

Human sewage identified as likely source of white pox disease of the threatened Caribbean elkhorn coral, *Acropora palmata*

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Summary

Caribbean elkhorn coral, *Acropora palmata*, has been decimated in recent years, resulting in the listing of this species as threatened under the United States Endangered Species Act. A major contributing factor in the decline of this iconic species is white pox disease. In 2002, we identified the faecal enterobacterium, *Serratia marcescens*, as an etiological agent for white pox. During outbreaks in 2003 a unique strain of *S. marcescens* was identified in both human sewage and white pox lesions. This strain (PDR60) was also identified from corallivorous snails (*Coralioophila abbreviata*), reef water, and two non-acroporid coral species, *Siderastrea siderea* and *Solenastrea bournoni*. Identification of PDR60 in sewage, diseased *Acropora palmata* and other reef invertebrates within a discrete time frame suggests a causal link between white pox and sewage contamination on reefs and supports the conclusion that humans are a likely source of this disease.

Introduction

Zoonotic diseases are routinely introduced from wildlife into human populations. While reverse zoonoses, where pathogens are passed from humans to animals, are known (Woolhouse and Gaunt, 2007), their occurrence between humans and marine invertebrates has not been reported. Part of this may be due to the lack of data

available for full investigations of outbreaks in marine organisms. In general, determining a source of infectious agent(s) during epidemiological investigations of disease outbreaks is difficult, especially in marine environments, due to several limiting factors including: (i) the likelihood of observing, documenting and identifying active outbreaks, (ii) the ability to rapidly mobilize collection efforts during a defined outbreak period, (iii) the ability to collect samples from a sufficient number and range of expected sources, and (iv) the inherently broad diversity of strains found in the environment, which makes the likelihood of finding a definitive source relatively small. Therefore, reports of marine epizootics are rarely followed up with documentation of the source of the outbreak. We report here on the investigation of a multi-reef outbreak of white pox disease in the threatened elkhorn coral, *Acropora palmata*, and our findings of a unique strain of the enterobacterium *Serratia marcescens* found in all white pox-diseased *A. palmata* and in corresponding human sewage samples. Previous investigations identified *S. marcescens* as an aetiological agent for white pox (Patterson *et al.*, 2002). This study identifies human sewage as a source of the pathogen in the Florida Keys.

The white pox host coral, *A. palmata*, is a key framework builder in Caribbean coral reefs and the extensive three-dimensional structure of this species provides critical habitat and shelter for many reef dwelling organisms. *Acropora palmata* has been decimated in recent years (Patterson *et al.*, 2002; Gardner *et al.*, 2003), resulting in the listing of this species as threatened under the United States Endangered Species Act (Precht *et al.*, 2002; Hogarth, 2006). A major contributing factor in the decline of *A. palmata* is disease (Gladfelter, 1982; Patterson *et al.*, 2002). White pox and white band are the most commonly reported diseases for *A. palmata* (Sutherland *et al.*, 2004). Between the 1970s and 1980s, epizootics of white band disease are believed to have resulted in a Caribbean-wide decline of acroporid corals (Gladfelter, 1982); however, there are few data on the rate and exact extent of loss. While many of the Caribbean acroporid populations were lost during the 1970s, 1980s and 1990s due to a combination of disease (Gladfelter, 1982; Aronson and Precht, 2001; Rodríguez-Martínez *et al.*, 2001; Patterson *et al.*, 2002; Sutherland and Ritchie,

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2004), bleaching (Harvell *et al.*, 1999; Hoegh-Guldberg, 1999), and hurricane damage (Hughes, 1994; Fong and Lirman, 1995; Lirman and Fong, 1997; Bythell *et al.*, 2000; Lirman, 2003; Sommerfield *et al.*, 2008), in the Florida Keys, living *A. palmata* coral cover remained at 11.3% by 1996 when wide-scale observations of white pox disease were first recorded (Patterson *et al.*, 2002). Between 1996 and 2002, white pox disease was observed affecting *A. palmata* populations throughout the Florida Keys (Porter *et al.*, 2001) and corresponded to an 88% decline in *A. palmata* cover (Patterson *et al.*, 2002; Sutherland and Ritchie, 2004). Over the same period, white band disease was rare with reports of its prevalence in Florida and elsewhere in the Caribbean ranging from 0 to 6% (Weil *et al.*, 2000; Porter *et al.*, 2001; Miller *et al.*, 2002). Recent reports indicate that, Caribbean-wide, white band disease is less prevalent than white pox (Mayor *et al.*, 2006; Muller *et al.*, 2008; Zubillaga *et al.*, 2008). Prevalence of white pox disease on Caribbean reefs has declined in recent years coincident with declines in host coral populations. *Acropora palmata* populations are threatened by a lack of large colonies, low sexual recruitment (Quinn and Kojis, 2005; Grober-dunsmore *et al.*, 2006; Zubillaga *et al.*, 2008), and biological stressors including white pox disease and predation by the corallivorous snail *Coralliophila abbreviata* (Grober-dunsmore *et al.*, 2006; Mayor *et al.*, 2006). Throughout the Caribbean, white pox disease has contributed to a flattening of the architectural complexity of coral reefs primarily based on the loss of branching acroporid corals (Alvarez-filip *et al.*, 2009). Severe reductions of acroporid corals have also contributed to the gathering impression of a system-wide phase shift from reef surfaces dominated by scleractinian corals to substrates dominated by soft corals and macroalgae (Maliao *et al.*, 2008).

In 2002, we reported a definitive agent of white pox disease as the bacterium *S. marcescens* and proposed that when *S. marcescens* is found in association with disease lesions, the disease be referred to as acroporid serratiosis to reflect its aetiology (Patterson *et al.*, 2002). Since June 2002, we have conducted targeted studies to elucidate the origin of the acroporid serratiosis pathogen and the prevalence and diversity of *S. marcescens* in human sewage and the marine environment. In July 2003 outbreaks of white pox occurred at two reefs in the Florida Keys, Grecian Rocks reef in the upper Keys and Rock Key reef in the lower Keys. Disease signs on affected *A. palmata* colonies manifested as large, coalescing, irregularly shaped distinct white patches of tissue loss located randomly and colony-wide on host corals. Before, during and after the 2003 white pox outbreaks, samples were collected from white pox-affected and -unaffected *A. palmata*, other marine organisms, nearshore and offshore water and sewage in the Florida

Keys and screened for the presence of *S. marcescens* using a three-step method: differential growth on MacConkey Sorbitol agar (MCSA) amended with colistin (Grasso *et al.*, 1988), differential growth on DNase with Toluidine Blue agar (DTC) amended with cephalothin (Farmer *et al.*, 1973) and amplification by *Serratia*-specific PCR. 16S rDNA PCR amplicons from a sub-sample [20% (85/413)] of the environmental isolates identified as *S. marcescens* using the three-step method were sequenced to confirm identity. *Serratia marcescens* isolated from all sources were compared using pulsed-field gel electrophoresis (PFGE) genotypic fingerprinting (by endonuclease restriction) in order to identify the origin and environmental diversity of the white pox disease pathogen. PFGE is considered the superior method for molecular strain typing of *S. marcescens* and other *Enterobacteriaceae* (Tenover *et al.*, 1995; Miranda *et al.*, 1996; Shi *et al.*, 1997; Olive and Bean, 1999; Alaidan *et al.*, 2009) and is widely used in epidemiological investigations of *S. marcescens* outbreaks (Steppberger *et al.*, 2002; van der Vorm and Woldring-Zwaan, 2002; Alfizah *et al.*, 2004; Milisavljevic *et al.*, 2004; Maragakis *et al.*, 2008).

Results

The three-step method (MCSA–DTC–PCR) was specific for the identification and isolation of *S. marcescens* from environmental sources in the Florida Keys (Fig. 1). Only *S. marcescens* showed characteristic growth on MCSA and DTC and were PCR positive (Table 1). While all *Serratia* spp. tested were positive in the *Serratia*-specific PCR assay, none of the other species showed characteristic ('positive') growth on both MCSA and DTC (Table 1). Of the non-*Serratia* control species tested, none was amplified by the *Serratia*-specific PCR assay and none

Table 1. Specificity of two-step culture method for *Serratia marcescens* and other bacterial strains using MacConkey Sorbitol agar with colistin (MCSA) (overnight at 37°C) and DNase with Toluidine Blue agar (DTC) amended with cephalothin (up to 48 h at 41°C).

Species	ATCC #	MCSA	DTC	PCR
<i>Serratia marcescens</i>	13880	+	+	+
<i>Serratia liquefaciens</i>	27592	+	–	+
<i>Serratia odorifera</i>	33077	+	–	+
<i>Serratia rubidaea</i>	33670	–	–	+
<i>Serratia plymuthica</i>	27593	+	–	+
<i>Escherichia coli</i>	15597	+	–	–
<i>Klebsiella pneumoniae</i>	13883	–	–	–
<i>Shewanella algae</i>	51181	–	–	–
<i>Vibrio vulnificus</i>	27562	–	+	–

For MCSA '+' indicates growth in the presence of colistin and fermentation of sorbitol (red colony); for DTC '+' indicates growth in the presence of cephalothin and production of DNase (red halo). ATCC, American Type Culture Collection.

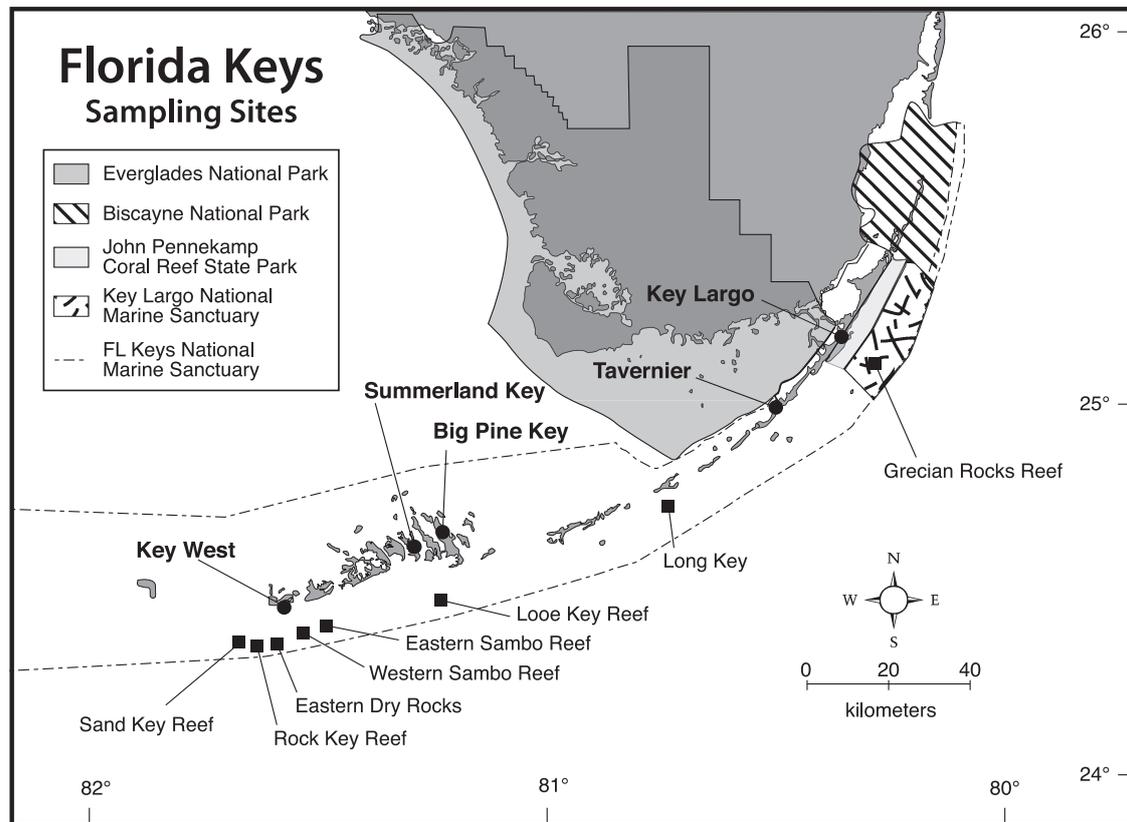


Fig. 1. Map of the Florida Keys National Marine Sanctuary showing sampling sites. *Acropora palmata*, other invertebrate, vertebrate (faeces) and seawater samples were collected at nearshore sites near Key Largo, and at Grecian Rocks, Long Key, Eastern Sambo, Western Sambo, Eastern Dry Rocks, Rock Key and Sand Key reefs. Water samples were collected at beaches in Key West and at canals on Big Pine Key and Summerland Key. Seabird faeces were collected in Tavernier and Key West. Raw sewage and treated effluent were collected at the Key West wastewater treatment plant. The 1999 acroporid serratosiis outbreak strain, *S. marcescens* PDL100, was collected at Looe Key Reef.

showed characteristic growth on both MCSA and DTC (Table 1). Of the subset of isolates (16S rDNA PCR amplicons) that were sequenced, all (85/85) were confirmed to be *S. marcescens*.

A total of 504 samples were collected from environmental sources in the Florida Keys from which 413 isolates of *S. marcescens* were identified with the three-step method (Tables 2 and 3). The majority of *S. marce-*

Table 2. Environmental sample types from which *Serratia marcescens* strain PDR60 was isolated in the Florida Keys (June 2002–August 2006).

Sample type	Location of collection	Date of collection	No. of samples	No. of <i>S. marcescens</i> (PDR60) isolates
Pre-outbreak				
<i>Siderastrea siderea</i> apparently healthy	Long Key	June 2002	4	3
Outbreak				
<i>Acropora palmata</i> white pox-affected	Rock Key	July 2003	15	18
	Grecian Rocks	July 2003	10	5
<i>Solenastrea bourmoni</i> apparently healthy	Key Largo	July 2003	6	4
<i>Coralliophila abbreviata</i> snails	Rock Key	July 2003	8 ^a	3
Reef water	Rock Key	July 2003	1	6
Raw sewage	Key West	September 2003	1	1
Post-outbreak				
Raw sewage	Key West	July 2004	1	1
<i>Coralliophila abbreviata</i> snails	Sand Key	August 2006	18	54
Total			64	95

a. Mantle fluid from eight snails were pooled for analysis.

Location and date of sample collection, number of samples collected from each sample type, and number of PDR60 isolated from each of these sample types are shown.

Table 3. Environmental sample types from which *Serratia marcescens* strains, excluding PDR60, were isolated in the Florida Keys (June 2002–August 2006).

Sample type	Location of collection	Date of collection	No. of samples	No. of <i>S. marcescens</i> (non-PDR60) isolates	No. of PFGE Patterns
Beach water	Key West	September 2003, April and July 2004, August 2006	7	21	13
Canal water	Big Pine Key	September 2003, April and July 2004, August 2006	20	109	48
Canal water	Summerland Key	April 2004	1	3	3
Seabird guano	Key West	September 2003, April and July 2004, August 2006	67	20	11
Seabird guano	Tavernier Key	July 2004	3	4	4
Raw sewage	Key West	September 2003, April and July 2004, August 2006	6	160	53
Treated effluent	Key West	April and July 2004, August 2006	4	1	1
Total			108	318	117 ^a

a. Not a sum: 16 PFGE patterns were demonstrated by isolates from more than one sample type. Location and date of sample collection, number of samples collected from each sample type, number of *S. marcescens* isolated from each of these sample types, and the number of pulsed-field gel electrophoresis (PFGE) genotypic fingerprint patterns exhibited by the isolates are shown.

scens isolates were collected from human sewage [39.2% (162/413)], canal water [27.1% (112/413)], and the coral predatory snail *C. abbreviata* [13.8% (57/413)] (Tables 2 and 3). *Serratia marcescens* was never recovered from parrotfish faeces [sample number (n) = 28], the predatory fireworm *Hermodice carunculata* (n = 3), predation lesions, caused by *H. carunculata* or *C. abbreviata*, on otherwise apparently healthy *A. palmata* (n = 59), or the surface mucus layer (SML) from apparently healthy colonies of *A. palmata* (n = 238). Additionally, *S. marcescens* was not recovered from apparent white pox lesions at Western Sambo (n = 2) or Rock Key (n = 2) reefs in August 2006.

Twenty-three *S. marcescens* isolates were collected from white pox-affected colonies of *A. palmata* during the two outbreaks at offshore reefs in the Florida Keys in July 2003 (Table 2). All (23/23) of these isolates have the same PFGE banding pattern (100% similarity), likely representing a single bacterial strain (Tenover *et al.*, 1995).

This strain, designated PDR60, was also identified in raw sewage from the Key West wastewater treatment facility between September 2003 and July 2004. Additionally, PDR60 was found in reef water and *C. abbreviata* during the July 2003 outbreak at Rock Key, in *C. abbreviata* at Sand Key reef in August 2006, and in two non-acroporid coral species, *Solenastrea bournoni* (Key Largo, July 2003) and *Siderastrea siderea* (Long Key, June 2002). Collectively, coral reef environments (corals, snails, reef water) yielded a total of 93 *S. marcescens* isolates and all (93/93) of these demonstrated the PDR60 PFGE pattern (Table 2). *Serratia marcescens* PDR60 was never found in canal water, beach water or seabirds. *Serratia marcescens* strains from these environments were only 80.0%, 52.6% and 45.6% similar to PDR60 respectively (Fig. 2).

PFGE typing of the 413 *S. marcescens* isolates demonstrated 118 different PFGE patterns (Tables 2 and 3), and each of these PFGE patterns represents a single *S.*

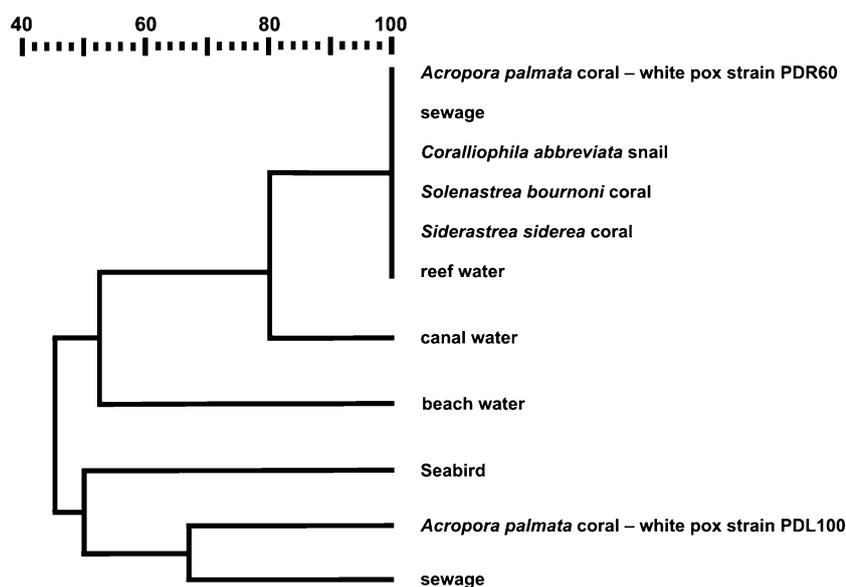


Fig. 2. Relatedness among *Serratia marcescens* isolates from reef organisms, seabirds, nearshore and offshore water, and sewage. Sources of *S. marcescens* isolates are shown on the vertical axis. Per cent similarity between pulsed-field gel electrophoresis (PFGE) patterns is shown along the horizontal axis. *Serratia marcescens* PDR60 from white pox-affected *Acropora palmata* showed 100% similarity to *S. marcescens* isolates collected during the outbreak period from human sewage, the coral predator *Coralliophila abbreviata*, two non-acroporid coral species *Solenastrea bournoni* and *Siderastrea siderea*, and reef water, and all of these isolates were identified as strain PDR60. *Serratia marcescens* from canal water, beach water and seabirds were only 80.0%, 52.6% and 45.6% similar to PDR60 respectively. The 1999 outbreak strain, *S. marcescens* PDL100, was only distantly related (45.5%) to PDR60.

marcescens strain. PDR60 was one of 54 strains of *S. marcescens* isolated from human sewage (Tables 2 and 3). While only two isolates of strain PDR60 were recovered from sewage (Table 2), this frequency of recovery was consistent for all 54 sewage strains. The mean and median number of *S. marcescens* isolates recovered per sewage PFGE strain were 2.6 and 1.0 respectively.

PFGE pattern comparisons for the two strains of *S. marcescens* isolated from white pox disease (acroporid serratiosis) lesions, PDR60 (collected in this study) and PDL100 (a 1999 outbreak strain described by Patterson *et al.*, 2002), indicate that PDR60 is only distantly related (45.5% similar) to PDL100 (Fig. 2). PDL100 was most similar (66.7%) to an *S. marcescens* strain isolated from human sewage (Fig. 2).

Discussion

A total of 118 PFGE strains of *S. marcescens* were recovered from the marine environment and human sewage (Tables 2 and 3), demonstrating genomic diversity among isolates. High levels of PFGE pattern diversity have also been reported for human disease outbreaks caused by *S. marcescens* (Miranda *et al.*, 1996; Shi *et al.*, 1997; Chetoui *et al.*, 1998; Milisavljevic *et al.*, 2004; Alaidan *et al.*, 2009) and *Escherichia coli* (McLellan *et al.*, 2001; Kahali *et al.*, 2004). To our knowledge this is the first report of the prevalence and diversity of *S. marcescens* in marine waters, coral reef invertebrates, seabirds and human sewage.

During white pox outbreaks in 2003, PDR60, a unique strain of the coral pathogen *S. marcescens*, was identified in both sewage and white pox-affected *A. palmata*. The outbreaks occurred at Grecian Rocks reef near Key Largo and at Rock Key reef near Key West. Offshore reefs near Key Largo and Key West, the largest human population centres in the Keys, have been shown to have higher levels of sewage contamination (Lipp *et al.*, 2007). During the 2003 outbreaks, PDR60 was circulating within human sewage and the reef environment. At affected reefs, this strain was found in white pox-affected *A. palmata*, the water column and the corallivorous snail *C. abbreviata*. At nearshore sites, near sewage-contaminated canals (Paul *et al.*, 2000; Lipp *et al.*, 2002), PDR60 was isolated from two non-acroporid coral species, *S. bournoni* and *S. siderea* (Table 2). In August 2006, 3 years after the white pox outbreaks at Grecian Rocks and Rock Key, PDR60 was recovered from *C. abbreviata* at Sand Key reef, with no observed signs of white pox.

Collectively, all reef isolates yielded the PDR60 PFGE pattern (93/93 *S. marcescens* isolates). Furthermore, PDR60 is the only strain of *S. marcescens*, other than PDL100 (Patterson *et al.*, 2002), reported from the reef environment. Other than coral reefs, the only environment

where this strain was found is raw sewage (Table 2). Of the environments where *S. marcescens* was recovered, PDR60 was never identified in beach water, canal water, seabirds or treated sewage (Fig. 2). Identification of PDR60 in sewage, diseased *A. palmata* and other reef invertebrates within a discrete time frame suggests a causal link between coral disease and sewage contamination on reefs.

Serratia marcescens (all strains) was consistently found in human sewage (100% of influent samples) and canals (77.2%) previously shown to be contaminated with human waste (Griffin *et al.*, 1999). The bacterium was also recovered from seabird guano and from Key West beaches with a history of closures to public use due to elevated faecal indicator bacteria levels (Griffin *et al.*, 1999; Nobles *et al.*, 2000). Encouragingly, our results indicate that the Key West wastewater treatment facility is effective at removing *S. marcescens* from wastewater; only one isolate of the bacterium was recovered from treated effluent during the entirety of our study (Table 3).

The SML samples from apparently white pox-affected corals, collected in August 2006 from Western Sambo and Rock Key reefs, did not contain this coral pathogen. The finding that *S. marcescens* cannot be isolated from all apparent white pox disease lesions suggests that either *S. marcescens* are present, but in a viable but non-culturable state, which was not revealed by our culture-based detection methods, or other pathogen(s) can cause white pox lesions indistinguishable from those caused by *S. marcescens*. However, it is important to note that white pox was rare ($n = 4$) and the apparent virulence of the disease was less pronounced in August 2006. In contrast to the disease signs produced during the July 2003 outbreaks, apparent disease lesions in August 2006 were smaller and multifocal and did not coalesce.

Our investigation of the coral predators, *C. abbreviata* and *H. carunculata*, were motivated by the hypothesis that these mobile marine invertebrates might play a role in transmission of the coral pathogen (Sussman *et al.*, 2003; Williams and Miller, 2005). *Hermodice carunculata* is a reservoir and vector of the coral pathogen, *Vibrio shiloi*, which induces bleaching in the Mediterranean coral *Oculina patagonica* (Sussman *et al.*, 2003). *Coralliophila abbreviata* has been shown to transmit an unidentified disease of Caribbean acroporid corals (Williams and Miller, 2005), and this gastropod preferentially feeds on *A. palmata* (Knowlton *et al.*, 1990; Miller, 2001) and threatens the recovery of decimated *A. palmata* populations in the Caribbean (Grober-dunsmore *et al.*, 2006). The identification of *S. marcescens* PDR60 in *C. abbreviata* at both a white pox-affected reef (Rock Key, 2003) and an apparently unaffected reef (Sand Key, 2006) combined with our finding that PDR60 is associated with other coral species indicates evidence for secondary transmission during a

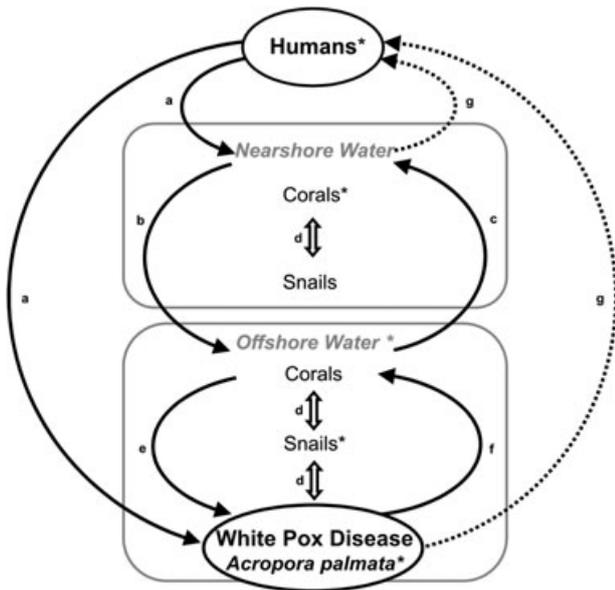


Fig. 3. Potential mechanisms of white pox pathogen transport from human source to nearshore and offshore marine waters and pathogen transmission from invertebrate reservoirs and vectors to the disease host, *Acropora palmata*. Environmental sources from which *Serratia marcescens* strain PDR60 has been isolated (*) and probable (solid arrows) and possible (dashed arrows) pathways of pathogen transport and transmission in the marine environment are indicated. (a) Human sewage is introduced to nearshore and offshore marine waters via septic systems, direct pump-out of sewage from boats and/or discharge of contaminated groundwater. (b) Nearshore marine waters transport the pathogen to offshore marine waters and to nearshore marine invertebrate reservoirs including non-host corals and *Coralliophila abbreviata*. (c) The pathogen circulates between invertebrate reservoirs and nearshore waters and between offshore and nearshore marine waters. (d) *Coralliophila abbreviata* function as vectors when snails that harbour the pathogen feed on corals. The pathogen may be transmitted from coral surface mucus layers to snails when snails feed on reservoir corals. (e) Offshore marine waters transport the pathogen to offshore marine invertebrates including the *A. palmata* host, and non-host coral and *C. abbreviata* reservoirs. The pathogen circulates between (f) host, reservoirs and offshore waters. (g) Humans could be infected with *S. marcescens* while recreating in nearshore or offshore waters.

white pox outbreak and the potential for biotic reservoirs to exist in the reef environment.

Potential mechanisms of primary and secondary disease transmission were derived from our results that identify human sewage as a likely source and coral reef invertebrates as potential vectors and reservoirs of white pox disease (Fig. 3). Leaking septic systems, and other on-site waste disposal receptacles, are a direct source of *S. marcescens* to nearshore marine waters. Alternatively, boats that pump-out sewage directly into nearshore and offshore waters may also serve as a direct pathogen source. *Serratia marcescens* in nearshore waters is transported to marine invertebrate reservoirs including non-host corals, where the pathogen becomes a resident in the SML, and *C. abbreviata*, where the pathogen

becomes a resident on or within snail tissues. The pathogen may be transmitted from coral SML to snails or vice versa when snails feed on reservoir corals. Within the nearshore marine environment, *S. marcescens* circulates between invertebrate reservoirs and nearshore waters. These nearshore waters transport *S. marcescens* directly to offshore coral reefs where the pathogen encounters the coral host, *A. palmata*, and causes white pox disease. Likewise, *S. marcescens* can be transported offshore via discharge of groundwater contaminated by the extensive use of in-ground wastewater disposal methods. *Serratia marcescens* may also be transported to non-host coral SML and *C. abbreviata*. These offshore reservoirs may function in secondary transmission of *S. marcescens* and/or as interepizootic reservoirs as the pathogen circulates between host, reservoirs and offshore waters. *Coralliophila abbreviata* may play an additional role as disease vectors when snails that harbour the pathogen feed on host corals. Furthermore, as the coral pathogen circulates within the marine environment, humans recreating (e.g. swimming, scuba diving) in nearshore or offshore waters could be exposed to this common human pathogen and develop respiratory infections or other signs or symptoms of *S. marcescens* infection (Hazen, 1988; Hejazi and Falkiner, 1997).

The July 2003 multi-reef outbreak was the only white pox outbreak encountered in the Florida Keys during the 4-year study period (June 2002–August 2006). In fact, this outbreak and a 2006 outbreak in St. John, US Virgin Islands (Muller *et al.*, 2008) are the most recent reported outbreaks of the disease. This low white pox disease prevalence may be due to the decimation of susceptible *A. palmata* populations during previous epizootics (Patterson *et al.*, 2002) and physical disturbances (e.g. hurricanes); however, observations of small recruits (Groberdunsmore *et al.*, 2006; Zubillaga *et al.*, 2008) and isolated large colonies of *A. palmata* suggest that strains of *A. palmata* that are resistant to white pox may exist (Reschef *et al.*, 2006). Low disease prevalence is a limitation of epidemiological investigations in wildlife populations. In order to identify a pathogen source, the outbreak must be witnessed by or reported to investigators equipped for a rapid response that includes sample collection from both host organisms and an array of appropriate potential sources.

Investigating the epidemiology of an outbreak, especially in the environment, requires that sampling occur within a relatively short time frame given that random genetic changes over time will alter PFGE patterns (Tenover *et al.*, 1995). Here, rapid mobilization of collection efforts during the 2003 multi-reef outbreak facilitated the identification of a source for white pox disease in the Florida Keys. Collection of samples from multiple sources within a defined outbreak period allowed us to effectively

describe the distribution of PDR60 in white pox diseased corals and in sewage. There was no match for the 1999 outbreak strain, PDL100, which was only distantly related to PDR60. This is likely due to the long interval between that outbreak and collection of samples during this study. However, it indicates that distinct strains are capable of causing disease.

Human activities can result in the direct or indirect introduction of novel pathogens into previously uninfected wildlife populations. Our results suggest that human sewage is a direct source of the white pox pathogen in the Florida Keys. Contamination of nearshore waters in the Keys has been clearly demonstrated (Lapointe *et al.*, 1990; 2004; Paul *et al.*, 1995a,b; 1997; 2000; Griffin *et al.*, 1999; Nobles *et al.*, 2000; Lipp *et al.*, 2002) and microbes found in human sewage can be detected in corals of the outer reef tract (Lipp *et al.*, 2007). Currently the majority of the Florida Keys and much of the Caribbean utilize on-site waste disposal including septic systems, injection wells and illegal cesspools (Paul *et al.*, 2000). Because of its large population and current low level of advanced wastewater treatment, the Florida Keys may be considered a bell-weather region and information gained from this system can be used to avoid similar fates in other potentially less polluted regions of the Caribbean which may eventually face similar outbreaks. Our results indicate that local action to improve wastewater disposal and treatment will be effective at reducing introduction of this pathogen and facilitating the recovery of an iconic coral species.

Experimental procedures

Sample collection

Samples were collected from nearshore and offshore marine environments in the Florida Keys before (June 2002), during (July and September 2003) and after (July 2004 and August 2006) the 2003 white pox disease outbreaks at Grecian Rocks and Rock Key reefs, Florida Keys. SML from apparently healthy colonies of *S. siderea* and *S. bourmoni* were collected from Long Key in June 2002 and from Key Largo in July 2003 respectively. *Acropora palmata* SML from white pox-affected colonies (from apparently healthy coral tissue and from margins of disease lesions) and reef water were collected from Rock Key and Grecian Rocks during the July 2003 white pox outbreaks. Corallivorous snails (*C. abbreviata*) were also collected from Rock Key during the 2003 outbreak. In September 2003, July 2004 and August 2006, *A. palmata* SML was collected from healthy corals and from predation lesions caused by *C. abbreviata* and fireworms (*H. carunculata*) at five reef sites in the lower Florida Keys National Marine Sanctuary: Rock Key, Sand Key, Eastern Dry Rocks, Eastern Sambo and Western Sambo (Fig. 1). Also collected at these sites during the same time periods were seawater, parrotfish faeces and *C. abbreviata*. Additionally, *A. palmata* SML was collected from apparently white pox-affected corals at Rock Key and Western Sambo in August 2006.

Water was collected from two beaches in Key West, Higgs Beach and Fort Zachary Taylor, and five canals on Big Pine Key in September 2003, April and July 2004, and August 2006. Additionally, water was collected from one canal on Summerland Key in April 2004. Guano was collected from gulls and other seabirds in Key West in September 2003 and April and July 2004 and from pelicans at the Florida Keys Wild Bird Rehabilitation Center, Tavernier in July 2004. Sewage influent (September 2003, April and July 2004, August 2006) and treated wastewater effluent (April and July 2004, August 2006) were collected from an advanced wastewater treatment plant in Key West.

Surface mucus layer was collected using sterile needleless 60 ml syringes. Reef, beach and canal water samples were collected from just below the surface of the water column by hand filling 1 l sterile polypropylene bottles. *Coralliophila abbreviata*, *H. carunculata* and freshly excreted parrotfish faeces were collected in sterile 50 ml plastic tubes. Freshly excreted seabird guano was collected using sterile cotton swabs and transferred to sterile 15 ml plastic tubes. Raw sewage and treated wastewater effluent were collected using 1 l sterile polypropylene bottles. All samples were stored on ice until processing.

Sample processing

All samples were processed within 3 h of collection. Subsamples of SML (10 ml), reef water (50 ml), canal water (5 ml and 50 ml), beach water (50 ml) and treated wastewater effluent (50 ml) were filtered through 47 mm 0.45- μ m-pore-size mixed cellulose ester filters. Filters were then plated on MCSA (Becton Dickinson, Franklin Lakes, NJ) with colistin (200 U ml⁻¹; MP Biomedicals, Aurora, OH) (Grasso *et al.*, 1988). Sewage influent (10 μ l and 50 μ l) and parrotfish faeces (100 μ l) were directly spread onto MCSA. Swabs containing seabird guano were streaked onto MCSA. Mantle fluid from *C. abbreviata* collected from Rock Key in July 2003 was pooled and spread onto MCSA. *Coralliophila abbreviata* collected between September 2003 and August 2006 were processed by swabbing the animal tissue with sterile cotton swabs and streaking onto MCSA. Whole *H. carunculata* were placed on MCSA plates, rolled on the agar and removed. Inoculated MCSA plates were incubated overnight at 37°C. All bacterial colonies that fermented sorbitol and were resistant to colistin, appearing pink to red on MCSA (characteristic of *S. marcescens*), were picked and plated onto DTC (Becton Dickinson) amended with cephalothin (0.1 mg ml⁻¹; ICN Biomedicals, Aurora, OH) (Farmer *et al.*, 1973). Inoculated DTC plates were incubated overnight at 41°C. Presumptive *S. marcescens*, determined by a DNase-positive reaction (appearance of a red halo on DTC) and resistance to cephalothin (characteristic of *S. marcescens*), were streaked onto non-selective Caso agar (EM Science, Gibbstown, NJ) and incubated overnight at 25°C for confirmation of pure colonies. Known controls were used to verify the accuracy of these culture methods (Table 1).

PCR assay

Presumptive *S. marcescens* identified by differential growth on MCSA and DTC were also assayed with *Serratia*-specific

PCR to confirm the identity of isolates as *S. marcescens*. The commercially available DNeasy Tissue Kit (Qiagen, Valencia, CA) was used to extract the DNA from pure cultures of presumptive *S. marcescens* (i.e. positive on both MCSA and DTC media, Table 1). Primers Smar 16SV (GGGAGCTTGCTCACTGGGTG) and Smar 16SWR (GCGAGTAACGTCAGTTGATGAGCGTATTA) described by Wilson and colleagues (1999) (Sigma-Genosys, Woodlands, TX) targeted a ~410 bp region of the 16S rRNA gene, specific for *Serratia* spp. When applied only to isolates that were identified as presumptive *S. marcescens* with the two-step culture method, this primer set was specific for *S. marcescens*. Each PCR mixture (25 µl final volume) contained 1 µl of purified genomic DNA (10–100 ng), 1× PCR buffer [providing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂ and 0.001% gelatin (w/v); Sigma-Aldrich, St. Louis, MO], a mixture of dNTPs (Fisher Scientific, Fair Lawn, NJ) at 0.2 mM each, 0.8 µM of each primer, and 0.625 U *Taq* DNA Polymerase (Sigma-Aldrich). DNA from *S. marcescens* control cultures (ATCC 13880) and reactions lacking DNA template served as positive and negative controls respectively. Reactions were performed in a thermal cycler (PTC-200, MJ Research, Watertown, MA) under the following conditions: initial denaturation at 94°C for 4 min; 40 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 30 s; and a final extension step at 72°C for 5 min. Amplified PCR products were separated by Tris-Acetate-EDTA (TAE)-agarose gel electrophoresis using 1.5% OmniPur agarose (EMD Chemicals, Gibbstown, NJ), stained with ethidium bromide and viewed by UV transillumination.

Sequencing

A subset of the PCR-positive amplicons (85 of 413) were purified and directly sequenced using primer extension, by a commercial vendor (Northwoods DNA, Bemidji, MN), for final confirmation. All sequences were searched in GenBank for identification using the BLASTN tool (Altschul *et al.*, 1997) and manually aligned with known sequences of *S. marcescens* and other *Serratia* spp. using PHYDIT (version 3.1) (Chun, 2001).

PFGE plug preparation

Confirmed (MCSA+, DTC+ and PCR+) environmental isolates of *S. marcescens* were transferred to 10 ml of LB broth (EM Science, Gibbstown, NJ) and incubated overnight at 37°C with gentle shaking. Overnight cultures were placed on ice with gentle shaking (10 min) and centrifuged for 10 min at 2400 g (Marathon 21000R, Fisher Scientific). The supernatant was removed and the cells were washed two times in 5 ml of PFI buffer [10 mM Tris (pH 7.6) and 1 M NaCl] with centrifugation (2400 g, 10 min). Cells were resuspended in 1 ml of PFI buffer, stained with crystal violet, and counted with the aid of a haemocytometer. Cell concentrations were diluted to 10⁶ cells ml⁻¹ in PFI buffer. Cell suspensions were mixed 1:1 with 1% InCert agarose (Cambrex Bio Science, Rockland, ME) and dispensed into PFGE plug molds (Bio-Rad, Hercules, CA). Cells within plugs were lysed by incubating them 2:1 in lysis buffer [6 mM Tris (pH 7.6), 1 M NaCl,

100 mM EDTA (pH 8.0), 0.5% Brij 58, 0.2% deoxycholate, 0.5% sarkosyl, 1 mg lysozyme ml⁻¹] with gentle shaking at 37°C (16 h). Lysis buffer was removed and plugs were incubated (1:1) two times in ESP buffer [0.5 M EDTA (pH 8.0), 1% sarkosyl, 1 mg Proteinase K ml⁻¹] with gentle shaking at 50°C (24 h). Plugs were washed three times in T₁₀E₁ buffer [10 mM Tris (pH 7.6) and 1 mM EDTA (pH 8.0)] at 25°C (30 min) and incubated 2:1 in 1.5 mM Pefabloc proteinase inhibitor [0.0350 mg 98% AEBSF hydrochloride (Acros Organics, NJ) in 1 ml of T₁₀E₁ buffer] with gentle shaking at 37°C (2.5 h). Plugs were washed three times in T₁₀E₅₀ buffer [10 mM Tris (pH 7.6) and 50 mM EDTA (pH 8.0)] with gentle shaking at 50°C (1 h) and stored in a final change of T₁₀E₅₀ buffer.

Restriction endonuclease digestion and PFGE

Plugs were washed three times in 1 ml of T₁₀E₁ buffer at 25°C (1 h), incubated in 1× restriction endonuclease buffer (Promega, Madison, WI) at 25°C (2 h), and digested with *Spe*I (Promega) at 37°C (20 h) (Marty *et al.*, 2002). Digested plugs were incubated in T₁₀E₁ buffer with gentle shaking at 50°C (2 h). PFGE was performed on a CHEF-DR II PFGE apparatus (Bio-Rad) using the following parameters: separation on a 0.8% agarose gel (Seakem GTG; Cambrex Bio Science) in 1× Tris-Borate-EDTA and switch times from 5 to 60 s over 16 h at 14°C and 6 V cm⁻¹. A Lambda ladder (Bio-Rad) was used to determine fragment sizes. Gels were stained in 1× SYBR Gold nucleic acid stain (Molecular Probes, Eugene, OR) and photographed under UV transillumination. The photographic image was captured digitally using Kodak 1D LE software (version 3.6.1; Kodak, New Haven, CT). PFGE patterns were analysed using GelCompar II software (version 4.01, Applied Maths, Austin, TX). Per cent similarities between *S. marcescens* isolates were identified with Dice correlation coefficient and unweighted pair group using arithmetic averages (UPGMA). Optimal settings for band position tolerance and optimization were automatically determined using GelCompar II. Two or more isolates were considered identical when their PFGE patterns exhibited similarity coefficients ≥ 95%.

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References

Alaidan, A., Bohol, M.F., Al-Thawadi, S.I., Al-Suwaine, A., Al-Qahtani, A.A., and Al-Ahdal, M.N. (2009) Rapid PFGE

- method for fingerprinting of *Serratia marcescens* isolates. *J Microbiol Methods* **78**: 238–241.
- Alfizah, H., Nordiah, A.J., and Rozaidi, W.S. (2004) Using pulsed-field gel electrophoresis in the molecular investigation of an outbreak of *Serratia marcescens* infection in an intensive care unit. *Singapore Med J* **45**: 214–218.
- Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**: 3389–3402.
- Alvarez-Filip, L., Dulvy, N.K., Gill, J.A., Cote, I.M., and Watkinson, A.R. (2009) Flattening of Caribbean coral reefs: region-wide declines in architectural complexity. *Proc R Soc* **276**: 3019–25.
- Aronson, R.B., and Precht, W.F. (2001) White-band disease and the changing face of Caribbean coral reefs. *Hydrobiologia* **460**: 25–38.
- Bythell, J.C., Hillis-Starr, Z.M., and Rogers, C.S. (2000) Local variability but landscape stability in coral reef communities following repeated hurricane impacts. *Mar Ecol Prog Ser* **204**: 93–100.
- Chetoui, H., Delhalle, E., Melin, P., Struelens, M.J., De Ryck, R., Osterrieth, P., and De Moi, P. (1998) Typing of nosocomial strains of *Serratia marcescens*: comparison of pulsed-field gel electrophoresis of macrorestriction fragments with biotyping, esterase typing, and ribotyping. *Res Microbiol* **149**: 137–143.
- Chun, J. (2001) PHYDIT version 3.1 [WWW document]. URL <http://plaza.snu.ac.kr/~jchun/phydit/>
- Farmer, J.J., Silva, F., and Williams, D.R. (1973) Isolation of *Serratia marcescens* on deoxyribonuclease-toluidine blue-cephalothin agar. *Appl Microbiol* **25**: 151–152.
- Fong, P., and Lirman, D. (1995) Hurricane caused population expansion of the branching coral *Acropora palmata* (Scleractinia): wound healing and growth patterns of asexual recruits. *Mar Ecol Prog Ser* **16**: 317–335.
- Gardner, T.A., Côté, I.M., Gill, J.A., Grant, A., and Watkinson, A.R. (2003) Long-term region-wide declines in Caribbean corals. *Science* **301**: 958–960.
- Gladfelter, W.B. (1982) White-band disease in *Acropora palmata*: implications for the structure and growth of shallow reefs. *Bull Mar Sci* **32**: 639–643.
- Grasso, G.M., D'Errico, M.M., Schioppa, F., Romano, F., and Montanaro, D. (1988) Use of colistin and sorbitol for better isolation of *Serratia marcescens* in clinical samples. *Epidemiol Infect* **101**: 315–320.
- Griffin, D.W., Gibson, C.J., III, Lipp, E.K., Riley, K., Paul, J.H., and Rose, J.B. (1999) Detection of viral pathogens by reverse transcriptase PCR and of microbial indicators by standard methods in the canals of the Florida Keys. *Appl Environ Microbiol* **65**: 4118–4125.
- Grober-Dunsmore, R., Bonito, V., and Frazer, T.K. (2006) Potential inhibitors to recovery of *Acropora palmata* populations in St. John, US Virgin Islands. *Mar Ecol Prog Ser* **321**: 123–132.
- Harvell, C.D., Kim, K., Burkholder, J.M., Colwell, R.R., Epstein, P.R., Grimes, J., et al. (1999) Emerging marine diseases – climate links and anthropogenic factors. *Science* **285**: 1505–1510.
- Hazen, T.C. (1988) Fecal coliforms as indicators in tropical waters: a review. *Toxic Assess* **3**: 461–477.
- Hejazi, A., and Falkiner, F.R. (1997) *Serratia marcescens*. *J Med Microbiol* **46**: 903–912.
- Hoegh-Guldberg, O. (1999) Climate change, coral bleaching and the future of the world's coral reefs. *Mar Freshw Res* **50**: 839–866.
- Hogarth, W.T. (2006) Endangered and threatened species: final listing determinations for elkhorn coral and staghorn coral. *Fed Regist* **71**: 26852–26861.
- Hughes, T.P. (1994) Catastrophes phase shifts and large-scale degradation of a Caribbean coral reef. *Science* **265**: 1547–1551.
- Kahali, S., Sarkar, B., Chakraborty, S., Macaden, R., Deokule, J.S., Ballal, M., et al. (2004) Molecular epidemiology of diarrhoeagenic *Escherichia coli* associated with sporadic cases and outbreaks of diarrhea between 2000 and 2001 in India. *Eur J Epidemiol* **19**: 473–479.
- Knowlton, N., Lang, J.C., and Keller, B.D. (1990) Case study of natural population collapse: post-hurricane predation on Jamaican staghorn corals. *Smithson Contrib Mar Sci* **31**: 1–25.
- Lapointe, B.E., O'Connell, J.D., and Garrett, G.S. (1990) Nutrient coupling between on-site sewage disposal systems, groundwaters, and near shore surface waters of the Florida Keys. *Biogeochemistry* **10**: 289–307.
- Lapointe, B.E., Barile, P.J., and Matzie, W.R. (2004) Anthropogenic nutrient enrichment of seagrass and coral reef communities in the Lower Florida Keys: discrimination of local versus regional nitrogen sources. *J Exp Mar Biol Ecol* **308**: 23–58.
- Lipp, E.K., Jarrell, J.L., Griffin, D.W., Jacukiewicz, J., Lukasik, J., and Rose, J.B. (2002) Preliminary evidence for human fecal contamination in corals of the Florida Keys, USA. *Mar Poll Bull* **44**: 666–670.
- Lipp, E.K., Futch, J.C., and Griffin, D.W. (2007) Analysis of multiple enteric viral targets as sewage markers in coral reefs. *Mar Poll Bull* **54**: 1897–1902.
- Lirman, D. (2003) A simulation model of the population dynamics of the branching coral *Acropora palmata*: effects of storm intensity and frequency. *Ecol Modell* **161**: 167–180.
- Lirman, D., and Fong, P. (1997) Patterns of damage to the branching coral *Acropora palmata* following Hurricane Andrew: damage and survivorship of hurricane-generated asexual recruits. *J Coast Res* **13**: 67–72.
- McLellan, S.L., Daniels, A.D., and Salmore, A.K. (2001) Clonal populations of thermotolerant Enterobacteriaceae in recreational water and their potential interference with fecal *Escherichia coli* counts. *Appl Environ Microbiol* **67**: 4934–4938.
- Maliao, R.J., Turingan, R.G., and Lin, J. (2008) Phase-shift in coral reef communities in the Florida Keys National Marine Sanctuary (FKNMS), USA. *Mar Biol* **154**: 841–853.
- Maragakis, L.L., Winkler, A., Tucker, M.G., Cosgrove, S.E., Ross, T., Lawson, E., Carroll, K.C., and Perl, T.M. (2008) Outbreak of multidrug-resistant *Serratia marcescens* infection in a neonatal intensive care unit. *Infect Control Hosp Epidemiol* **29**: 418–423.
- Marty, K.B., Williams, C.L., Guynn, L.J., Benedik, M.J., and Blanke, S.R. (2002) Characterization of a cytotoxic factor in culture filtrates of *Serratia marcescens*. *Infect Immun* **70**: 1121–1128.

- Mayor, P.A., Rogers, C.S., and Hillis-Starr, Z.M. (2006) Distribution and abundance of elkhorn coral, *Acropora palmata*, and prevalence of white-band disease at Buck Island Reef National Monument, St. Croix, US Virgin Islands. *Coral Reefs* **25**: 239–242.
- Milisavljevic, V., Wu, F., Larson, E., Rubenstein, D., Ross, B., Drusin, L.M., et al. (2004) Molecular epidemiology of *Serratia marcescens* outbreaks in two neonatal intensive care units. *Infect Control Hosp Epidemiol* **25**: 719–721.
- Miller, M.W. (2001) Corallivorous snail removal: evaluation of impact on *Acropora palmata*. *Coral Reefs* **19**: 293–295.
- Miller, M.W., Bourgue, A.S., and Bohnsack, J.A. (2002) An analysis of the loss of acroporid corals at Looe Key, Florida, USA: 1983–2000. *Coral Reefs* **21**: 179–182.
- Miranda, G., Kelly, C., Solorzano, F., Leanos, B., Coria, R., and Patterson, J.E. (1996) Use of pulsed-field gel electrophoresis typing to study an outbreak of infection due to *Serratia marcescens* in a neonatal intensive care unit. *J Clin Microbiol* **34**: 3138–3141.
- Muller, E.M., Rogers, C.S., Spitzack, A.S., and van Woesik, R. (2008) Bleaching increases likelihood of disease on *Acropora palmata* (Lamarck) in Hawksnest Bay, St. John, US Virgin Islands. *Coral Reefs* **27**: 191–195.
- Nobles, R.E., Brown, P., Rose, J.B., and Lipp, E.K. (2000) The investigation and analysis of swimming-associated-illness using the fecal indicator enterococcus in southern Florida's marine waters. *Fla J Environ Health* **169**: 15–19.
- Olive, D.M., and Bean, P. (1999) Principles and applications of methods for DNA-based typing of microbial organisms. *J Clin Microbiol* **37**: 1661–1669.
- Patterson, K.L., Porter, J.W., Ritchie, K.B., Polson, S.W., Mueller, E., Peters, E.C., et al. (2002) The etiology of white pox, a lethal disease of the Caribbean elkhorn coral, *Acropora palmata*. *Proc Natl Acad Sci USA* **99**: 8725–8730.
- Paul, J.H., Rose, J.B., Brown, J., Shinn, E.A., Miller, S., and Farrah, S.R. (1995a) Viral tracer studies indicate contamination of marine waters by sewage disposal practices in Key Largo, Florida. *Appl Environ Microbiol* **61**: 2230–2234.
- Paul, J.H., Rose, J.B., Jiang, S., Kellogg, C., and Shinn, E.A. (1995b) Occurrence of fecal indicator bacteria in surface waters and the subsurface aquifer in Key Largo, Florida. *Appl Environ Microbiol* **61**: 2235–2241.
- Paul, J.H., Rose, J.B., Jiang, S.C., Zhou, X., Cochran, P., Kellogg, C., et al. (1997) Evidence for groundwater and surface marine water contamination by waste disposal wells in the Florida Keys. *Water Res* **31**: 1448–1454.
- Paul, J.H., McLaughlin, M.R., Griffin, D.W., Lipp, E.K., Stokes, R., and Rose, J.B. (2000) Rapid movement of wastewater from on-site disposal systems into surface waters in the lower Florida Keys. *Estuaries* **23**: 662–668.
- Porter, J.W., Dustan, P., Jaap, W.C., Patterson, K.L., Kosmynin, V., Meier, O.W., et al. (2001) Patterns of spread of coral disease in the Florida Keys. *Hydrobiologia* **460**: 1–24.
- Precht, W.F., Bruckner, A.W., Aronson, R.B., and Bruckner, R.J. (2002) Endangered acroporid coral of the Caribbean. *Coral Reefs* **21**: 41–42.
- Quinn, N.J., and Kojis, B.L. (2005) Patterns of sexual recruitment of acroporid coral populations on the West Fore Reef at Discovery Bay, Jamaica. *Rev Biol Trop* **53**: 83–90.
- Reschef, L., Koren, O., Loya, Y., Zilber-Rosenberg, I., and Rosenberg, E. (2006) The coral probiotic hypothesis. *Environ Microbiol* **8**: 2068–2073.
- Rodríguez-Martínez, R.E., Banaszak, A.T., and Jordán-Dahlgren, E. (2001) Necrotic patches affect *Acropora palmata* (Scleractinia: Acroporidae) in the Mexican Caribbean. *Dis Aquat Organ* **47**: 229–234.
- Shi, Z.-Y., Liu, P.Y.-F., Lin, Y.-H., and Hu, B.-S. (1997) Use of pulsed-field gel electrophoresis to investigate an outbreak of *Serratia marcescens*. *J Clin Microbiol* **35**: 325–327.
- Sommerfield, P.J., Jaap, W.C., Clarke, K.R., Callahan, M., Hackett, K., Porter, J., et al. (2008) Changes in coral reef communities among the Florida Keys: 1996–2003. *Coral Reefs* **27**: 951–965.
- Stepberger, K., Walter, S., Claros, M.C., Spencker, F.B., Kiess, W., Rodloff, A.C., and Vogtmann, C. (2002) Nosocomial neonatal outbreak of *Serratia marcescens* – analysis of pathogens by pulsed-field gel electrophoresis and polymerase chain reaction. *Infection* **30**: 277–281.
- Sussman, M., Loya, Y., Fine, M., and Rosenberg, E. (2003) The marine fireworm *Hermodice carunculata* is a winter reservoir and spring-summer vector for the coral-bleaching pathogen *Vibrio shiloi*. *Environ Microbiol* **5**: 250–255.
- Sutherland, K.P., and Ritchie, K.B. (2004) White pox disease of the Caribbean elkhorn coral *Acropora palmata*. In *Coral Health and Disease*. Rosenberg, E., and Loya, Y. (eds). Heidelberg, Germany: Springer-Verlag, pp. 289–297.
- Sutherland, K.P., Porter, J.W., and Torres, C. (2004) Disease and immunity in Caribbean and Indo-Pacific zooxanthellate corals. *Mar Ecol Prog Ser* **266**: 273–302.
- Tenover, F.C., Arbeit, R.D., Goering, R.V., Mikelsen, P.A., Murray, B.A., Parsing, D.H., and Swaminathan, B. (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* **33**: 2233–2239.
- van der Vorm, E.R., and Woldring-Zwaan, C. (2002) Source, carriers, and management of a *Serratia marcescens* outbreak on a pulmonary unit. *J Hosp Infect* **52**: 263–267.
- Weil, E., Urreiztieta, I., and Garzón-Ferreira, J.G. (2000) Geographic variability in the incidence of coral and octocoral diseases in the wider Caribbean. *Proc 9th Int Coral Reef Symp, Bali, Indonesia* **2**: 23–27.
- Williams, D.E., and Miller, M.W. (2005) Coral disease outbreak: pattern, prevalence and transmission in *Acropora cervicornis*. *Mar Ecol Prog Ser* **301**: 119–128.
- Wilson, V.L., Tattford, B.C., Yin, X., Rajki, S.S., Walsh, M.M., and LaRock, P. (1999) Species-specific detection of hydrocarbon-utilizing bacteria. *J Microbiol Methods* **39**: 59–78.
- Woolhouse, M., and Gaunt, E. (2007) Ecological origins of novel human pathogens. *Crit Rev Microbiol* **33**: 231–242.
- Zubillaga, A.L., Marquez, L.M., Croquer, A., and Bastidas, C. (2008) Ecological and genetic data indicate recovery of endangered coral *Acropora palmata* in Los Roques, Southern Caribbean. *Coral Reefs* **27**: 63–72.