Human Pathogen Shown to Cause Disease in the Threatened Eklhorn Coral *Acropora palmata*

Kathryn Patterson Sutherland1*, Sameera Shaban1, Jessica L. Joyner2, James W. Porter3, Erin K. Lipp4

1 Department of Biology, Rollins College, Winter Park, Florida, United States of America, 2 Odum School of Ecology and Department of Environmental Health Science, University of Georgia, Athens, Georgia, United States of America, 3 Odum School of Ecology, University of Georgia, Athens, Georgia, United States of America, 4 Department of Environmental Health Science, University of Georgia, Athens, Georgia, United States of America

Abstract

Coral reefs are in severe decline. Infections by the human pathogen *Serratia marcescens* have contributed to precipitous losses in the common Caribbean elkhorn coral, *Acropora palmata*, culminating in its listing under the United States Endangered Species Act. During a 2003 outbreak of this coral disease, called acroporid serratioasis (APS), a unique strain of the pathogen, *Serratia marcescens* strain PDR60, was identified from diseased *A. palmata*, human wastewater, the non-host coral *Siderastrea siderea* and the corallivorous snail *Coralliophila abbreviata*. In order to examine humans as a source and other marine invertebrates as vectors and/or reservoirs of the APS pathogen, challenge experiments were conducted with *A. palmata* maintained in closed aquaria to determine infectivity of strain PDR60 from reef and wastewater sources. Strain PDR60 from wastewater and diseased *A. palmata* caused disease signs in elkhorn coral in as little as four and five days, respectively, demonstrating that wastewater is a definitive source of APS and identifying human strain PDR60 as a coral pathogen through fulfillment of Koch’s postulates. *A. palmata* inoculated with strain PDR60 from *C. abbreviata* showed limited virulence, with one of three inoculated fragments developing APS signs within 13 days. Strain PDR60 from non-host coral *S. siderea* showed a delayed pathogenic effect, with disease signs developing within an average of 20 days. These results suggest that *C. abbreviata* and non-host corals may function as reservoirs or vectors of the APS pathogen. Our results provide the first example of a marine “reverse zoonosis” involving the transmission of a human pathogen (*S. marcescens*) to a marine invertebrate (*A. palmata*). These findings underscore the interaction between public health practices and environmental health indices such as coral reef survival.

Citation: Sutherland KP, Shaban S, Joyner JL, Porter JW, Lipp EK (2011) Human Pathogen Shown to Cause Disease in the Threatened Elkhorn Coral *Acropora palmata*. PLoS ONE 6(8): e23468. doi:10.1371/journal.pone.0023468

Editor: Steve Vollmer, Northeastern University, United States of America

Received February 28, 2011; Accepted July 18, 2011; Published August 17, 2011

Copyright: © 2011 Sutherland et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This research was supported by Mote Marine Laboratory Protect Our Reefs grant POR-2008-23 (www.mote.org) to KPS and EKL, the Rollins College Student-Faculty Collaborative Research Program and Edward W. and Stella C. Van Houten Memorial Fund to S and KPS, and by a Rollins College Critchfield Research Grant (www.rollins.edu) to KPS. Additional partial support was provided by National Science Foundation (NSF) grants EF-1015032 to KPS and EF-1015342 to JWP and EKL as part of the joint NSF-National Institutes of Health Ecology of Infectious Disease program (www.nsf.org) and by the US Environmental Protection Agency South Florida Water Quality Protection Program (www.epa.gov) to JWP. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: kpsutherland@rollins.edu

Introduction

Once the most common coral in the Caribbean, elkhorn coral *Acropora palmata* was listed for protection under the United States Endangered Species Act in 2006 [1], largely due to a disease unique to this coral species (Fig. 1). This disease, termed white pox or acroporid serratioasis (APS), is caused by the bacterium *Serratia marcescens* [2]. *S. marcescens* is an opportunistic human pathogen, causing respiratory, wound and urinary tract infections, meningitis and pneumonia [3–10]. Human diseases caused by this bacterium have been associated with waterborne infections in tropical freshwater lakes [11], but are most often associated with nosocomial infections of neonates and immuno-compromised patients [12]. During a 2003 outbreak, a unique strain of *S. marcescens*, strain PDR60, was identified from both diseased *A. palmata* and untreated human sewage, suggesting a causal link between wastewater and APS [13]. In addition, strain PDR60 was isolated from potential vectors and reservoirs including the non-host corals *Siderastrea siderea* and *Solenastrea bonnioni* and the coral predatory snail *Coralliophila abbreviata* [13].

Koch’s postulates, the preferred method for proving disease causation in humans and wildlife, require inoculation of a pure culture of a suspected pathogen isolated from a diseased host onto an apparently healthy host followed by development of disease signs and resolution of the pathogen. Koch’s postulates have proven difficult to fulfill for a number of coral diseases due in part to difficulty isolating and identifying potential pathogens and growing these organisms in pure culture. To date, the postulates have been satisfied for only five coral diseases, including APS with strain PDL100 isolated from a 1999 outbreak [2,14]. Unlike strain PDR60, isolated from host and non-host corals and snails, strain PDL100 has been identified only from diseased *Acropora palmata* [13]. Here we hypothesize that strain PDR60 isolated from two distinct environments, one terrestrial (human wastewater) and one marine (APS-affected *A. palmata*, apparently healthy *Siderastrea siderea* and *Coralliophila abbreviata*) causes APS in *A. palmata*. To examine this hypothesis we conducted challenge experiments by inoculating eight isolates of *Serratia marcescens* representing three strains onto *A. palmata* fragments maintained in closed seawater aquaria. Our results confirm strain PDR60 as a coral pathogen...
through fulfillment of Koch’s postulates. These results are also consistent with the hypothesis that non-host corals and predatory snails may function as interepizootic reservoirs or vectors of the APS pathogen. Furthermore, we show that *S. marcescens* isolated from human wastewater causes APS in as little as four days, unequivocally verifying humans as a source of a marine invertebrate disease.

**Materials and Methods**

**Challenge Experiment Design**

Experiments were conducted in June 2009 at the Mote Tropical Research Laboratory (MTRL), Summerland Key, FL. Eleven aquaria (20 L) were placed in a wet-table raceway with flowing 20 μm filtered groundwater and filled with 10 μm filtered seawater. A pump was placed in each aquarium, approximately 1 cm below the water surface, to maintain aeration. The raceway was covered with a plastic tarp and this shading plus water circulating around aquaria maintained aquaria temperatures at 28°C. Aquaria containing seawater acclimated for 24 hours before corals were added.

Small *Acropora palmata* fragments (25 cm²) were collected from 19 apparently healthy colonies at Western Sambo Reef, FL. Prior to fragment collection, the surface mucopolysaccharide layer (SML) was removed from each of the 19 coral colonies using sterile needleless 20 cm³ syringes and placed in a cooler on ice. Coral fragments were placed in 1 gallon zip-lock plastic bags containing seawater and stored in a cooler without ice. SML and coral samples were transported to the MTRL. Within three hours of collection, SML samples were spread plated (100 μl) on MacConkey Sorbitol agar (Becton Dickinson, Franklin Lakes, NJ, USA) with colistin (200 U ml⁻¹; MP Biomedicals, Aurora, OH, USA) (MCSA) [15]. Inoculated MCSA plates were incubated overnight at 37°C.

The number of coral fragments collected per coral colony ranged from one to four (Table 1). Within three hours of collection, these fragments were distributed into aquaria, three fragments per aquarium, with the goal of limiting replication of fragments from any one colony in a single aquarium. For colonies from which more than one fragment was collected, this distribution of fragments allowed for genotype comparisons across treatments (Table 1). Clonal replication within an aquarium was measured in aquariums 4 and 10 (Table 2) to determine genotype response to a single
treatment. Coral fragments acclimated in seawater aquaria for 48 hours prior to commencement of inoculation experiments.

Challenge Experiment Inoculations

Test isolates used in the challenge experiments were collected between June 2002 and August 2006 from offshore coral reefs and wastewater in the Florida Keys (Table 3). These Serratia marcescens isolates were cultured and identified from environmental samples by Sutherland et al. [13] using a three-step method: culture on CSIA (characteristic of S. marcescens) followed by culture on DNase with Toluidine Blue agar by Sutherland et al. [13] using a three-step method: culture on MCSA followed by culture on DNase with Toluidine Blue agar 

Table 1. Acropora palmata colonies used in the challenge experiments.

| Colony | Number of Fragments | Tissue Loss | No Tissue Loss |
|--------|---------------------|-------------|---------------|
| 1      | 2                   | F1 Isolate 2 A. palmata | F1 Isolate 8 wastewater |
| 2      | 1                   | F3 Isolate 4 S. siderea | F1 Isolate 5 C. abbreviata |
| 3      | 1                   | F3 Isolate 2 A. Palmata | F1 Isolate 7 PDL100 |
| 4      | 3                   | F3 Isolate 2 A. Palmata | F2 Isolate 4 S. siderea |
| 5      | 3                   | F2 Isolate 4 S. siderea | F3 seawater control |
| 6      | 2                   | F3 vehicle control | F3 Isolate 3 A. palmata |
| 7      | 4                   | F1 Isolate 1 A. Palmata | F1 Isolate 4 S. siderea |
| 8      | 1                   | F1 seawater control | F2 E. coli control |
| 9      | 1                   | F1 vehicle control | F1 Isolate 3 A. Palmata |
| 10     | 2                   | F3 Isolate 5 C. abbreviata | F2 Isolate 7 PDL100 |
| 11     | 2                   | F3 Isolate 8 wastewater | F2 E. coli control |
| 12     | 1                   | F1 Isolate 6 wastewater | F1 Isolate 3 A. Palmata |
| 13     | 3                   | F3 Isolate 1 A. Palmata | F2 Isolate 5 C. abbreviata |
| 14     | 2                   | F3 Isolate 4 S. siderea | F3 seawater control |
| 15     | 1                   | F2 vehicle control | F1 E. coli control |
| 16     | 1                   | F1 E. coli control | F2 Isolate 3 A. Palmata |
| 17     | 1                   | F2 Isolate 3 A. Palmata | F1 seawater control |
| 18     | 1                   | F3 Isolate 6 wastewater | F2 seawater control |
| 19     | 1                   | F3 seawater control | F2 seawater control |

Number of fragments per A. palmata colony, the challenge for each fragment (control or inoculation with a Serratia marcescens test isolate), and the result of each challenge (tissue loss or no tissue loss) are included. Three coral fragments (F1, F2, F3) were used for each challenge.

doi:10.1371/journal.pone.0023468.t001

Data Collection

The number of days to tissue loss for each coral fragment was calculated from the first experimental inoculation (Table 2). When tissue loss was observed, the fragment was photographed and SML (containing necrotic coral tissue) was collected from the lesion margin using a sterile needle-less 20 cm syringe. SML was transferred to a sterile 15 ml plastic tube and centrifuged (5 min, 1000 × g) and supernatant discarded. The pellet was resuspended in ASW (500 ml) and spread plated (100 μl) in triplicate on MCSA. Inoculated MCSA plates were incubated overnight at 37°C. MCSA positive bacterial colonies, appearing pink to red on MCSA (characteristic of Serratia marcescens), were picked and plated onto DTC. 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), were streaked onto non-
selective TS agar and incubated overnight at 25°C for confirmation of pure colonies. Experiments were monitored several times daily. Partial water changes were conducted at least once daily and salinity was maintained at 35 (measured by refractometry). After 26 days experiments were concluded. All fragments remaining alive were apparently healthy. Each fragment was photographed and then SML was collected and processed as described above. At the conclusion of experiments, or after development of disease signs and examination of SML, each Acropora palmata fragment was fixed with Z-Fix concentrate (ANATECH, LTD, Battle Creek, MI, USA) diluted 1:4 with ASW and stored for future study.

Strain Typing of APS Pathogens
Isolates recovered from APS-affected Acropora palmata fragments in inoculation experiments and identified as presumptive Serratia marcescens by differential growth on MCSA and DTC were assayed with Serratia-specific PCR as described above and in Sutherland et al. [13] to confirm the identity of isolates as S. marcescens. PCR-confirmed S. marcescens were assayed with PFGE as described in Sutherland et al. [13]. Band patterns were used to determine relatedness between isolates in order to confirm that bacteria collected from APS lesions produced during inoculation experiments were the same strain of bacterium inoculated in each inoculation experiment, thereby fulfilling Koch’s postulates.

Results
Serratia marcescens were not identified from SML of any Acropora palmata fragments prior to use in challenge experiments or from any apparently healthy fragments remaining alive at the end of the

Table 2. Treatments and controls used in the challenge experiments.

| Aquarium | Coral Fragment | Coral Colony | Challenge | No. Days To Tissue Loss |
|---|---|---|---|---|
| 1 | F1 | 8 | Seawater Control | No Tissue Loss |
| | F2 | 19 | No Tissue Loss |
| | F3 | 5 | No Tissue Loss |
| 2 | F1 | 16 | E.coli Control | No Tissue Loss |
| | F2 | 11 | No Tissue Loss |
| | F3 | 13 | No Tissue Loss |
| 3 | F1 | 9 | Vehicle Control | 17 |
| | F2 | 15 | 15 |
| | F3 | 6 | 17 |
| 4 | F1 | 7 | Isolate 1 Acropora palmata | 10 |
| | F2 | 7 | 10 |
| | F3 | 14 | 9 |
| 5 | F1 | 1 | Isolate 2 A. palmata | 7 |
| | F2 | 7 | 5 |
| | F3 | 4 | 7 |
| 6 | F1 | 13 | Isolate 3 A. palmata | No Tissue Loss |
| | F2 | 17 | No Tissue Loss |
| | F3 | 6 | No Tissue Loss |
| 7 | F1 | 7 | Isolate 4 Siderastrea siderea | 19 |
| | F2 | 5 | 23 |
| | F3 | 2 | 19 |
| 8 | F1 | 3 | Isolate 5 Coralliophila abbreviata | No Tissue Loss |
| | F2 | 13 | No Tissue Loss |
| | F3 | 10 | 13 |
| 9 | F1 | 12 | Isolate 6 Wastewater | 4 |
| | F2 | 5 | 4 |
| | F3 | 18 | 4 |
| 10 | F1 | 4 | Isolate 7 PDL100 A. palmata | 19 |
| | F2 | 10 | 18 |
| | F3 | 4 | 19 |
| 11 | F1 | 1 | Isolate 8 Wastewater | 11 |
| | F2 | 14 | 12 |
| | F3 | 11 | 12 |

A total of eleven challenge experiments were conducted each within a single aquarium and each with three Acropora palmata coral fragments (F1, F2, F3). Coral colony of origin and number of days to tissue loss is included for each coral fragment. Treatments and controls for which there was no tissue loss are also noted.
doi:10.1371/journal.pone.0023468.t002
study. Seawater circulating around aquaria, seawater used for water changes in aquaria, and deionized water used to adjust salinity were all negative for *S. marcescens*. *Acropora palmata* fragments in the *E. coli*-plus-vehicle control, the seawater-alone control, and the isolate 3-plus-vehicle treatment aquaria remained apparently healthy and were maintained for the duration of the 26 day study. In the vehicle control aquarium, coral tissue loss was noted by day 15 (Table 2). Therefore, test inocula were considered virulent if disease signs occurred prior to day 15, or within 14 days of the first inoculation, and attenuated if disease signs developed after 14 days.

When disease occurred, the process began with the distinct smell of *Acropora palmata* mucus followed by decreased water clarity, presumably due to the release of mucus by the coral into the water. Seawater in the affected aquaria was changed two to five hours once these signs developed. Mucus release was followed by a tissue bleb adjacent the region of inoculation. Finally, the tissue lifted off the skeleton and was sloughed in stringy fragments. This process of disease progression, once begun, was completed within 24 hours.

Koch’s postulates were satisfied for *Serratia marcescens* strain PDR60 from APS-affected corals (isolates 1, 2). *Acropora palmata* fragments also developed APS signs when inoculated with two *S. marcescens* strains from wastewater [strain PDR60 (isolate 6) and strain WWI31 (isolate 8)] and with strain PDR60 from healthy non-host corals (isolate 4) and snails (isolate 5); however, individual strains showed varying degrees of pathogenic effect. Strain PDR60 from APS-affected corals and wastewater (isolates 1, 2, 6) caused disease signs within four to ten days (Table 2, Fig. 1). Strain WWI31 (isolate 8) from wastewater caused disease signs within 12 days. Isolate 4 (strain PDR60 from *Siderastrea siderea*) and strain PDL100 (isolate 7), were attenuated, and only caused disease signs at 23 and 19 days, respectively (Table 2, Fig. 1). PFGE analyses demonstrated that the original inocula were recovered from all APS lesions.

### Discussion

*Serratia marcescens* strain PDR60 isolated from wastewater and diseased *Acropora palmata* caused APS in as little as four days, confirming humans as a source of this disease. Although the scope of this study was limited to three replicate *A. palmata* fragments in a single aquarium for each treatment and each control, our results with strain PDR60 from wastewater, predatory snails, and non-host corals contribute to the understanding of the mechanisms of transmission of APS and of the factors that drive the emergence and maintenance of this marine epizoic.

**Table 3. Bacterial treatments inoculated onto the *Acropora palmata* fragments in the challenge experiments.**

| Isolate | Strain | Source of Collection | Location of Collection | Date of Collection |
|---------|--------|----------------------|------------------------|-------------------|
| 1       | PDR60  | APS-affected *Acropora palmata* coral | Rock Key                | July 2003         |
| 2       | PDR60  | APS-affected *Acropora palmata* coral | Grecian Rocks            | July 2003         |
| 3       | PDR60  | *Siderastrea siderea* coral           | Grecian Rocks            | July 2003         |
| 4       | PDR60  | *Coralliophila abbreviata* snail      | Long Key                | June 2002         |
| 5       | PDR60  | wastewater                | Sand Key                | August 2006       |
| 6       | PDR60  | wastewater                | Key West                | September 2003    |
| 7       | PDL100 | APS-affected *Acropora palmata* coral | Looe Key                | May 1999          |
| 8       | WWI31  | wastewater                | Key West                | September 2003    |

Eight isolates of *Serratia marcescens* representing three pulsed-field gel electrophoresis (PFGE) strains (PDR60, PDL100, and WWI31) were used. The source, location, and date of collection are included for each treatment. doi:10.1371/journal.pone.0023468.t003

Acropora palmata inoculated with strain PDR60 from the non-host coral *Siderastrea siderea* (isolate 4) and strain PDL100 from APS-affected host corals (isolate 7) showed an attenuated pathogenic affect (Fig. 1). These two isolates caused APS signs after the fragments in the vehicle control showed tissue loss. All other treatments that exhibited tissue loss, did so by day 15, two days before the vehicle control (Table 2). For this reason, results for isolates 4 and 7, may indicate that stressors other than *Serratia marcescens* infection contributed to tissue loss; however, the maintenance of the *E. coli*-plus-vehicle control and the isolate 3-plus-vehicle treatment for the duration of the 26 day study lends support to the pathogenic effects of isolate 4 and isolate 7. Results with isolate 4 indicate that *S. siderea*, and possibly other non-host corals (*e.g., S. siderea*) [13], may serve as a reservoir for the acropod pathogen in the reef environment. Previous work suggests that PDL100 is poorly adapted for survival in the marine environment, persisting in seawater for only 15 h [26], but survival and proliferation of this strain is enhanced when it is grown in *A. palmata* SML and *S. siderea* SML [26,27]. Our results indicate that PDL100 survived for at least 11 days in association with *A. palmata*; PDL100 was recovered from an APS lesion on day 19 of the experiment (Table 2), 11 days after the second experimental inoculation. *S. marcescens* resident in non-host SML may be transmitted to neighboring *A. palmata* by sloughing of SML or movement by *Coralliophila abbreviata* vectors [13].

*Coralliophila abbreviata* preys preferentially on *Acropora palmata* [28] and has been implicated in the transmission of an unknown disease of *Acropora cervicornis* [29]. Strain PDR60 from *C. abbreviata* (isolate 5) was virulent, but only in one of three fragments (Table 2). Colony 13, a colony for which genotype comparisons across treatments were conducted, did not develop APS signs when inoculated with isolate 5 (Table 2) or with any other treatment (Table 1). The limited virulence of isolate 5 may be due to the apparent APS resistance of colony 13 or to the specific origin of this isolate, collected in 2006 at Sand Key reef when no sign of white pox was apparent at that reef [13]. These experimental and field observations support the limited virulence of isolate 5, but virulence of this isolate against one *A. palmata* fragment is consistent with the hypothesis that *C. abbreviata* may function as an interepizooic reservoir of the APS pathogen or as a vector when snails that harbor the pathogen feed on host corals.

*Acropora palmata* inoculated with isolate 3 from APS-affected corals remained apparently healthy for the entirety of the experiment, indicating that either this isolate of strain PDR60 is non-virulent or that the corals on to which it was inoculated were resistant to APS. Evidence in support of the former conclusion is
that the three *Acropora palmata* fragments inoculated with isolate 3 were each a different genotype and all remained apparently healthy for the duration of the 26 day study (Table 2). The later conclusion is supported by the apparent APS resistance of colony 13 (Table 1). Observations of *A. palmata* populations suggest that colonies of this species that are resistant to APS may exist. Prevalence of white pox disease has declined in recent years coincident with declines in host coral populations. The most recent reported outbreaks of the disease occurred in the Florida Keys in 2005 [13] and 2009 (Fig. 1) and in St. John, US Virgin Islands in 2006 [30]. Lower disease prevalence may be due to the decimation of susceptible *A. palmata* populations during previous white pox epizootics [2,31], other disease outbreaks [32,33], or may be an indication of reduced pathogen virulence [34].

*Serratia marcescens* strain PDL100 exhibited attenuated virulence in this study, and this strain’s reduced pathogenic effect, combined with the virulence of strain PDR60 and the wastewater strain WWI31, raises the question of whether unique strains exist for each a different genotype and all remained apparently healthy for the duration of the 26 day study (Table 2). These results may explain variability in manifestation of APS lesions on *A. palmata* colonies in the field including time to infection, rate of tissue loss, and likelihood of recovery versus whole-colony death. Future studies will investigate host susceptibility and resistance and will evaluate genetic variation between *S. marcescens* isolates and strains confirmed to be pathogenic and those that do not cause disease or cause disease at a slower rate. There is a need to define key differences between *S. marcescens* strains (PDL100 and PDR60) as well as among isolates of the same strain (PDR60, e.g., isolates 1–6). Important differences may exist in their ability to adapt to marine conditions (i.e., ability to persist and/or grow in seawater) and in genetic differences leading to increased virulence. *S. marcescens* vary widely in their survival in seawater with strain PDL100 surviving for 15 h or less at 30°C [26] and marine-derived isolates of the PDR60 pathogen strain persisting for up to 20 days under the same conditions (KP Sutherland and EK Lipp unpublished data). Fitness of different *S. marcescens* strains in seawater may also shed light on the mode of APS transmission. For example, saltwater tolerant strains may be more likely to become persistent or even resident on the reef whereas less halo-tolerant strains may either die off before reaching the reef or may be responsible for discrete outbreaks over short time periods. Our data from microcosm studies suggest that PDL100 (isolated in 1999) follows the latter pattern [26] and is supported by the fact that this strain has never been re-isolated from the marine environment [13]. On the other hand, strain PDR60, which has been recovered from multiple marine sources [13], is highly persistent in marine waters (KP Sutherland and EK Lipp unpublished data), suggesting that this may be an endemic disease strain.

Human culpability in the demise of a threatened species necessitates an immediate response and supports ongoing mitigation to improve wastewater treatment in the Florida Keys, and elsewhere, in order to protect the health and biodiversity of coral reef ecosystems. Advanced wastewater treatment successfully removes *Serratia marcescens* to undetectable levels [13]. However, most wastewater in the Keys and wider Caribbean is not treated, but rather is disposed of through in-ground receptacles [35] within porous limestone substrate that permits leakage from these systems into near-shore waters [36]. Human fecal contamination of near-shore and off-shore coral reef environments has been clearly demonstrated in the Florida Keys [35–38] and elsewhere in the Caribbean [39] and is associated with waterborne disease in humans [40]. In response, the state of Florida passed legislation to improve water quality in the Florida Keys by requiring the upgrade of all wastewater facilities, including in-ground receptacles, to the best available technology or to advanced wastewater treatment at an estimated cost of $939 million [41].

Coral reefs are amongst the most critically endangered habitats on earth. Because population declines of *Acropora palmata* are caused, in part, by a human strain of the common fecal enteric bacterium, *Serratia marcescens*, our findings address not only environmental protection, but also the socioeconomic and socio-ecological determinants of coastal zone protection and the cost of wastewater treatment infrastructure. This study brings to light a disease system dynamic, from humans to wildlife, which is the opposite of the traditional wildlife-to-human disease transmission model. The passage of pathogens from wildlife to humans is well documented, but the movement of disease-causing microbes from humans to marine invertebrates has not been shown [42]. This “reverse zoonosis” is all the more interesting because it involves the jump of a pathogen from vertebrate to invertebrate and from terrestrial to marine. Furthermore, disease incidence or severity may increase with rising temperatures [26,43,44], reinforcing the importance of near-shore water quality under climate change scenarios. Given a reliance on tourism by most Caribbean countries and the widespread lack of consistent wastewater treatment in the region, the APS disease system is of great economic interest and public health concern to developing nations and has particular significance for sustainable development activities and coastal-zone carrying capacity studies world-wide, especially under changing climatic conditions.

**Acknowledgments**

We thank the Mote Tropical Research Laboratory, Summerland Key, FL for boat support, field assistance, and facilities. Research at Florida Keys National Marine Sanctuary was conducted under permit no. FKNMS-2009-050.

**Author Contributions**

Conceived and designed the experiments: KPS EKL JWP. Performed the experiments: KPS SS JLJ EKL. Analyzed the data: KPS SS EKL. Wrote the paper: KPS SS EKL JWP.

**References**

1. Hogarth WT (2006) Endangered and threatened species: final listing determinations for elkhorn coral and staghorn coral. Fed Register 71(89):26532–26561.
2. Patterson KL, Porter JW, Ritchie KB, Polson SW, Mueller E, 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.
3. Graber CD, Higgins LS, Davis JS (1965) Seldom-encountered agents of bacterial meningitis. JAMA 192: 956–960.
4. Magnuson EW, Elston HR (1966) Infections caused by non-pigmented *Serratia marcescens*. Ann Intern Med 65: 409–418.
5. Ringrose RE, McKown B, Felton FG, Barclay BO, Muchmore HG, et al. (1968) A hospital outbreak of *Serratia marcescens* associated with ultrasonic nebulizers. Ann Intern Med 69: 719–729.
6. Cabrera HA (1969) An outbreak of *Serratia marcescens* and its control. Arch Intern Med 123: 650–655.
7. Tillotson JR, Finland M (1969) Bacterial colonization and clinical superinfection of the respiratory tract complicating antibiotic treatment and pneumonia. J Infect Dis 119: 597–624.

8. Miranda G, Kelly C, Solorzano F, Lezanos B, Coria R, et al. (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.

9. Shi ZY, Lu PYF, Lin YH, Hu BS (1997) Use of pulsed-field gel electrophoresis to investigate an outbreak of Serratia marcescens. J Clin Microbiol 35: 323–327.

10. Grimont F, Grimont PAD (2006) The Genus Serratia. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E, eds. The Prokaryotes, vol. 6. New York: Springer. pp 219–244.

11. Hazen TC (1988) Fecal coliforms as indicators in tropical waters: a review. Tox Assess 3: 461–477.

12. Hejaai A, Falkiner FR (1997) Serratia marcescens. J Med Microbiol 46: 903–912.

13. Sutherland KP, Porter JW, Turner JW, Thomas BJ, Looney EE, et al. (2010) Use of pulsed-field gel electrophoresis and polymerase chain reaction. Infection 30: 277–281.

14. Sutherland KP, Porter JW, Torres C (2004) Disease and immunity in Caribbean Serratia marcescens. J Med Microbiol 53: 388–397.

15. Grasso GM, D’Errico MM, Schioppa F, Romano F, Montanaro D (1988) Use of 23S rDNA oligonucleotide probe for identification of Serratia marcescens. J Clin Microbiol 35: 325–327.

16. Hazen TC (1988) Fecal coliforms as indicators in tropical waters: a review. Tox Assess 3: 461–477.

17. Wilson VL, Tatford BC, Yin X, Rajki SS, Walsh MM, et al. (1999) Species-level identification of Serratia marcescens isolates by ribotyping. J Clin Microbiol 37: 3625–3630.

18. Miranda G, Kelly C, Solorzano F, Lezanos B, Coria R, et al. (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.

19. Alfizah H, Nordiah AJ, Rozaidi WS (2004) Using pulsed-field gel electrophoresis in the setting of Serratia marcescens infection outbreak. J Hosp Infect 55: 231–236.

20. van der Vorm ER, Woldring-Zwaan C (2002) Source, carriers, and transmission in Acropora corals. Environ Microbiol 4: 1122–1131.

21. Sutherland KP, Porter JW, Torres C (2004) Disease and immunity in Caribbean Serratia marcescens. J Med Microbiol 53: 388–397.

22. van der Vorm ER, Woldring-Zwaan C (2002) Source, carriers, and transmission in Acropora corals. Environ Microbiol 4: 1122–1131.

23. Steppberger K, Walter S, Claros MC, Spencker FB, Kiess W, et al. (2002) In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E, eds. The Prokaryotes, vol. 6. New York: Springer. pp 219–244.

24. Ligozzi M, Fontana R, Aldegheri M, Scalet G, Lo Cascio G (2010) Comparative evaluation of an automated repetitive-sequence-based PCR instrument versus pulsed-field gel electrophoresis in the setting of Serratia marcescens nosocomial infection outbreak. J Clin Microbiol 48: 1690–1695.

25. Bayramoglu G, Burak K, Dinc U, Muelu M, Yilmaz G, et al. (2011) Investigation of an outbreak of Serratia marcescens in a neonatal intensive care unit. J Microbiol Immunol 44: 111–115.

26. Looney EE, Sutherland KP, Lipp EK (2010) Effects of temperature, nutrients, organic matter and coral mucus on the survival of the coral pathogen, Serratia marcescens. PLoS ONE 119: 597–624.

27. Kriedt CJ, Ritchie KB, Cohen M, Lipp E, Sutherland KP, et al. (2009) Utilization of mucus from the coral Acropora palmata by the pathogen Serratia marcescens, environmental and communal correlates. Appl Environ Microbiol 75: 3851–3858.

28. Miller MW (2003) Coral fibroblast cell removal: evaluation of impact on Acropora palmata. Coral Reefs 19: 293–295.

29. Williams DE, Miller MW (2005) Coral disease outbreak: pattern, prevalence and transmission in Acropora corals. Mar Ecol Prog Ser 301: 119–128.

30. Muller EM, Rogers CS, Spitzaack AS, van Woestik R (2006) Bleaching increases likelihood of disease on Acropora palmata (Lamarrk) in Hawkemnest Bay St. John, US Virgin Islands. Coral Reefs 27: 191–195.

31. Rodriguez-Martinez RE, Banaszak AT, Jordan-Dahlgren E (2001) Necrotic patches affect Acropora palmata (Scleractinia: Acroporidae) in the Mexican Caribbean. Dis Aquat Org 47: 229–234.

32. Gladcicler VB (1982) White-band disease in Acropora palmata: implications for the structure and growth of shallow reefs. Bull Mar Sci 32: 639–643.

33. Aronson RB, Precht WF (2001) White-band disease and the changing face of Caribbean coral reefs. Hydrobiologia 460: 25–38.

34. Reschel L, Koron O, Loya Y, Gilbre-Rosenberg I, Rosenberg E (2006) The coral probiotic hypothesis. Environ Microbiol 8: 2068–2073.

35. Paul JL, Rose JB, Jiang SC, Zhou X, Cochran P, et al. (1997) Evidence for groundwater and surface marine water contamination by waste disposal wells in the Florida Keys. Water Res 31: 1449–1454.

36. Lapointe RE, O’Connell JD, Garrett GS (1990) Nutrient coupling between onsite sewage disposal systems groundwaters and nearshore surface waters of the Florida Keys. Biogeochemistry 10: 289–307.

37. Griffin DW, Gibson CJ, 3rd, Lipp EK, Riley K, Paul NL, et al. (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.

38. Futch JC, Griffin DW, Lipp EK (2010) Human enteric viruses in groundwater indicate offshore transport of human sewage to coral reefs of the upper Florida Keys. Environ Microbiol 12: 964–974.

39. Boussky M, Hernandez-Delgado EA, Sandoz B, Robledo IE, Norat-Ramirez J, et al. (2009) Detection of spatial fluctuations of non-point source fecal pollution in coral reef surrounding waters in southwestern Puerto Rico using PCR-based assays. Mar Poll Bull 58: 45–54.

40. Nobles RE, Brown P, Rose JB, Lipp EK (2000) The investigation and analysis of swimming-associated-illness using the fecal indicator enterococcus in southern Florida Keys. Environ Microbiol 2: 411–4125.

41. CH2MHILL (2000) Monroe County Sanitary Wastewater Master Plan. Monroe County, FL. Available: http://www.monroecounty-fl.gov/DocumentView.aspx?DID=477. Accessed: 2011 Aug 01.

42. Woodhouse M, Gaunt E (2007) Ecological origins of novel human pathogens. Crit Rev Microbiol 33: 211–242.

43. Harvell CD, Kin K, Burkholder JM, Colwell RR, Epstein PR, et al. (1999) Emerging marine diseases: climate links and anthropogenic factors. Science 285: 1505–1510.

44. Ritchie KB (2006) Regulation of microbial populations by coral surface mucus-associated bacteria. Mar Ecol Prog Ser 322: 1–14.