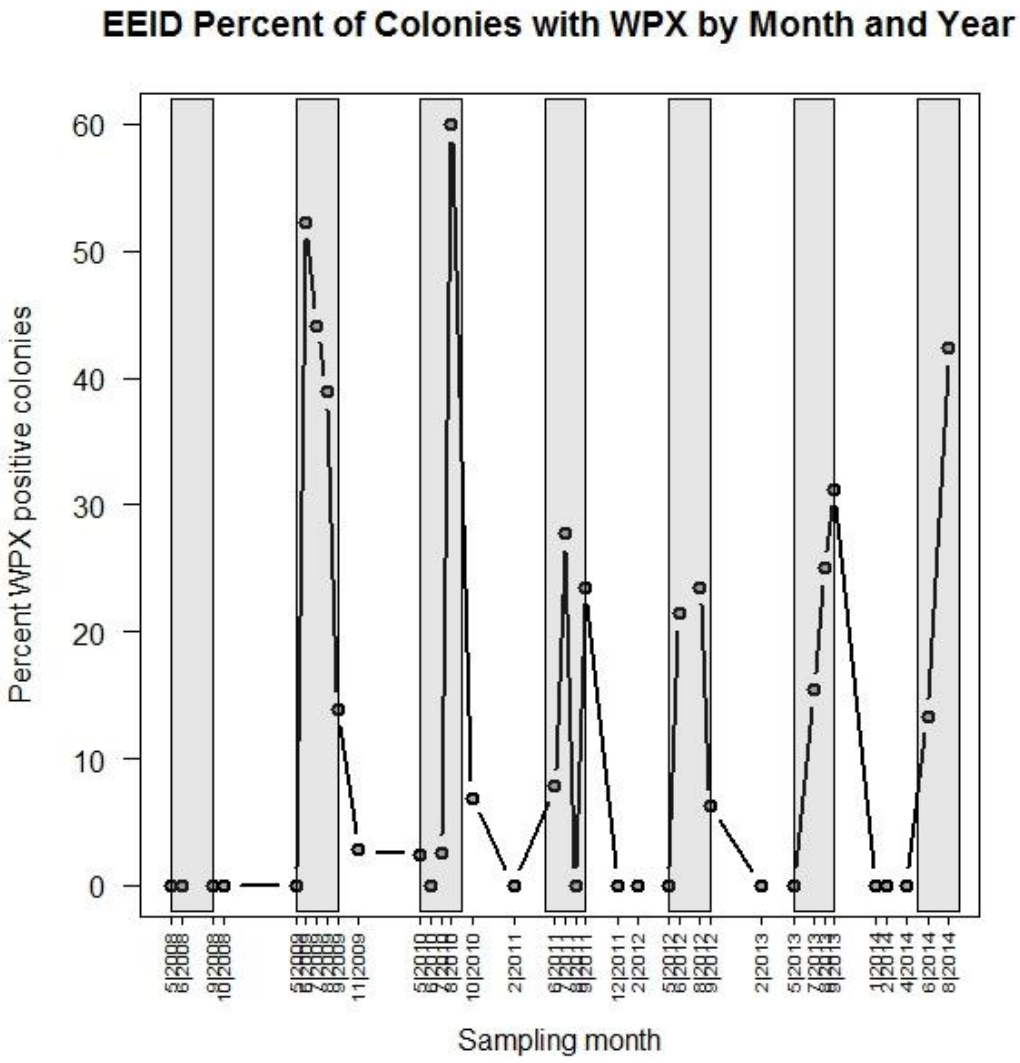

Figure 11: EEID WPX Prevalence with Seasonality

Boxes accentuate summer seasonality, when surveys were performed between May-August.

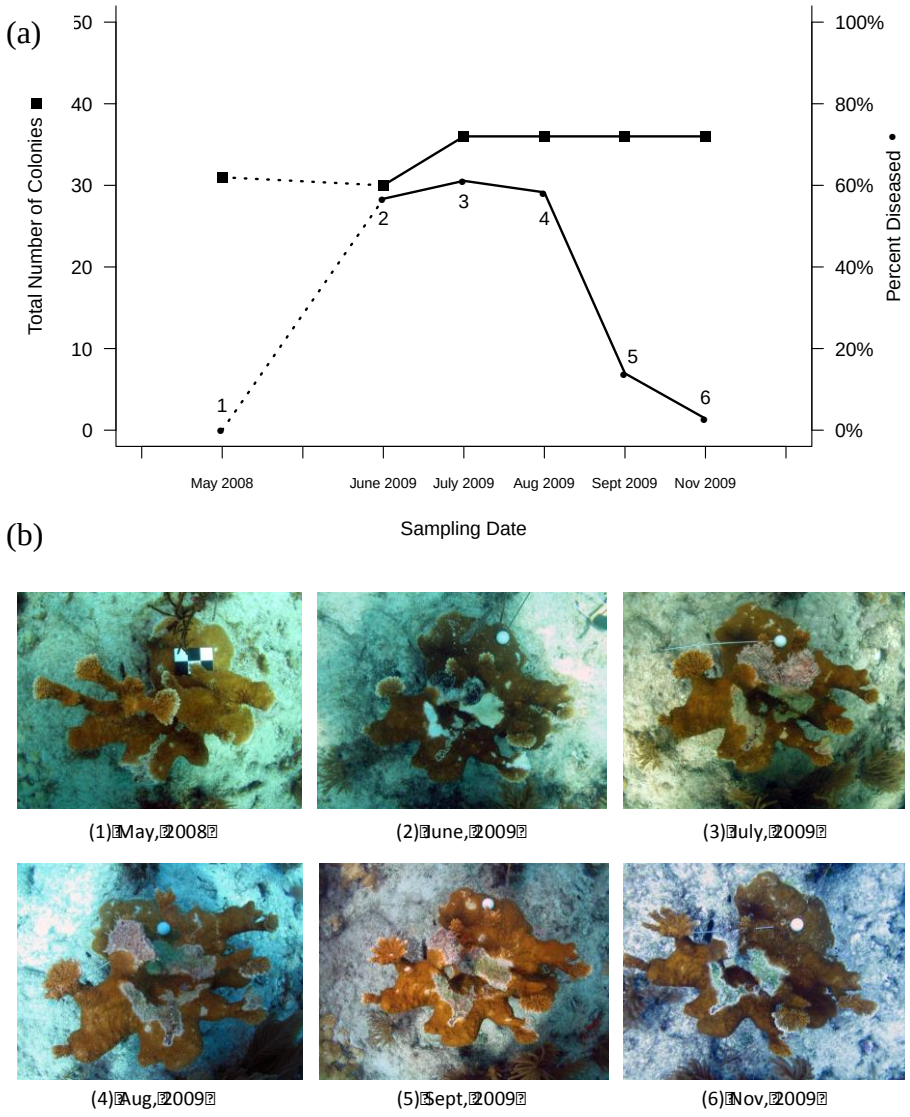

Figure 12: Partial Mortality Monthly survey of A. *palmata* at Looe Key, Florida, summer 2009

(a) Total number of A. *palmata* colonies (left axis) and percentage of A. *palmata* colonies with WPX (prevalence) in May 2008 and June, July, August, September and November 2009 (right axis).

(b) Onset and progression of WPX in a single A. *palmata* colony for all six dates plotted in (a). WPX is present June through September 2009.

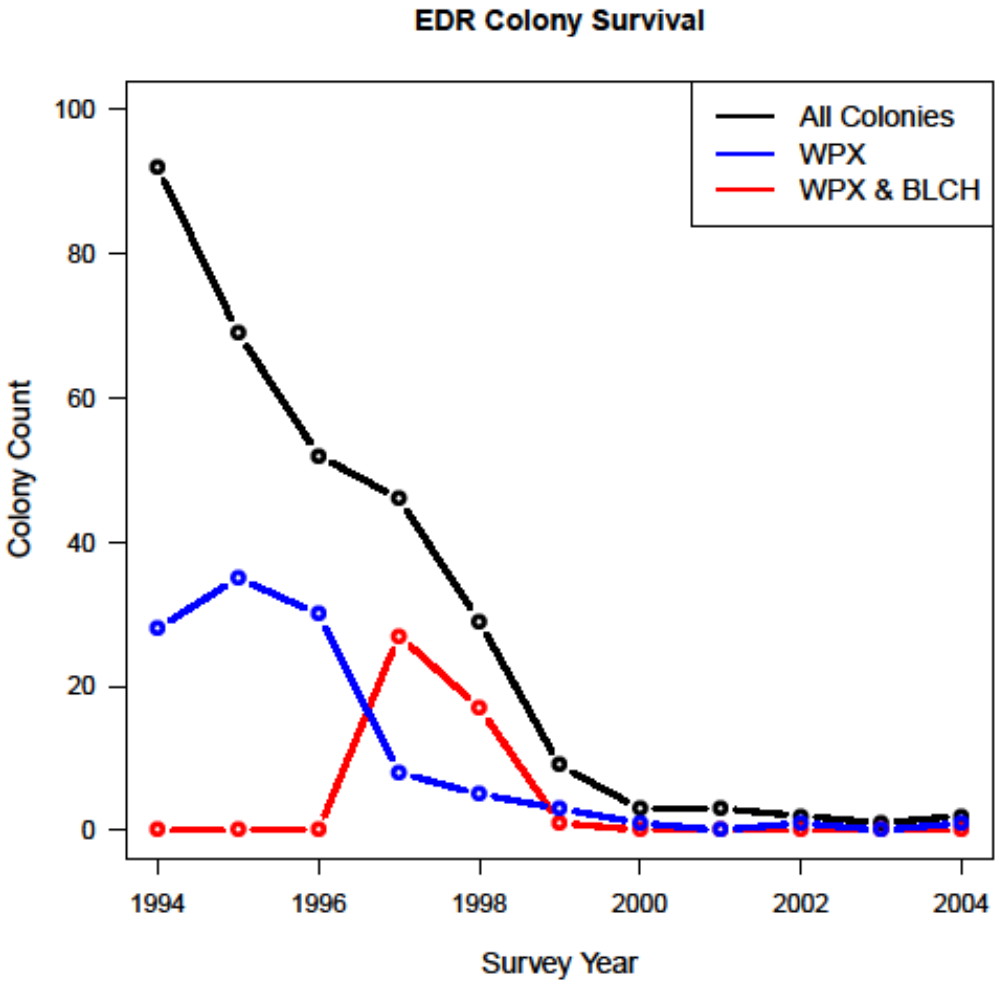

Figure 13: EDR BLCH and WPX Co-Occurrence Survival: All Colonies

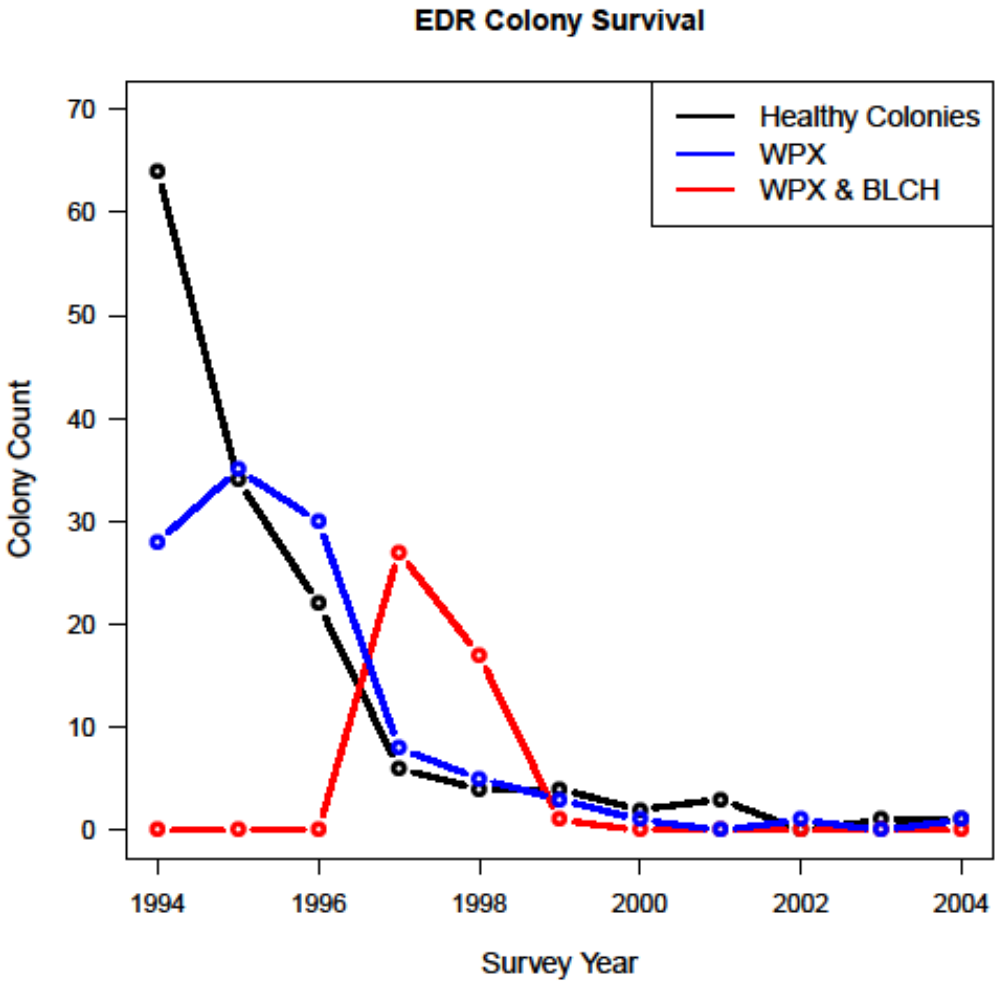

Figure 14: EDR BLCH and WPX Co-Occurrence Survival Healthy Colonies

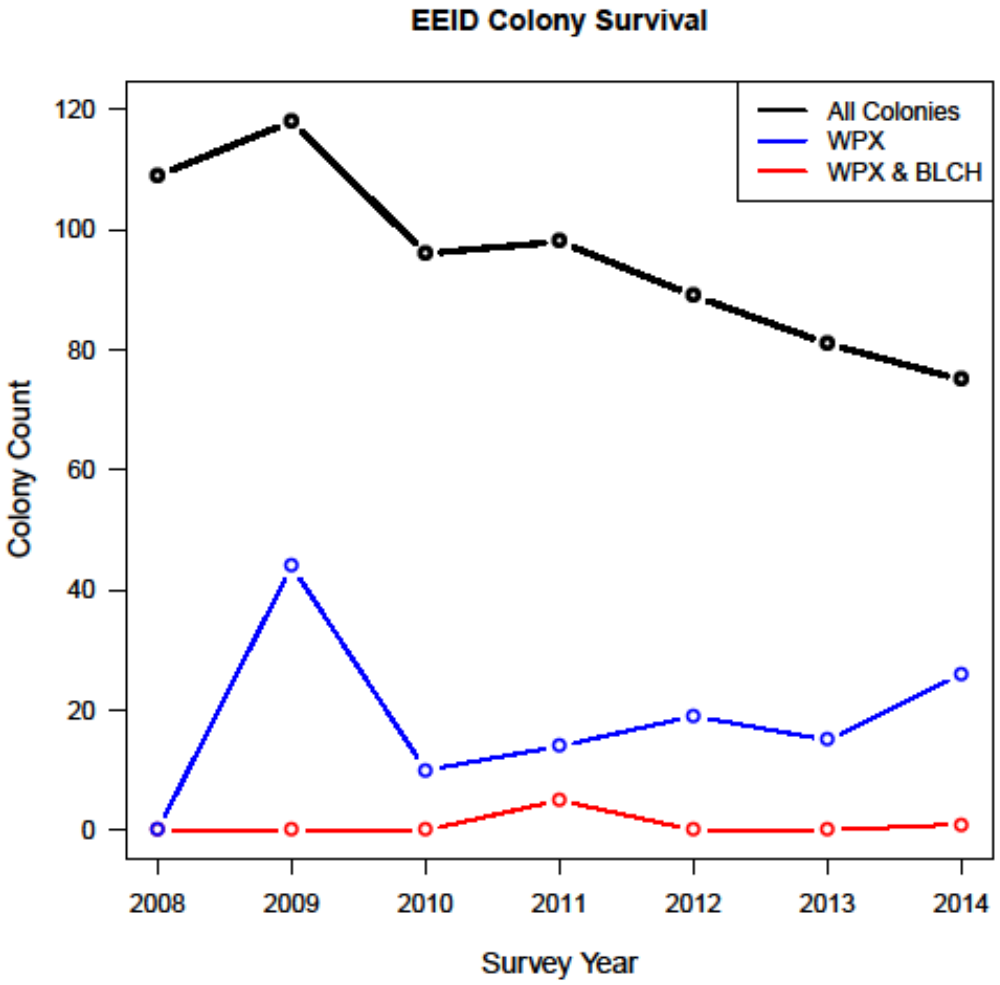

Figure 15: EEID BLCH and WPX Co-Occurrence Survival All Colonies

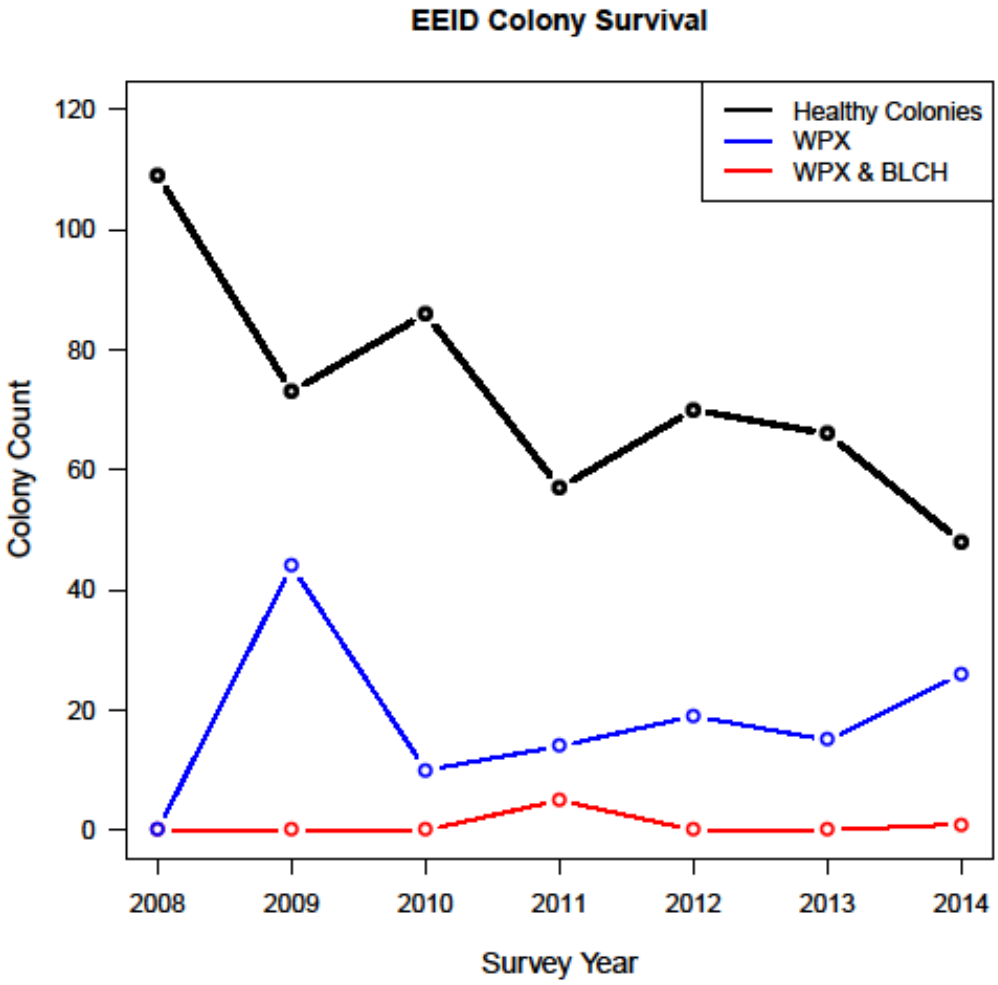

Figure 16: EEID BLCH and WPX Co-Occurrence Survival Healthy Colonies

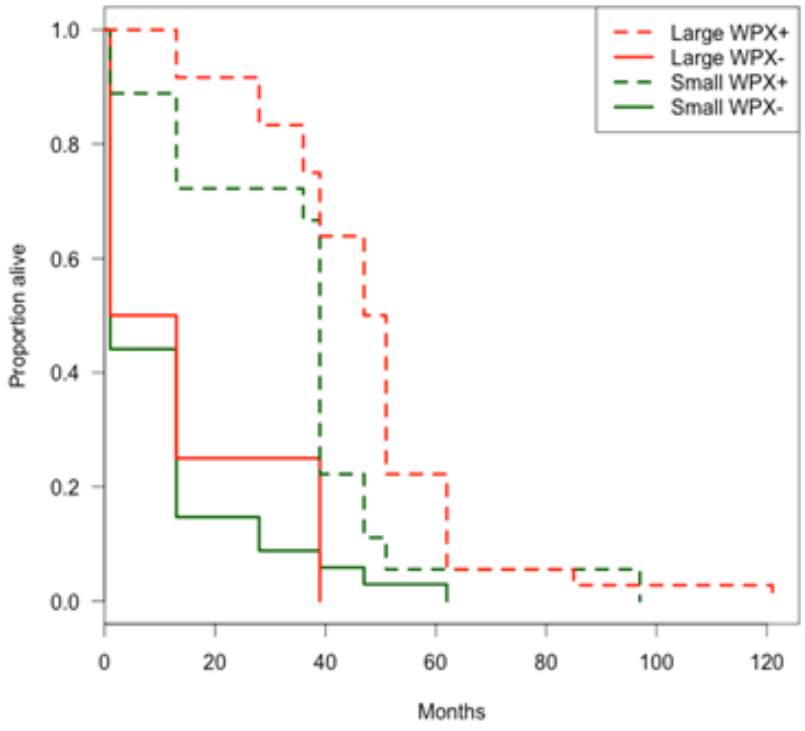

Figure 17: EDR Survival Curves

Dashed lines correspond to colonies that were ever WPX positive during the survey period, and solid lines correspond to those colonies never exhibiting WPX. Red and green lines are either "large" or "small", respectively, with the size cut-off at the median projected surface area of living tissue (33.2 cm^2).

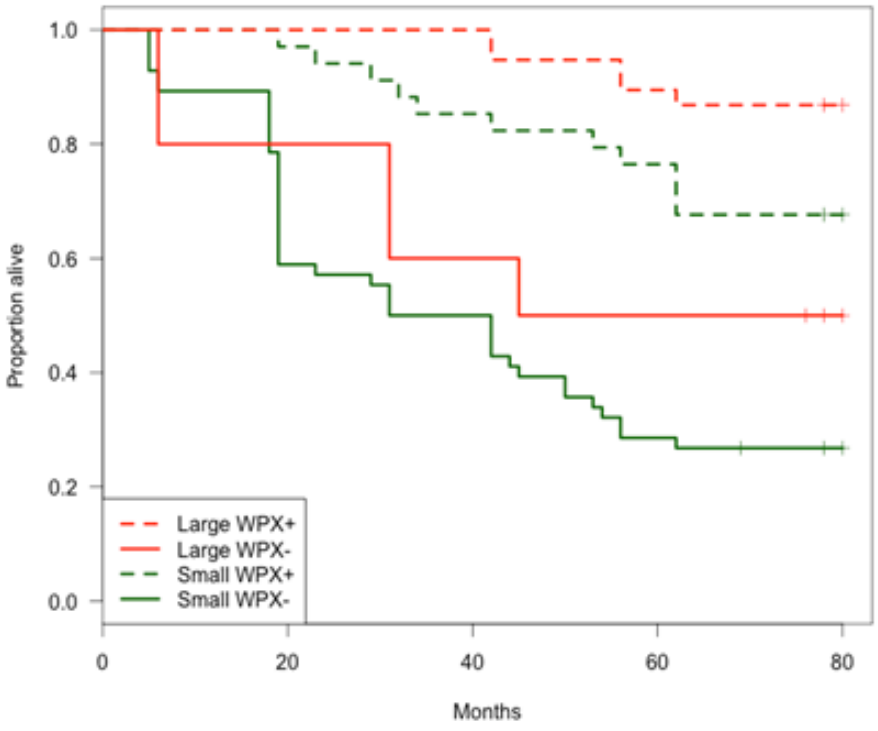

Figure 18: EEID Survival Curves

Dashed lines correspond to colonies that were ever WPX positive during the survey period, and solid lines correspond to those colonies never exhibiting WPX. Red and green lines are either "large" or "small", respectively, with the size cut-off at the median projected surface area of living tissue (710.5 cm^2).

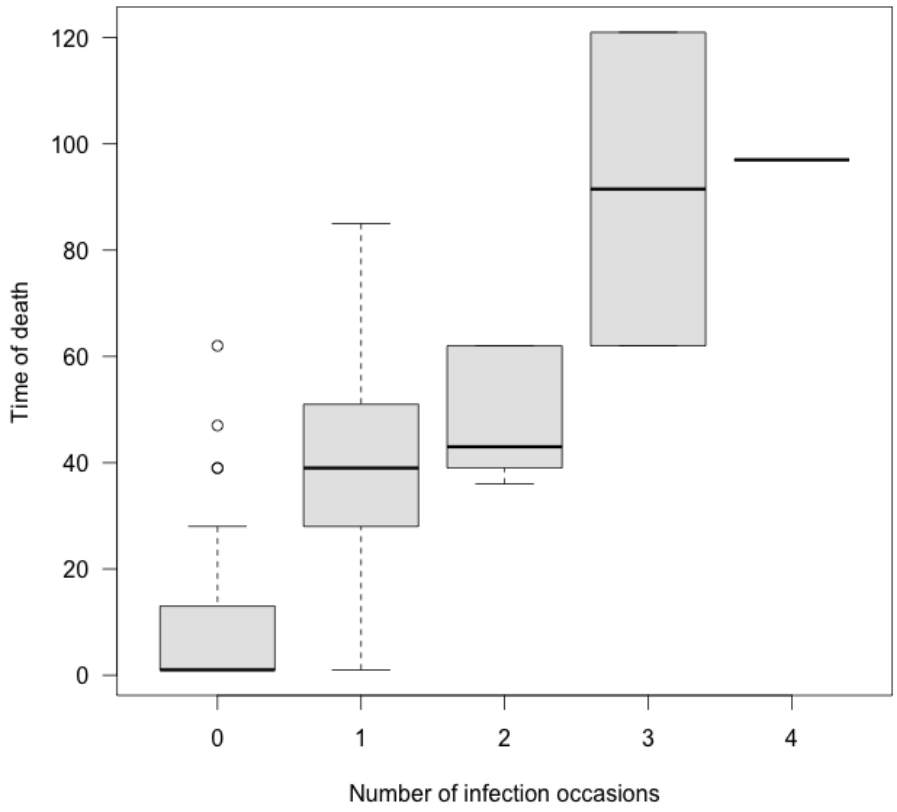

Figure 19: EDR Number of Colony Infections and Time of Death

Number of infection occasions (x-axis) refers to the number of times an individual colony became infected with WPX, cleared the infection, and subsequently became infected by WPX again in a following survey. Time of death (y-axis) is on a month scale, beginning from the first survey time.

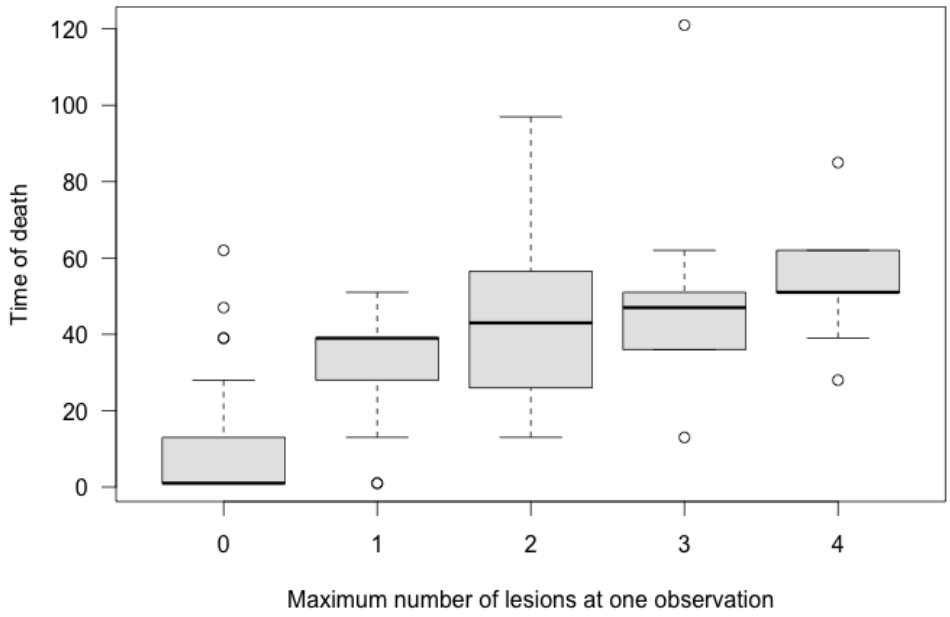

Figure 20: EDR Maximum Number of Lesions Observed per Survey and Time of Death

The x-axis refers to the maximum number of lesions found on an individual colony during one observation during the study (maximum of 4). The y-axis refers to the time of death on a month scale, beginning from the start of the first survey.

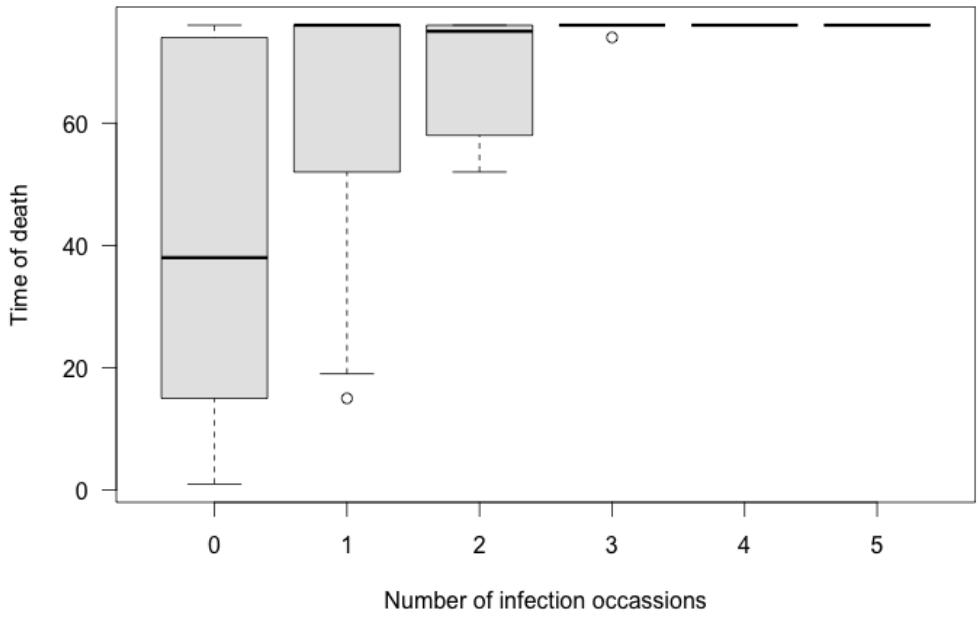

Figure 21: EEID Number of Colony Infections and Time of Death

Number of infection occasions (x-axis) refers to the number of times an individual colony became infected with WPX, cleared the infection, and subsequently became infected by WPX again in a following survey. Time of death (y-axis) is on a month scale, beginning from the start of the first survey.

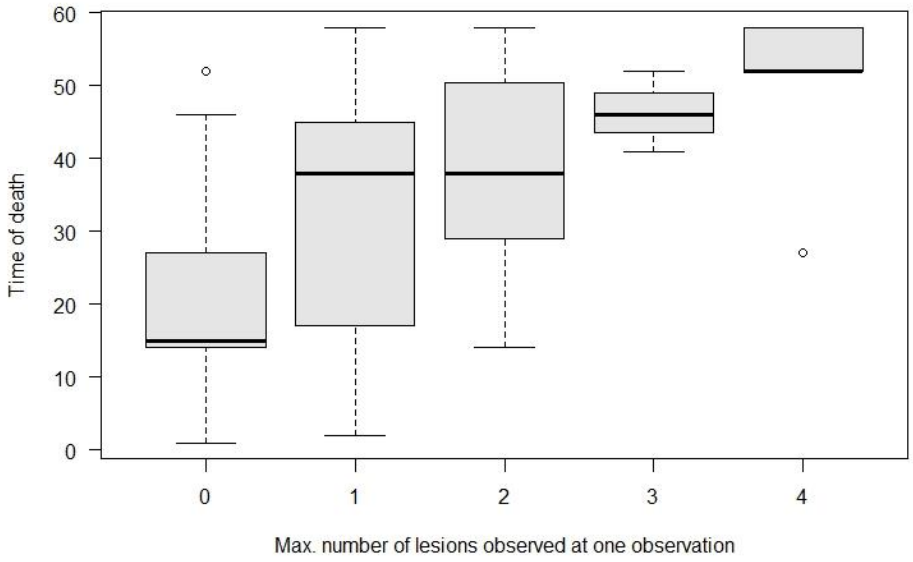

Figure 22: EEID Maximum Number of Lesions

The x-axis refers to the maximum number of lesions found on an individual colony during one observation during the study (maximum of 4). The y-axis refers to the time of death on a month scale, beginning from the start of the first survey.

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