

Ecology of pathogens

Studies on pathogens in the environment have been initiated relatively recently at Texas State University in collaboration with Dr. Michael Forstner (genetics, herpetology), Dr. Francis Rose (herpetology), Dr. Tim Bonner (ichthyology) and Dr. Ivan Castro-Arellano (wildlife ecology). Two different organisms are currently investigated:

- Bacteria of the genus *Salmonella*, a large group of enteric bacteria that are associated with a broad range of hosts including humans, and
- Fungi (*Batrachochytrium dendrobatidis*) that have been implicated as major contributor for significant declines in populations of amphibians worldwide.

Distribution, dissemination and fate of salmonellae in a pristine headwater ecosystem

Salmonellae are a large group of enteric bacteria with a broad range of hosts. Although not all cause disease, salmonellae are recognized worldwide as major zoonotic pathogens for both animals and humans. The majority of infections of humans with salmonellae result from exposure to undercooked animal products or cross contamination of vegetables, fruit and ready-to-eat products; however, salmonellosis can also result from contact with contaminated waters. Infection with salmonellae is often related to their presence in a broad range of animal reservoirs that includes invertebrates, birds, reptiles and mammals. The intestinal tract of vertebrates is assumed to be the native habitat of salmonellae, from which the feces then contaminate environments such as fresh- or marine waters, estuarine environments, vegetables, compost, or soils and sediments. The occurrence of salmonellae in these environments is therefore frequently linked to environmental contamination through, e.g. manure or wastewater discharges. Yet, this

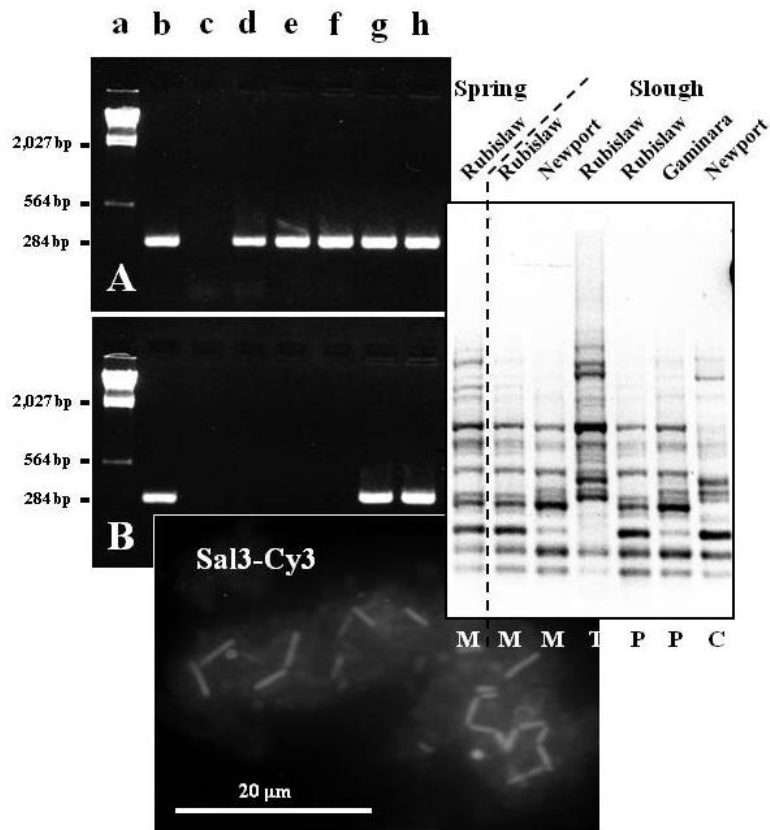
organism's ecology must be more complex than this scenario as salmonellae have been recovered from rivers and streams in remote areas without any significant human impacts and without any detectable impact from potential host animals.

Indeed, we now know that salmonellae released by animals in the vicinity of aquatic systems represent a potent non-point source of contamination for water and sediments when transported into the aquatic system by strong rainfall events and associated runoff. Non-point sources include agricultural run-off, contaminated soils surrounding the



system, and from fecal droppings from wildlife, cattle, goats and sheep. Released from animal reservoirs into the environment, salmonellae have been shown to survive, e.g. in cattle manure or in soils for time periods that exceeded a month. It is the overall ecosystem ecology of salmonellae that remains unknown. Our studies are aimed at refining the current paradigm that defines salmonellae as a contaminant to an updated version that includes salmonellae as an ecosystem component with concomitant consequences for recreational (e.g. swimming, diving, and fishing) and/or commercial users (e.g. municipal water and agriculture).

Using traditional enrichment culture techniques in combination with molecular detection and identification tools for salmonellae (i.e. polymerase chain reaction (PCR), rep-PCR and *in situ* hybridization), we demonstrated that salmonellae known to cause salmonellosis in humans were established members of the microbial community in, and on, free-living turtles in a supposedly clean habitat such as Spring Lake, the spring-fed headwaters of the San Marcos River, Texas. Salmonellae were detected in the cloacae as well as in biofilms on the carapace of about 50% of all turtles that included common musk turtles (*Sternotherus odoratus*), red-eared sliders (*Trachemys scripta elegans*), Texas river cooters (*Pseudemys texana*), and snapping turtles (*Chelydra serpentina serpentina*) however, could not be detected in water and sediment samples at the same sites, except after significant rainfall events.



Salmonellae were also found from the gut of ecologically distinct fishes (i.e. in largemouth bass (*Micropterus salmoides*), channel catfish (*Ictalurus punctatus*), common carp (*Cyprinus carpio*), and suckermouth catfish (*Hypostomus plecostomus*)). Detection of salmonellae was usually limited to one location of the gut, with serovars being highly variable among individuals, and normally associated with particulate material in the gut, in highly variable numbers ranging from an occasional cell to comprising the major population in these associations. These observations suggest that salmonellae are not components of the indigenous microbial community in fish guts, but are rather taken up with particulate material.

The detection of salmonellae on the carapaces of turtles that are generally covered with algal mats suggested that salmonellae can at least persist in a viable state on the body surface, most

likely in algal mats or biofilms. Analysis of the diversity and distribution of salmonellae in biofilms on concrete in Spring Lake, covered with water after prolonged drought conditions without any rainfall for about 3 months frequently detected them in enrichment cultures, with small-scale differences in occurrence and abundance. Replicate samples from the same site showed significant micro-heterogeneity with differences in detection, but also in diversity of salmonellae, suggesting the presence of different strains in a small sample.

***Batrachochytrium dendrobatidis* in amphibians of the Texas Hill Country**

During the last two decades significant declines and extinctions of amphibians have been observed worldwide. A substantial number of populations have suffered declines even in seemingly pristine habitats. These declines are often associated with the presence of a fungus, *Batrachochytrium dendrobatidis* that is now recognized as a serious contributor to amphibian declines in Australia and Europe as well as all of the Americas. Studies have indicated that *B. dendrobatidis* can have variable effects on amphibian populations with potential impacts ranging from persistence with no mortalities to mass die-offs without recovery. The prevalence and pathogenicity of *B. dendrobatidis* on host organisms can vary across different species, and developmental stages, and is affected by environmental factors such as temperature, precipitation, and pollutants.

During 2007, molecular tools were standardized and used to evaluate and document the presence of *B. dendrobatidis* in central Texas. *B. dendrobatidis* was detected on samples of Houston Toad (*Bufo houstonensis*) as well as on each of five species of salamanders, i.e. the Edwards Plateau (*Eurycea neotenes*), Fern Bank (*E. pterophila*), the threatened San Marcos (*E. nana*), the endangered Barton Springs (*E. sosum*), and the Jollyville (*E. tonkawae*) salamander which has recently been petitioned to be added to the endangered list. These tools are now used to monitor and study outbreaks of *B. dendrobatidis* in water and sediment samples, and to confirm the absence of *B. dendrobatidis* in Houston Toads released into the environment in a “headstarting program” (a project in collaboration with Dr. Forstner and the Houston Zoo).

Selected publications

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8. **Sha, Q., Forstner, M.R.J., Hahn, D.** 2013. Diversity of *Salmonella* in biofilms and water in a headwater ecosystem. *FEMS Microbiology Ecology* **83**, 642-649.
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10. **Gaertner, J.P., Brown, D.J., Mendoza, J.A., Forstner, M.R.J., Bonner, T., Hahn, D.** 2012. Geographic variation in *Batrachochytrium dendrobatidis* occurrence among populations of *Acris crepitans blanchardi* in Texas, USA. *Herpetological Review* **43**, 150-159.
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