Project Update: September 2015

To determine infection status and to avoid cross contamination, animals were collected using clean, decontaminated equipment, individually handled with fresh disposable gloves, and placed on individual bags prior to obtaining the skin swab samples. Each animal was sampled by running a sterile synthetic cotton swab (Medical Wire Equipment MW100) over the ventral surface, the inner thigh area and the plantar surface of the hind feet webbing for a total of 50 strokes. Skin swabs were preserved either in 96% ethanol or were air dried and stored at -20˚C until processed. Infection intensity (as zoospores equivalents) was determined through Real-time TaqMan PCR – qPCR. To determine if the microbial-associated community changes among life stages and between sympatric species that co-occur, we collected skin swabs from 17 tadpoles, 10 juveniles and 17 adults, we also took 10 samples of pond water. All individuals were collected using a dip net or a new plastic bag. Prior to sampling, individuals were rinsed twice with 50 mL of sterile water in order to remove transient microbes and sediments ensuring that samples correspond mostly to skin-associated bacteria. Whole community DNA was extracted from each of 54 samples using the Qiagen DNeasy blood & tissue kit (Valencia, CA, USA) following manufacturer's protocol slightly modified. We obtain 16S rRNA gene amplicons amplifying the V3 and V4 regions of the gene obtaining a single amplicon of approximately 460 bp. In total we have 54 samples to describe the skin microbiome using Ilumina MiSeq, and 24 samples to describe the culturable microbial community and to identify strains with antifungal activity. We are still processing the data from Illumina miSeq and performing the tests to determine bacteria with probiotic potential.


Pond where *Rheobates palmatus* and *Dendropsophus labialis* were surveyed. Photos by Laura A. Escobar