

# Diversity, Patterns, and Benefits of Lionfish Skin-associated Bacteria

## Diversidad, Patrones y Beneficios de la Flora Bacteriana Cutánea del Pez León

## Modèles, Diversités, et Bénéfices des Bactéries Dermatologiques Associé au Rascasse

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### EXTENDED ABSTRACT

Widespread success of lionfish throughout the Western Atlantic, Caribbean Sea, and Gulf of Mexico resulted in their persistence as an invasive species. Not only has the range of the lionfish expanded, but estimates predict the population density of lionfish in the invaded range to be approximately five times greater than that observed in their native range (Darling et al. 2011, Green and Côté 2009). In other instances of successful invasion, the invader is released from pathogen pressure but retains a close relationship with beneficial symbionts (Vermeij et al. 2005, Crowl et al. 2008). When an invasion occurs through the ornamental or pet trade, as is assumed to be the case with lionfish, these interactions with microbial symbionts are likely even stronger as only the healthiest, strongest individuals are selected (Padilla and Williams 2004). Therefore, we were interested in characterizing the diversity of bacterial communities associated with the skin surfaces of lionfish throughout their native and invaded ranges and assessing the potential roles of these bacteria in facilitating invasion.

Fish can acquire their associated bacteria vertically from a parent, horizontally through environmental transmission, or a combination of both mechanisms (Hansen and Olafsen 1989). Depending on the mode of transmission, it was possible that lionfish could introduce non-native microorganisms into the invaded range. To determine whether lionfish skin-associated bacterial communities were different than those of native Atlantic fishes, we compared the bacterial communities of lionfish to those of three species of native fishes (squirrelfish *Holocentrus adscensionis*, white grunt *Haemulon plumierii*, and lane snapper *Lutjanus synagris*). We used a genetic fingerprinting technique, terminal restriction fragment-length polymorphism (T-RFLP) analysis, and subsequent sequencing to characterize the communities. Lionfish supported a significantly different bacterial community than the three native fish species, and all fish hosted bacterial communities that were significantly different from the ambient bacterioplankton. Although lionfish skin-associated bacteria were different from bacterioplankton, inspection of lionfish eggs did not reveal the presence of bacteria, minimizing the possibility of vertical transmission. When the bacteria on lionfish and squirrelfish skin were identified through sequencing of the 16S rRNA gene, lionfish did not host any known opportunistic or pathogenic bacteria, while the native fish species hosted multiple pathogens. Because known pathogens were not identified and vertical transmission was unlikely, lionfish are probably not serving as vectors of disease.

Because host-associated microbial communities often reflect the biogeographical patterns of their hosts (Pinhassi et al. 2003, Martiny et al. 2006), we evaluated whether lionfish-associated bacteria from both the native and invaded ranges displayed this pattern. Based on genetic testing, it appears that Indonesia served as the source population for invasion. Although *P. volitans* is restricted to the Pacific Ocean and *P. miles* to the Indian Ocean, populations of both are found in Indonesia where the oceans meet (Kochzius and Blohm 2005, Kulbicki et al. 2012). In the Atlantic, two distinct populations of lionfish have been identified: a northern population with higher genetic diversity and a southern population with lower genetic diversity (Betancur et al. 2011, Butterfield et al. 2015). Lionfish fin clips were collected from nine locations within the invaded Atlantic and eight locations in the native Indo-Pacific. Bacterial communities from lionfish in the native and invaded ranges were not significantly different, indicating that lionfish retained a core bacterial community. Within this core community, we identified 17 operational taxonomic units that were found on 90% of lionfish in both ranges. Local-level effects, however, generated significant differences in bacterial communities based on collection location, but were not reflective of proposed genetic differences within lionfish populations.

Fish-associated microorganisms serve a variety of beneficial functions, including reducing drag (Bernadsky and Rosenberg 1992), aiding in digestion (Ganguly and Prasad 2012), and enhancing disease resistance (Chabrilón et al. 2005). Protection from disease can occur from direct (e.g., competition for space) and indirect (e.g., antibiotic production) mechanisms (Chabrilón et al. 2005). To identify potential roles of the lionfish skin-associated core bacteria, isolates were cultured from lionfish collected throughout both ranges and their ability to aid in disease resistance was tested against six strains of known fish pathogens. Of 149 bacterial isolates cultured from lionfish, 36% produced antibacterial metabolites effective against at least one of the pathogens tested. The taxonomic diversity of active isolates was similar between the native and invaded ranges, and included members of the core community identified previously. Gram-positive and gram-negative isolates demonstrated activity, indicating that individual lionfish likely host a variety of bacteria capable of

producing antibacterial metabolites against a suite of pathogens.

In summary, we have shown that lionfish retained a core beneficial bacterial community upon introduction to the invaded range that differs from the bacteria associated with native Caribbean fishes. This community appears capable of withstanding infection by known fish pathogens. Thus, the bacterial communities associated with lionfish likely contributed to the invasive success of lionfish over the last three decades.