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A REASSESSMENT OF THE CONSERVATION STATUS OF A CRITICALLY ENDANGERED NEOTROPICAL FROG, *MANNOPHRYNE OLMONAE*, USING OCCUPANCY MODELING TECHNIQUES

Department of Biology

Senior Independent Study Thesis

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ABSTRACT

Amphibian species worldwide are threatened with decline and extinction, making species monitoring an important scientific endeavor. The Bloody Bay Poison Frog, *Mannophryne olmonae*, a Tobago island endemic, was identified as critically endangered by the IUCN in 2004. Recent evidence suggests that a less severe conservation status may be appropriate for *M. olmonae*. This study employs acoustic calling surveys, land-use information, and multi-year (2011 and 2012) occupancy modeling techniques to propose an appropriate conservation status for this species. This study suggests that *M. olmonae* occupies a larger geographic range than was previously thought, and is not experiencing population declines. These findings, in conjunction with other data, suggest that this species does not require the conservation status of critically endangered and should be re-classified as vulnerable.

INTRODUCTION

Conservation biologists are increasingly concerned with the issue of species loss and seek to preserve local, regional, and global biodiversity. A number of circumstances, such as habitat loss and fragmentation, global climate change, non-native species introduction, and human population density, contribute to what is arguably a sixth mass extinction- the Holocene extinction (Wake and Vredenburg, 2008; Santos *et al.*, 2006). Amphibians are the vertebrate group most devastated by these extinctions, with as many as 43% of species experiencing population declines, and therefore, in the greatest need of conservation assessment (Stuart *et al.*, 2004; Wake and Vredenburg, 2008). Amphibian populations are particularly sensitive to chemical pollutants, collection for trade, and increased ultraviolet radiation (Santos *et al.*, 2006; Crossland *et* al, 2005).

The tropics have a high biodiversity, especially in amphibians, as well as numerous rare species. Rare species, which are at higher risk of extinction than common species, are those with small population sizes and small geographic ranges. Additionally, habitat specialization and life history traits of these organisms often limit how quickly they can reproduce and disperse (Santos *et al.*, 2006). The tropics are continuously subject to extreme habitat alteration for agriculture and development. These factors cause a higher rate of extinction for amphibians in the tropics than any other place on earth.

Amphibians are also highly threatened by the emerging infectious aquatic disease chytridiomycosis, caused by the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Bd). Chytridiomycosis, a skin infection, is the probable cause of over 200 amphibian declines and extinctions worldwide (Johnson and Speare, 2003; Stuart *et al.*, 2004). Recent research shows that global temperature shifts and unusually warm years are directly linked with amphibian declines and the incidence of infectious diseases such as chytridiomycosis (Pounds *et al.*, 2006). As temperatures rise, the thermal optima for Bd may be reached in more areas (i.e. at higher latitudes or altitudes), leading to more amphibian declines and extinctions; this has been proposed as the ‘climate-chytrid paradox’ (Pounds *et al.*, 2006). The thermal optima for Bd, the temperature at which it grows best, is between 17-25 degrees Celsius (Piotrowsky *et al.*, 2004).

The International Union for Conservation of Nature (IUCN) is an organization dedicated to protecting nature and natural resources. They accomplish this goal in part by first identifying individual species and species groups that are at risk of extinction. The IUCN then assess how to best conserve these groups. The IUCN Red List assigns a conservation status- vulnerable, endangered, or critically endangered- on a global scale, based on a five major criteria. Criterion A requires a population reduction of between 30% (vulnerable) and 90% (critically endangered) over the previous three generations or ten years. Criterion B considers occupied range reduction and fluctuation based on the area of occupancy and habitat fragmentation. Criterion C assesses population size and fluctuation based on three subcategories: estimates of continued population decline, total number of mature individuals in a population, or fluctuations in the number of mature individuals. Criterion D is similar to criterion C; however, this criterion is met if fewer than 50 (critically endangered), 250 (endangered), or 1,000 (vulnerable) mature individuals compose the population. Lastly, criterion E assesses the probability of extinction in the wild within the next ten to 100 years (Baillie *et al.*, 2004). Only one of the above criteria must be met in order for a species to be classified as threatened (IUCN, 2012).

These assessments must be made over the entirety of the range in which a species exists; if a species is endemic to an island then the assessment would only take place on this island. For a species that occurs on a number of landmasses, an assessment must include all associated landmasses (IUCN, 2012). In order to make these assessments, researchers must determine with an appropriate level of certainty that the species is in danger of extinction. In some unique cases, such as a species that only occurs over a restricted range (i.e. small island endemics), less evidence may be used to assign a conservation status. Some of the most powerful assessment methods include multi-year population studies, ecological-niche factor analyses, and ecological and occupancy modeling techniques (IUCN Red List, 2012; Santos *et al.*, 2006; Novick, 2012).

In 2004, the Global Amphibian Assessment (GAA), a study of all described amphibian species and their conservation status based on the IUCN Red List criteria, was published (Stuart *et al*, 2004). During the preceding three decades, researchers noted that mass amphibian declines were occurring globally. Major declines were first noted in the United States, Australia, and Puerto Rico in the 1970's. The assessment of amphibian conservation is particularly difficult due to natural population fluctuations. Research shows that the majority of global amphibian declines are non-random in distribution, meaning that multiple species in a region may decline simultaneously. Many amphibian declines are linked to environmental issues, and climatic changes in temperature and precipitation patterns likely contributed to the decline of 2,454 amphibian species between 1980 and 2004 (Stuart *et al.*, 2004; Sodhi *et al.*, 2008).

Through the GAA, researchers determined that 43% of global amphibian species are experiencing some form of population decline and only 27% are confirmed as stable populations (Stuart *et al.*, 2004). A mere 0.5% of amphibian populations are experiencing a continuous upward population trend. In many cases, habitat alteration, destruction, and overexploitation can be blamed for these on-going declines. The processes threatening 22.5% of amphibians have yet to be determined due to data deficiencies and an inability to identify the threats. A total of 32.5% of amphibians have been assigned a conservation status of vulnerable or even more at-risk under the IUCN criteria. Only 12% of birds and 23% of mammals have been assigned a conservation status of vulnerable or higher (Stuart *et al.,* 2004). When comparing these three groups, it becomes clear that amphibians are in fact the most at-risk vertebrate group.

Of the at-risk amphibian species, 7.4% are considered critically endangered by IUCN standards. Between 1980 and 2004 the number of critically endangered amphibian species nearly doubled from 4% (Stuart *et al.,* 2004). The GAA separated species that are considered critically endangered into three categories based on the causes of their declines: overexploited, reduced-habitat, or enigmatic-decline (Stuart *et al.*, 2004). Roughly half (207) of all critically endangered species fall into the third category, which is described as having severe declines without any clear reason. These types of declines often happen in habitats that appear pristine or undisturbed, but new data indicate that climate change or disease infection may be the cause (Stuart *et al.,* 2004). For species suffering from enigmatic declines, there is currently no clear option for conservation other than through captive breeding programs (Stuart *et al.,* 2004). Identifying the cause of decline is necessary to implement conservation strategies in an amphibian’s natural habitat.

Recent studies suggest that Bd is often more abundant in primary, undisturbed forests than secondary forests (Becker and Zamudio, 2011). This may be the cause of some enigmatic declines, and two main hypotheses regarding the abundance of Bd in pristine habitats have been proposed. First, pristine habitats share water sheds with disturbed habitats, and, since Bd is a waterborne pathogen, it is able to move freely between habitats and within the pristine environment. Furthermore, habitat fragmentation can limit the movement of Bd within a disturbed environment (Becker and Zamudio, 2011). The second hypothesis assesses a connection between the Bd thermal optima and habitat fragmentation. Disturbed forests often lack canopy cover and facilitate a microclimate which is slightly warmer than the microclimate of pristine forests which have more canopy cover. This results in the thermal optima of Bd being maintained in pristine forests, while disturbed forests are slightly warmer than the thermal optima (Becker and Zamudio, 2011). This is particularly likely in tropical and neotropical regions where the macroclimate is often warmer than the Bd thermal optima (Becker and Zamudio, 2011).

The GAA identified 122 possibly extinct amphibian species, 113 of which have disappeared since 1980. Only 34 species (including none of the above 122) have been confirmed extinct since the year 1500, which is largely due to the difficulty of proving that an animal no longer exists anywhere in nature (Stuart *et al.*, 2004). The GAA has estimated that between 9 and 122 amphibian species have gone extinct since 1980, but the number of extinctions cannot be confirmed without large-scale surveys (Stuart *et al.*, 2004).

Among the amphibian species have been identified as critically endangered is the frog *Mannophryne olmonae*- the Bloody Bay Poison Frog (Hardy, 2004). *Mannophryne olmonae* is an endemic species to the island of Tobago, off the coast of Venezuela. During his 2004 assessment of *M. olmonae* for the IUCN Red List, Hardy stated that the species had likely declined by 80% during the prior three generations, had a fragmented habitat, and could potentially be a victim of chytridiomycosis. Recent studies suggest that the population number and range of *M. olmonae* are likely not declining, since it has been determined that it occupies more of the island than was thought in 1997 (Murphy, 1997; Novick, 2012). Studies also show the species is having reproductive success, since is clear from the high number of juveniles observed in the population (Calkins, 2012). Alemu *et al*. (2008) identified Bd in 29.7% of *M. olmonae* tested, however no dead or dying frogs were observed during their study or by any other researchers studying amphibians in Tobago.

Ecological modeling

Ecological modeling is a tool that was developed to assess the habitat requirements of species and ecological relationships on multiple levels (Crossland *et al.,* 2005; Hernandez *et al.,* 2006; Breckling and Muller, 1994; Olson *et al.*, 2005). Ecological data is often imprecise due to variation between individuals and populations of a species (Breckling and Muller, 1994). Ecological modeling is able to accommodate this variation by using mathematical formulas to detect biologically relevant trends (Breckling and Muller, 1994; Crossland *et al.*, 2005; Jimenez-Alfaro *et al.,* 2012). As it becomes clearer that ecological relationships are affected by human environmental changes, models are being designed that also accommodate environmental data. The results from various forms of ecological modeling can be used to develop environmental and conservation protection and management plans (Breckling and Muller, 1994).

Ecological modeling can utilize data indicating how land is being used when assessing the habitat requirements of a species (Santos *et al*., 2006). Over 20% of terrestrial habitats have been converted to human applications, such as industry, agriculture, and urban development, globally (Ribeiro *et al.,* 2009). Urban land-use, road density, and habitat fragmentation have all been shown to affect species richness in a habitat (Lehtinen *et al.,* 1999). Land-use information is particularly important because the quality, structure, and composition of the landscape can affect wildlife on numerous ecological levels (Ribeiro *et al.,* 2009). Land-use can affect soil structure and cause erosion, alter water quality and movement, and disrupt food-chains by altering the flora and fauna assemblages of a habitat (Ribeiro *et al.,* 2009; Lehtinen *et al.,* 1999). These relationships should be considered when developing regional conservation plans (Lehtinen *et al.,* 1999).

Distribution modeling, a type of ecological modeling, provides accurate results regarding species with limited geographical ranges and environmental tolerance- rare species (Hernandez *et al.,* 2006). Distribution modeling is able to predict the ecological requirements of such species, and provide information about how climate change and habitat alteration may affect them (Hernandez *et al.,* 2006). Using the output from these models, researchers can produce a map of all areas, within the geographic range of a species, that meet the habitat requirements for this species. These models can also help identify potential locations for reintroduction efforts if the species is being transplanted from nature or in a captive breeding program (Hernandez *et al.,* 2006).

Occupancy modeling is another type of ecological modeling. This technique uses naïve data regarding the proportion of focal sites at which a given species is observed to determine the likelihood of not detecting the species when it is in fact present, or a false negative (Pearl *et al.*, 2009). A naïve data set is one that conveys presence or absence of a species based only upon the detection of the species on multiple visits, but does not correct for false negatives. This technique has been used to assess mammal, bird, invertebrate, and, most recently, amphibian species (Moritz *et al.,* 2008; Olson *et al.*, 2005; Tyre *et al.,* 2001;Crossland *et al.,* 2005).

When assessing a number of associated covariates in relation to these naïve presence data, occupancy models can provide information about which covariate or combination of covariates significantly affects the probability of detection of a species. Detection probability is the likelihood that a species will be observed (i.e. heard, seen, tracked, etc.) during the sampling period (Crossland *et al.,* 2005). Additionally, a number of covariates can be assessed to determine the likelihood that a given site is occupied irrespective of detection. These are variables that remain constant for a site during and between seasons, such as longitude and latitude, altitude, or distance from the ocean. If only one survey is done per site, the detection and occupancy probabilities are confounded, making them equal (Crossland *et al.,* 2005). In contrast, with the use of data from multiple years, occupancy models can give robust information about the population trends (i.e. declining or increasing) of a given species (Novick, 2012; Pearl *et al.*, 2009).

Occupancy modeling is a relatively new technique that has not yet been globally applied and has not been used extensively in the tropics. Occupancy modeling is an important tool for managing and conserving amphibian species in particular (Crossland *et al.*, 2005). The robust estimates of occupancy and detection probability produced by these models can provide valuable information regarding the status of declining species, including those amphibian species that are most at-risk (Pearl *et al.*, 2009). These types of models are often able to produce reliable information about the distribution and habitat requirements of such species with only limited sampling effort, making these tools both cost and time effective forms of conservation management (Crossland *et al.*, 2005). They are particularly effective because they produce an estimate of the number of sites occupied in an area instead of the number of individuals in a population. This means that researchers need to only find evidence of a single individual (which likely indicates that more individuals are present) instead of searching an entire area for as many individuals as possible (Crossland *et al.,* 2005).

A set of four assumptions are made in occupancy modeling. First, sites must not change in occupancy during the survey period. Secondly, the focal species must not be detected at the site when it is absent (false positive), thus researchers must be analyzing cues distinct to that species. Next, species detection at any site must be independent of all other sites. In the case of calling surveys, sites that overlap in acoustic range cannot be used. Finally, there must be an equal likelihood of detection at all sites and detection probabilities determined from sites where the species is present should be extendable to sites where the species was never detected (Crossland *et al.,* 2005).

Tobago

Tobago is a small island in the Caribbean Sea, roughly 300 km^2, located 36 kilometers northeast of Trinidad and 115 kilometers off the coast of Venezuela. At the interior of the island is a mountainous region called the Main Ridge, which includes the highest elevation on the island, 549m above sea level (Murphy, 1997).

The native flora is very similar to the flora of northern continental South America and nearby Trinidad due to several periods during which the three land masses were connected. Prior to colonization by humans, Tobago was covered by four main forest types: mangrove forest, littoral forest, seasonal forest, and rainforest (Beard, 1944). The mangrove forests were the nearest to the ocean and grew in swampy areas. The mangrove forests were largely composed of ferns and mangrove trees (Beard, 1944). The littoral forests fell slightly inland of the mangrove swamps and were composed of relatively short, fleshy plants with leaves that could withstand salt from the constant ocean spray. Some of the littoral vegetation still grows on cliffs around the coast (Beard, 1944). The seasonal forests no longer exist on mainland Tobago, but an analysis of the nearly Little Tobago island indicated that these forests were likely separated into an upper and lower deciduous canopy (Beard, 1944). The most inland forest is the rainforest, which is most abundant on the Main Ridge. The rainforest is made of a 40 to 60 foot canopy and an understory largely composed of palms (Beard, 1944).

The majority of the vegetation found on Tobago has been disturbed by humans for agricultural or urban use, save for the Tobago Forest Reserve (Beard, 1944; R. M. Lehtinen, personal communication, Feb. 28, 2012). In preparation for cultivation of the land into sugarcane and, eventually, cocoa plantations, most of the coastal swamps were drained and mangrove forests are now limited. In addition to the draining of the wetlands, settlers also destroyed the majority of the native low-land seasonal forests in search of cultivatable land (Beard, 1944). Consequently, non-native bamboo has invaded much of the island and can be found in nearly all disturbed forest (Murphy, 1997).

Study species

Mannophryne olmonae, or the Bloody Bay Poison Frog, is an Aromobatid (formerly Dendrobatid) frog endemic to the island of Tobago (Manzanilla *et al.*, 2009). Despite the common name, *M. olmonae* is not poisonous; Aromobatid frogs are the non-toxic, close relatives of the toxic Dendrobatid frogs (Lehtinen and Hailey, 2008; Murphy, 1997). This frog is largely terrestrial but typically remains within ten meters of a stream or pool of water (Alemu *et al.*, 2007). It is active during the day (diurnal) between the hours of 0600 to 0800h and 1500 to 1850h (Alemu *et al.*, 2007; Murphy, 1997).

Mannophryne olmonae tends to lay few eggs (11-19) compared to other amphibian species. After the tadpoles (ovoid in shape and colored dark brown) hatch, the males carry them on their backs to be distributed in safe bodies of water where the tadpoles can metamorphose (Alemu *et al.*, 2007; Murphy, 1997; Hardy, 1983; Lehtinen and Hailey, 2008). Tadpoles are not typically distributed directly into a river or other main body of water, but are instead deposited into small streams or pools, shallow rock crevices, or, sometimes, tire ruts that are filled with water (Alemu *et al.,* 2007; Lehtinen and Hailey, 2008). Some deposition locations hold high densities of tadpoles that are often of different developmental stages, suggesting that multiple clutches are deposited in a single location by multiple males (Lehtinen and Hailey, 2008). Physical characteristics of the tadpole deposition location are not significantly different from one another; it appears that pools are chosen based on a lack of predators compared to the main streams and rivers (Alemu *et al.,* 2007).

This frog is small in size when mature, maximum size 25.7 mm, and is distinguishable by its yellow throat (if female) or grey throat (if male) and black throat band (Alemu *et al*., 2007). Males turn black when calling to females and quickly return to their normal coloration when calling ceases (Murphy, 1997). The range of *M. olmonae* has been accepted as tributaries of all main rivers in the northeastern portion of Tobago, but a recent study shows that the range is larger than that noted in 1997 by Murphy (Novick, 2012; Alemu *et al.,* 2007). When immature, it is difficult to distinguish males from females- all juveniles have a yellow throat band- until they reach a critical size of 18.5 mm or larger (Alemu *et al.,* 2007). Males tend to call only in small groups, four or fewer individuals, but it is often difficult to distinguish how many males are calling due to their call- a prolonged series of individual peeps (Alemu *et al.,* 2007; Lehtinen *et al.,* 2010). Calling males tend to be positioned adjacent to streams and rivers in the forested vegetation lining these waterways (Alemu *et al.,* 2007).

Mannophryne trinitatis, a closely related Trinidadian species, and *M. olmonae* were distinguished as separate species based on face mask markings and other morphological features in 1983 (Hardy), but Murphy (1997) challenged this distinction. Murphy (1997) claimed that the morphological features used by Hardy to distinguish these two species have high variation within both populations, indicating that the frog populations are actually very similar and could potentially be a single species. However, more recent analysis of mitochondrial genes and chromosome banding, has since determined that these are two independent species, *M. olmonae* being endemic to Tobago, while *M. trinitatis* is endemic to Trinidad (Lehtinen *et. al.,* 2010; Kaiser *et al.,* 2003).

In 2012, Calkins found a negative correlation between the body condition of *M. olmonae* and forest canopy cover. Body condition is an indicator of the health of an animal and can be measured by comparing the weight of an animal to the length (Karraker and Welsh, 2006). Forest canopy cover can be used as a proxy measurement for whether a forest is undisturbed (primary) or disturbed (secondary) (Calkins, 2012). In places with greater canopy cover, such as the old growth primary forest in the Tobago Forest Reserve, frogs exhibited poorer body condition than in secondary forest. This suggests that *M. olmonae* uses secondary forests as a habitat and is successful in this environment. The Calkins study also found that populations were juvenile biased, a trend that was also observed in 2012. This population structure- more juveniles than adults-suggests that *M. olmonae* is continuously experiencing reproductive success. This finding may be biased due to the timing of the survey during the mating season, because tadpoles and recent metamorphs that may not reach maturity are expected to be in higher abundance than outside the mating season (Calkins, 2012).

The presence of non-native bamboo in secondary forests has been hypothesized to be a cause of potential declines in *M. olmonae* (Hardy, 2004; R. M. Lehtinen, personal communication, Feb. 21, 2013; Vanko, 2012). Previous studies found that the coloration of *M. olmonae* correlates with the substrate color in the immediate vicinity of the population (Lehtinen, unpublished data). Populations existing on different substrates exhibit different coloration patterns (Lehtinen, unpublished data). Non-native bamboo changes the leaf litter color to a light, tan shade rather than the dark colors typically found in Tobago forests (R. M. Lehtinen, personal communication, Feb. 21, 2013). In an attempt to identify potential relationships between bamboo and *M. olmonae* coloration, Vanko analyzed the coloration of populations found near large quantities of bamboo litter and in the absence of bamboo litter (2012). This study found that there was no measurable difference in the coloration of the frog when bamboo was present and suggests that the bamboo does not affect the coloration of the frog (Vanko, 2012). This trend may be due to the short time (about 150 years) that the bamboo has been present in Tobago compared to the millions of years of evolutionary history during which time this species was exposed to the rock substrate (Lehtinen, unpublished data). This study provides further support that the secondary forest is a suitable habitat for *M. olmonae*.

Despite the current conservation status of *M. olmonae* (critically endangered), recent studies indicate that the species is not on the brink of extinction, and its conservation status is currently being re-assessed (Novick, 2012; Calkins, 2012; Patrikeev, unpublished data). My study provides information on the distribution of *M. olmonae* and assesses potential temporal changes in populations. These results can be informative about which conservation status would be most appropriate for this species and how land-use, in the form of percent forest cover, may be affecting the distribution or success of this frog.

Methods

Formal chorusing surveys

A chorusing survey was performed between July 28, 2012 and August 3, 2012 at 34 sites in Tobago that were chosen and surveyed by Novick in 2011 (Figure 1; Appendix 1; site 24 was excluded from the current survey). These dates correspond with the rainy season which is the mating season for *M. olmonae.* Site selection was based upon two main criteria: 1). Ease of access from the main roads in Tobago 2). Size of stream (i.e. very large rivers were not sampled). Additionally, the distance between sites was determined by Novick to be sufficient to minimize pseudoreplication (Novick, 2012). Six major river drainage basins were represented in the surveyed sites. Nine of the 34 sites were not within the previously recorded known range of *M. olmonae*; however recent studies suggest that *M. olmonae* may occupy more of Tobago than noted previously (Hardy, 2004; Murphy, 1997; Novick, 2012).

The chorusing survey was conducted between the afternoon peak calling hours of 1500 and 1830h to increase chances of detecting frogs if present (Alemu *et al.*, 2007). Chorus strength (amphibian-calling index; ACI) was rated on a subjective four-point scale as follows (Dorcas *et al.*, 2010):

0 = no frogs calling
1 = at least one calling frog with no call overlap
2 = multiple calling frogs with some call overlap
3 = full chorus of frogs with constant call overlap

Upon arrival, Dr. R. Lehtinen and I (hereafter referred to as surveyors) waited for a one minute acclimation period before beginning the listening survey (Dorcas *et al.,* 2010). Typically, acoustic calling surveys are conducted for five minutes in order to increase the likelihood of detection (Dorcas *et al.,* 2010). Due to the unique call of *M. olmonae*, a long, continuous peeping, each site was surveyed for two minutes, allowing for a maximum number of surveys to be conducted (Novick, 2012). In addition to the ACI being recorded for each site visit, barometric pressure (millibars), wet bulb temperature (°C), humidity, air temperature (°C) were collected from a Kestrel 4000 instrument that had been placed in a shaded area at stream level during the calling survey. Wind speed (km/h) was measured at shoulder height at road level by a second Kestrel 4000 instrument (Novick, 2012). Stream width was only measured if the species was detected from an associated tributary of the main stream and all other stream widths were considered constant with the 2011 measurement. The extent of rain (none, light, heavy), time of day, and date for each survey were also recorded for analysis.

Each site was visited two (N=12) or three (N=22) times for a total of 90 visits in order to reduce the likelihood of detecting a false negative. Most sites were visited on different days, however due to unforeseen circumstances some sites (N=15) were visited multiple times in the same day. At sites where *M. olmonae* was not detected on one of the earlier visits, special effort was made to visit them a maximum number of times to decrease the chance that this was not a case of false negatives. The calling surveys all occurred within a sufficiently short time span to assume that if frogs were present during one of the three surveys, they were living at that location and no colonization or local extinction events occurred during the sampling period (seven days) (Novick, 2012).

Statistical analysis was conducted on data from 2011 and 2012. The same covariates were collected in both years using the same methods. One surveyor, Dr. Lehtinen, was consistent across both years and a consensus model was used when determining the presence or absence of *M. olmonae*.

Informal surveys

A total of six informal surveys were conducted on August 3, 2012 during the afternoon peak calling hours in order to further our understanding of the range of this species. While outside the formal survey area, surveyors listened for the call of *M. olmonae* from the Leeward Road. GPS coordinates were recorded for all streams at which the frog was present and no other variables were collected. Each site was only visited once, surveyors did not listen for a prescribed amount of time, and no acclimation period was practiced prior to listening.

Forest cover data

Percent forest cover was determined using a novel procedure that combined the modeling capacity of the program SketchUp and imaging data available through Google Earth Pro. Forest cover was obtained by visually assessing the most recent available imaging (1969-2008) data available through Google Earth Pro. For more detailed information about forest cover data, see Appendix 1.

Occupancy modeling

Using the program PRESENCE 5.3 (available at: http://www.mbr-pwrc.usgs.gov/software/presence.html), naïve presence-absence data and all covariates were analyzed. PRESENCE is a program designed to run occupancy models to determine occupancy (ψ) and detection (p) probabilities, as well as colonization (γ) and extinction (ε) statistics for either single or multi season surveys. These model types determine the likelihood of false negatives in detection and correct for them to produce robust estimates of occupancy. Using the model's Akaike's information criterion (ACI) and ΔACI, the program can organize models to determine which set of covariates can most significantly explain the likelihood of occupancy. All models within 2.0 ΔACI of the best model are considered to have equal explanatory power. Models are penalized for the number of covariates (K) used, thus simpler models are likely to have a smaller ACI than more complex models. The AIC weight is also determined for each covariate and can be used to determine the explanatory power of that particular covariate.

Due to the overwhelming number of covariate combinations possible when analyzing nine sampling covariates (and two potential quadratic relationships; n=11) and four site covariates, a null model can be a useful analysis tool. By determining the model performance (ACI; ΔACI) of each covariate individually compared to a model using no covariates, poorly performing covariates can be discarded from further analysis. This null model concept was used to analyze all covariates for this study.

All models were run using the "Init occ, local colonization, extinction, detection" option in the multi-season analysis pack. A null model was run and the AIC was compared to a model for occupancy (ψ) containing one covariate. This was compared for each of four covariates (longitude, latitude, stream width, percent forest cover). Stream width was not recorded for two sites, 20 and 22, and approximate measures were used for these sites. PRESENCE is unable to run models containing missing data for site covariates and, in the absence of these approximate measures, would have provided the option to remove stream width from the analysis or to remove sites 20 and 22. In order to maintain as many sites as possible in the analysis, it was determined that proxy measures would be appropriate.

The same procedure was conducted for all models containing sample covariates (year, date, time, rain during, wet bulb temperature, barometric pressure, air temperature, wind speed, humidity) to determine detection probability (P). As no models for ψ or P were more explanatory than the null model, no combinatory models were conducted. No covariates were collected for the specific analysis of γ or ε, however PRESENCE provides an output for these measures.

Results

A total of 38 populations of *M. olmonae* were identified through formal and informal sampling in 2011 and 2012 (Figure 1; Novick, 2012). The majority of these populations (N=24) were found inside the known range, but 14 populations were found farther west (Figure 1; Novick, 2012). Six populations were identified inside the Tobago Forest Reserve (Figure 1; Novick, 2012). Through formal surveys, four populations were identified in 2011 that were not found in 2012 (Figure 1; Novick, 2012). Conversely, in 2012 three populations were identified through formal surveys that were not found in 2011 (Figure 1). Finally, through informal surveys nine new populations were found west of the known range (2011 N=3; 2012 N=6; Figure 1; Novick, 2012). The western-most population was located at 11° 16.253 north and 60° 42.091 west, which is 4° 0.24 west of the known range for *M. olmonae* (Figure 1; Murphy, 1997). This represents a western range expansion of 59% compared to the previously known range (Murphy, 1997).

The sites measured during formal surveys exhibited a narrow range for each of the site covariates (Table 1). The range of climate dependent sampling covariates measured in 2012, such as air and wet bulb temperature, was variable and consistent with the known climate of Tobago (Table 1). Rain was not encountered as often as expected for a neotropical island during the rainy season (Table 1).

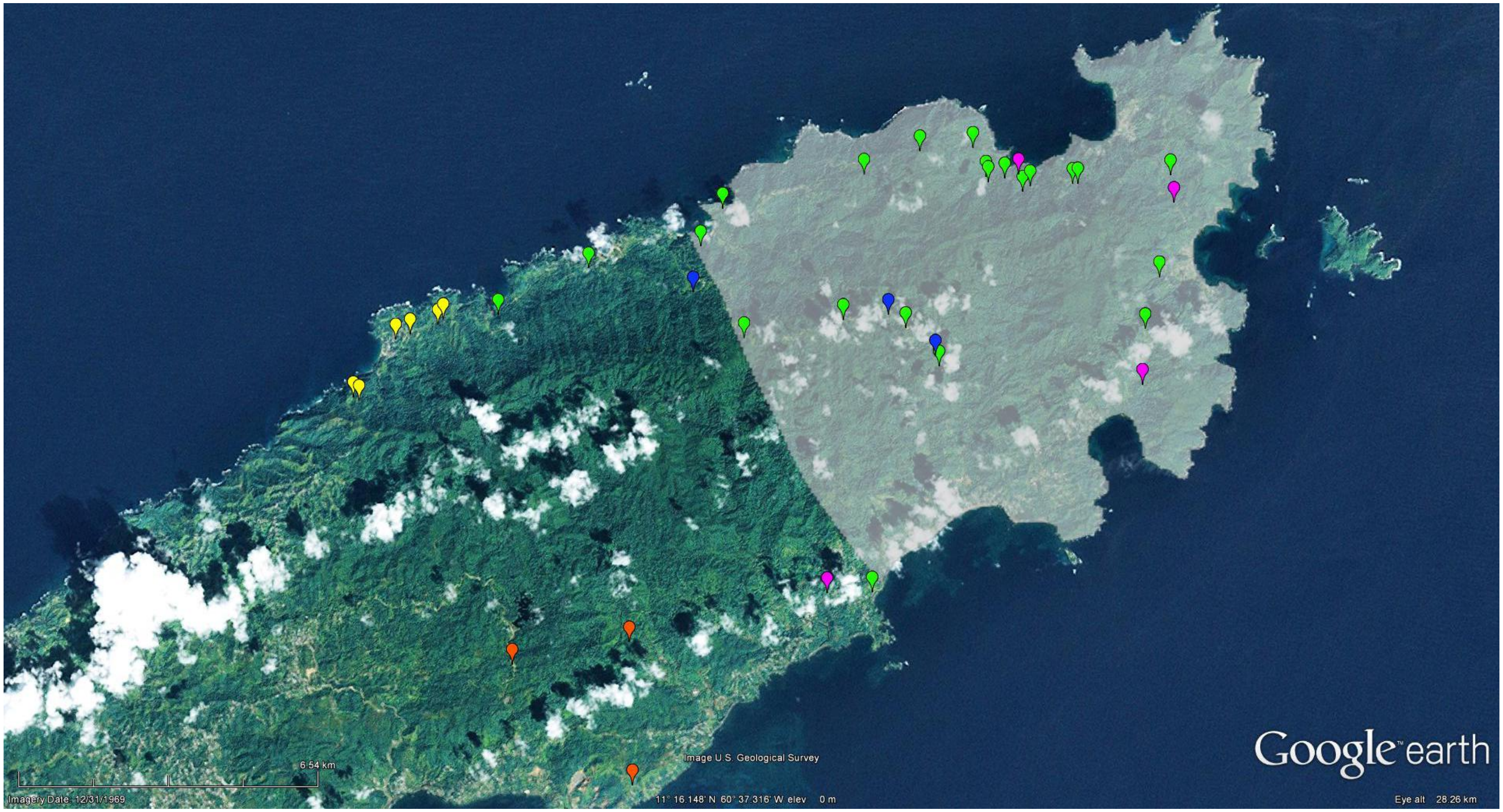

Figure 1: Distribution of *M. olmonae* in 2011 (Novick, 2012) and 2012 compared to known range (shaded region, on east side of island) (Hardy, 2004). Green, purple, and blue sites were formally surveyed in 2011 and 2012. Green sites represent populations found in both 2011 and 2012. Purple sites represent populations found only in 2011. Blue sites represent populations found only in 2012. Red and yellow sites represent populations identified through informal surveys in 2011 and 2012 respectively. Map made using Google Earth.

Table 1: The upper and lower range (with units of measurement) encountered (during 2012 survey periods) for each covariate and covariate type.

Covariate	Covariate Type	Lower Boundary	Upper Boundary	Units
Forest cover	Site	27.41	99.65	%
Longitude (West)	Site	32.25	40.85	Minutes
Latitude (North)	Site	13.951	19.186	Minutes
Stream Width	Site	0.48	3.85	Meters
Year	Sampling	0	1	Relative Scale
Date	Sampling	1	7	Relative Scale
Time	Sampling	14:59	18:07	Hours
Rain During	Sampling	0	2	Relative Scale
Wet Bulb Temperature	Sampling	23.3	30.2	°C
Barometric Pressure	Sampling	958.3	1011.1	Millibars
Air Temperature	Sampling	24.5	34.8	°C
Wind Speed	Sampling	0	5.4	Km/h
Humidity	Sampling	65.4	100	%

The strongest model for ψ and p was the null model (ACI=215.4; Table 2; Table 3). The detection probability (p) was 0.7577 (SE=0.0403). The naïve occupancy was 74.3% of sites in 2011 and 70.6% in 2012. PRESENCE calculated the robust occupancy estimate (ψ) across the two years to be 75.9% (SE=0.0779). The occupancy during this period was relatively static but, there is evidence of some population founding as the colonization (γ) estimate was 0.158 (SE=0.1555) and the local extinction (ε) was lower at 0.0834 (SE=0.0678). The lambda score for these analyses was 0.9668 (SE=0.0858).

Table 2: All occupancy models for site covariates (psi; ψ). The symbols (.) indicate that no covariates were assessed for those criteria, thus (.) for all criteria indicates a null model. Gamma (γ) represents colonization, eps (ε) represents extinction, and p represents detection. AIC (Akaike's information criterion) and ΔAIC assess the strength of each model individually and relative to one another. The AIC weight assesses relative likelihood of the model.

Site Covariate Model	AIC	ΔAIC	AIC Weight
psi(.),gamma(.),eps(.),p(.)	215.40	0	0.7862
psi(Longitude),gamma(.),eps(.),p(.)	219.73	4.33	0.0902
psi(Latitude),gamma(.),eps(.),p(.)	220.19	4.79	0.0717
psi(Stream Width),gamma(.),eps(.),p(.)	221.80	6.40	0.0320
psi(Forest Cover),gamma(.),eps(.),p(.)	222.83	7.43	0.0191

Table 3: All occupancy models for detection probability (p). The symbols (.) indicate that no covariates were assessed for those criteria, thus (.) for all criteria indicates a null model. Gamma represents colonization, eps represents extinction, and psi represents occupancy. Q preceding a covariate represents a quadratic model of these data. AIC (Akaike's information criterion) and ΔAIC assess the strength of each model individually and relative to one another. The AIC weight assesses relative likelihood of the model.

Detection Covariate Model	AIC	ΔAIC	AIC Weight
psi (.),gamma(.),eps(.),p(.)	215.40	0	0.9991
psi(.),gamma(.),eps(.),p(Year)	229.44	14.04	0.0009
psi(.),gamma(.),eps(.),p(Humidity)	243.20	27.80	0
psi(.),gamma(.),eps(.),p(Time)	243.23	27.83	0
psi(.),gamma(.),eps(.),p(Wind Speed)	243.36	27.96	0
psi(.),gamma(.),eps(.),p(Q Temp)	243.43	28.03	0
psi(.),gamma(.),eps(.),p(Air Temperature)	243.90	28.50	0
psi(.),gamma(.),eps(.),p(Q Time)	244.05	28.65	0
psi(.),gamma(.),eps(.),p(Barometric Pressure)	245.23	29.83	0
psi(.),gamma(.),eps(.),p(Rain During)	245.47	30.07	0
psi(.),gamma(.),eps(.),p(Wet Bulb Temperature)	246.34	30.94	0
psi(.),gamma(.),eps(.),p(Date)	246.53	31.13	0

The majority (n=29) of sites visited in the formal surveys had between 95.1% and 100% forest cover (Figure 2). Only two sites with less than 95% forest cover were confirmed as occupied sites through the acoustic calling survey (Figure 2). One site (site 25) had considerably less forest cover than any other site (Figure 2). This site was located inside of an agricultural research facility and there was no forest visible from the survey location.

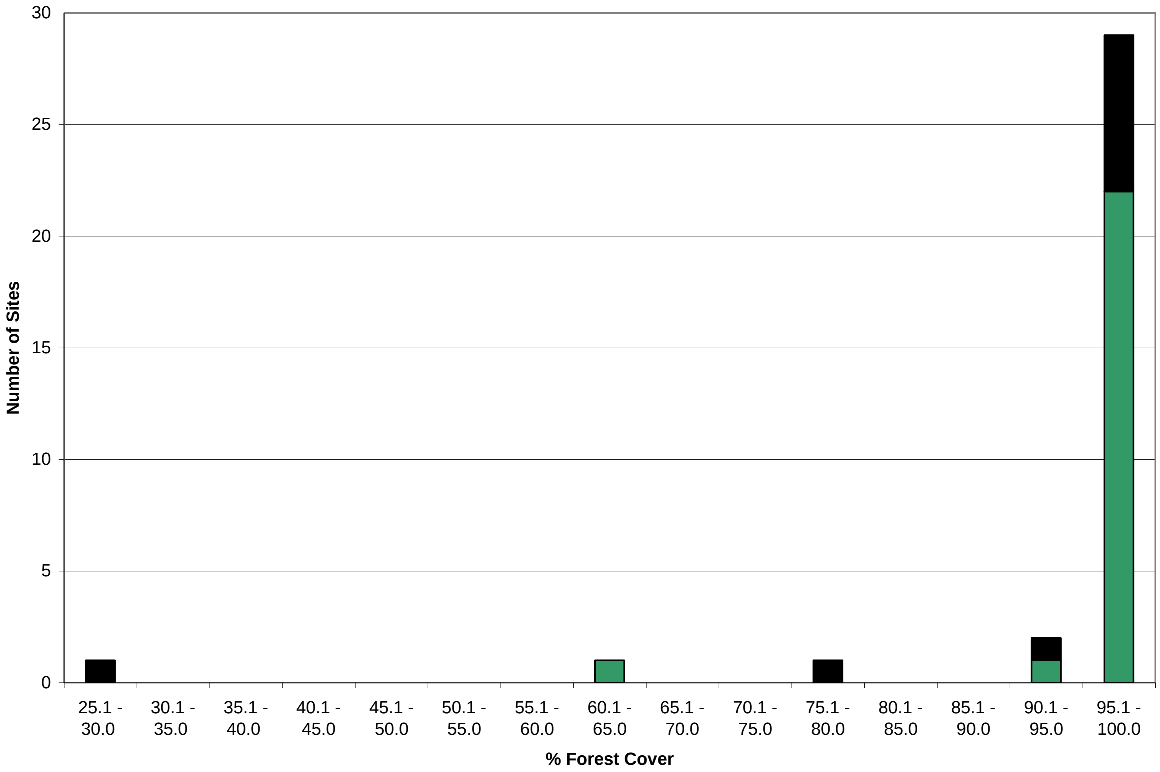

Figure 2: Number of sites found occupied (green) or unoccupied (black) compared to percent forest cover within a 200m radius circle. 85% of sites had between 95.1 and 100% forest cover. 76% of these sites were occupied. Percent forest cover was not an explanatory variable for the occupancy of *M. olmonae.*

Discussion

Occupancy covariates

None of the covariates for site occupancy (ψ) tested using PRESENCE were found to be explanatory in this multi-year analysis. This initially suggests that forest cover, stream width, longitude, and latitude may not affect whether *M. olmonae* is able to survive in a given habitat. However, these covariates may only be non-explanatory over the narrow range which they were encountered.

Mannophryne olmonae is a forest dwelling species which requires canopy cover to survive (Alemu *et al*, 2007; Calkins, 2012). For this reason, percent forest cover was investigated as a potential indicator of occupancy. The range of percent forest cover found within a radius of 200m from all formally surveyed sites was not very diverse; twenty-nine sites had greater than 95.1% forest cover (Figure 2). Of the remaining five sites, two had between 90.1% and 95% cover. Only one of the final three sites (site 26; forest cover=62.5%) was occupied. Due to site 26 being occupied and having considerably less forest cover than all other occupied sites, all occupancy models were conducted without site 26 in an attempt to identify outlier effects if they were present. Forest cover was not found to be explanatory (ΔAIC=5.26) in the absence of site 26, so in order to maintain the largest sample size possible, site 26 was retained in the final analyses.

My study used percent forest cover as a measure of land-use; a high percent forest cover indicated that the land was not being used for human applications. A previous study suggests that the American toad is affected by percent forest cover when considering a 500m radius circle and 2,500m radius circle around a habitat, but not when considering a 1,000m radius circle (Lehtinen *et al.*, 1999). This implies that percent forest cover may not be biologically relevant at all spatial scales, however, a 500m radius circle may be an appropriate focal range for land-use variables (Lehtinen *et al*., 1999). Unfortunately, due to the close proximity of some of my survey sites to one another, a 500m circle was not practical for my study.

My study analyzed the percent forest cover within a 200m radius circular boundary model around each formally surveyed site. This supplied data from the largest area possible without considerable overlap between sites (Appendix 1, Figure 1). The limited size of the circular boundary model used for this analysis may have reduced the likelihood of finding a biologically relevant connection between percent forest cover and occupancy. The circular boundary model for some sites (N=4) encompassed ocean as well as the terrestrial habitat, thus reducing the amount of land in the analysis for those sites even further (Appendix 1). The circular boundary model for site 26 encompassed more ocean than any other site, thus the percent forest cover was calculated for the smallest terrestrial habitat at this site. This may have caused the un-forested areas near the perimeter of the model to have a larger affect on percent forest cover for site 26 than they would have for other sites.

The stream widths surveyed in this study fell within a narrow range. Streams were only surveyed if they were initially thought to be a potential habitat for *M. olmonae*, and each survey site was on a different stream or tributary. Very large rivers, such as the Bloody Bay River, were not sampled because they were above the ideal size for this species to inhabit. *Mannophryne olmonae* typically inhabits small streams and pools that are predator free; large rivers can support fish as well as other potential predators (Alemu *et al,* 2007; personal observation, July 2012). As such, the narrow range of stream widths may be an explanation for the non-explanatory nature of that variable.

In 2011, stream width was found to have potential explanatory power over occupancy for *M. olmonae* (Novick, 2012). A potential explanation for the discrepancy between the first year of this survey and the multi-year analysis (2011 and 2012) is that *M. olmonae* was heard calling from tributaries associated with three of the streams in 2012. As such, the width of these tributaries was substituted for the stream width that was used in the 2011 analysis. These tributaries represented the actual habitat of *M. olmonae* and were considerably smaller than the initial streams.

The physical location, latitude and longitude, of the sites was also somewhat constrained in respect to the size of the island. The majority of sampled sites (N=25) were inside the known range as indicated by Murphy in 1997, which is located on the eastern half of the island. The other nine sites were located west of this known range; however the far western side of the island was not formally surveyed. All formal survey sites were chosen prior to the identification of several western populations during the 2011 and 2012 informal surveys (n=12; n=6; respectively), thus these western populations were not included in the formal surveying. The lack of sites on the western side of Tobago likely influences the explanatory power of longitude as a variable.

The use of more westerly sites in a future survey may influence more than just the explanatory power of longitude and latitude. The western region of Tobago is more urbanized than the eastern portion and contains less forested area. The presence of the Tobago Forest Reserve in the surveyed area ensured that the preferred habitat of *M. olmonae* was sampled. By surveying more western sites, a wider variety of percent forest cover and longitude and latitude could be tested. Finally, testing a wider variety of stream sizes (some larger and some smaller than those surveyed in this study) may offer better insight into the stream size preferred by *M. olmonae*.

Detection probability covariates

Similar to the site covariates, none of the sampling covariates were found to be explanatory. These results and a detection probability of 75.8% suggest that *M. olmonae* can be detected using an acoustic calling survey under variable conditions, and by extension, that the males of this species are calling for mates in a variety of environmental conditions. A variety of conditions were encountered for most of the sampling covariates, which suggests that *M. olmonae* may not be affected by climactic conditions over the range measured (Table 1; MacKenzie *et al*, 2006).

Barometric pressure, the covariate with the most explanatory power in the 2011 single-season models (Novick, 2012), did not prove to be explanatory in the multi-year models. This suggests that barometric pressure is likely not influential in the calling of *M. olmonae* despite the 2011 results, because the multi-year analysis employs more data. Having a larger data set increases the chance of finding truly biologically relevant connections (Hernandez *et al.,* 2006). Furthermore, this may suggest that altitude, for which barometric pressure acts as a rough proxy measure, may not be a strong determining factor in the habitat or calling of *M. olmonae* as has been suggested previously. *Mannophryne olmonae* was thought to live only between 95 and 360m above sea level (Angulo *et al*, unpublished data). The non-explanatory power of barometric pressure suggests that this may not be an accurate limitation for this species. Personal observation also indicates that this species is present at a wider range of altitudes. Site 26 was nearly at sea level and the frogs were not only detected acoustically, but tadpoles and calling males were seen at this site. This negates the claim that *M. olmonae* is preferentially found at higher altitudes.

The calling surveys were conducted between 1500h and 1800h in both 2011 and 2012 in order to maintain consistency between years (Novick, 2012). *Mannophryne olmonae* also calls during the morning hours, 0600h to 0800h, but these hours were not sampled (Alemu *et al.*, 2007; Murphy, 1997). Richard M. Lehtinen (personal communication, Jul. 27, 2012) observed that *M. olmonae* tended to call more toward the end of the evening calling period, so an attempt was made to survey each site at a different time during this census period. The time covariate was modeled in both the linear and quadratic form in order to determine whether there was an optimal time to detect *M. olmonae* if it was present at a site. The linear model assessed whether detection probability increased or decreased linearly during the survey period. The quadratic model assessed whether there may have been a peak detection interval in the middle of the survey period. The quadratic model also assessed the possibility of a peak detection interval at both the beginning and end of the survey period. Neither of these models had explanatory power.

Temperature was also modeled in a linear and quadratic form to determine if there was an optimal air temperature at which *M. olmonae* could be detected. Like the time covariate linear model, the temperature linear model assessed whether the detection probability increased or decreased linearly with respect to air temperature. The quadratic model assessed whether detection probability was highest or lowest in the middle temperature range. Neither form produced explanatory results.

Range extension

The formal and informal surveys (2011 and 2012) identified 14 populations outside the known range for *M. olmonae*. These populations increase the range of this species 4° 0.24 minutes farther west, which represents a 59% increase in geographic range. This could indicate that the range of *M. olmonae* is expanding, but it could also suggest that previous studies did not locate populations that were present in central Tobago. Additionally, this and related studies indicate that *M. olmonae* can inhabit a wider range of habitats than previously thought (Murphy, 1997; Angulo *et al.*, unpublished data); the species can inhabit secondary forest (Calkins, 2012) and altitudes outside the known altitudinal range. Future surveys which attempt to detect populations farther west than those found in this study would be valuable for further geographic range assessment.

Colonization and extinction

The colonization and extinction rates determined by PRESENCE (γ=0.158 SE=0.1555; ε=0.0834 SE=0.0678) indicate that local extinction is not currently threatening the populations surveyed. These numbers, in conjunction with the lambda value (0.9668 SE=0.0858), indicate that the number of populations is nearly static. A lambda value of 1.0 indicates that there is no net change in the number of populations due to colonization and extinction between years. However, since these values are derived from only two years of survey data, additional survey years are needed in order to be confident that this trend is biologically relevant.

These findings hold promise for the conservation status of *M. olmonae* because they indicate that there does not seem to be a current decrease in population number. In 2004, the

IUCN used an 80% population decline over three generations as an indicator that this species was at high risk of extinction (Hardy, 2004), but these data indicate that there has been no net change over the last two breeding seasons. Additionally, the populations observed in 2011 and 2012 were juvenile biased (Calkins, 2012). When visually assessing a population in 2012, it was clear that recent metamorphs were abundant in the population. Tadpoles and frogs that were metamorphosing were also observed at a number of sites, indicating that there was reproductive success during the 2012 wet season. Adult females were not often seen, but the adults (particularly the females) are very fast and often hard to observe (R. M. Lehtinen, personal communication, Aug. 1, 2012). Adult males were often seen in calling condition (completely black in color) along the stream banks and on rocks that were near secluded pools.

Conservation status

With these results as evidence, I suggest that *M. olmonae* does not require a conservation status of critically endangered under the IUCN criteria for conservation status. These results suggest that criterion A is not met due to a robust occupancy estimate of 75.9%. This suggests that there was likely not an 80% decline in population (Hardy, 2004) or that *M. olmonae* has recovered. My study expanded the known range of this species by 59% and has identified 38 populations, thus criterion B is not met. Observations during my study and the results of Calkins (2012) indicate the juvenile biased population suggests that this species is undergoing reproductive success. Furthermore, Calkins (2012) located 43 mature individuals and my study confirmed that mature males were present in at least 38 locations. These findings suggest that neither criterion C or D is met.

The final criterion (E) states that a species is critically endangered if there is a 50% or higher chance of extinction in ten years. A species is endangered if there is a 20% or higher chance of extinction in 20 years and threatened if there is a 10% or higher chance of extinction in 100 years (Baillie *et al.*, 2004). This is the second year of a three year survey, thus additional years of observation are crucial to effectively address this criterion.

I suggest that the conservation status of *M. olmonae* be revised to vulnerable. This is the lowest of conservation statuses, but does not suggest that *M. olmonae* should be left unmonitored entirely. Criteria A-D suggest that the species is not as threatened as was thought by Hardy (2004). However, this species is endemic to a small island, and thus the global range of *M. olmonae* is very small. This endemism may be reason to continue some population surveys, but the time, effort, and resources required to monitor a critically endangered species need not be applied to *M. olmonae.*

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APPENDIX 1

Forest Cover Data Collection

Using the program Google Earth Pro (available at http://www.google.com/earth) and SketchUp 3D modeling software (available at: http://www.sketchup.com), land use information was collected by using percent forest cover as a proxy measure. Circular boundary models were created using SketchUp by selecting the “Plan View-Meters” template and using the circle tool to draw a circle with a 200m radius (400m diameter). A second circular model with a 2m radius was placed inside the first by clicking the center of the original model (indicated by visible axes) and entering the appropriate radius measure when directed to. This smaller circle was used as visual verification (in addition to coordinate based centering) that the circular boundary model was centered precisely on each site (Figure 3) on a Google Earth Pro map. All default textures found in this circular boundary model were deleted and the color of the remaining circular boundary was changed to green to facilitate accurate use later. The circular boundary was saved and then sent to Google Earth Pro using the “Preview in Google Earth” option.

Google Earth Pro settings were adjusted to ensure that the latitude and longitude were displayed in degree minute decimal format to be in agreement with the format used for locating sites while in the field. Additionally, the option of “Do not automatically tilt while zooming” was selected to prevent the satellite imagery from tilting and potentially skewing forest cover measure due to inconsistency between tilt measures at different sites. The “Terrain” and “3D Buildings” settings were also turned off to allow optimal viewing of the circular boundary model. The “Roads” feature was turned on for future analysis.

The circular boundary model imported into Google Earth Pro from SketchUp was located on the world map (the default location for image placement is Boulder, Colorado) and, using the properties menu of the model, the absolute altitude was changed to 1m in order to have an unobstructed view of the model. The GPS coordinates of the site to be measured were entered into the model's properties menu and the circular boundary model was immediately moved to this location. After selecting a range view of 500m (i.e. a viewpoint that is 500m from earth), the model was copied, pasted, and relocated to each of 34 sites using the GPS coordinates to produce identical circular boundary models for each focal location.

In order to determine the percent of land that was forested inside each circular boundary model, the "Polygon" tool in the Google Earth Pro program was used. A polygon was drawn around all forested areas that could be visually identified using the most recent imaging data available for the site in question. As all sites were directly adjacent to a road, a minimum of two polygons was required for each site; roads were never included in a polygon. At sites which contained ocean in the circular boundary model, the ocean was marked with a different colored polygon to indicate that this was not part of the terrestrial habitat. The area of each polygon, as automatically calculated by Google Earth Pro, was recorded in meters squared. The total area covered by forest for each site was divided by $124{,}233\text{m}^2$ as this was the total area of the circular boundary model automatically determined by SketchUp (SketchUp uses short, straight line segments to produce a circle so the total area of the model was slightly different than the calculated area of a non-computer generated 200m radius circle). Sites which contained an ocean polygon were divided by the total terrestrial area within the circular boundary model (i.e. the ocean polygon was subtracted from the total area because only terrestrial area was being considered).

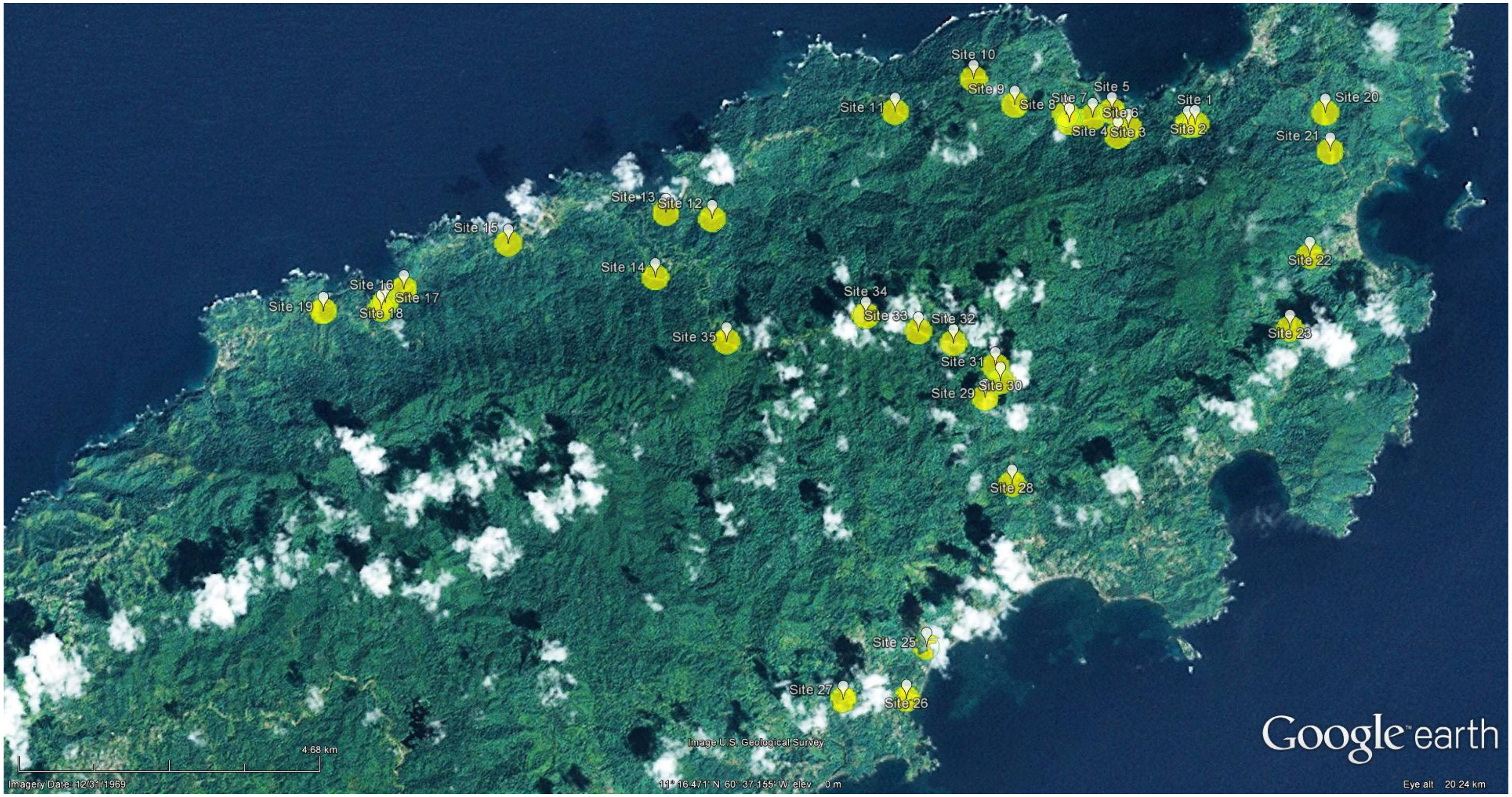

Figure 1: Formal survey site locations with 200m radius circle boundary models. Map made using Google Earth and SketchUp.

APPENDIX 2

Site locations

Table 1: Road name and GPS coordinates for the formal survey sites. Shaded site was not used in this analysis but has been included for consistency with Novick, 2012.

Site #	Road	GPS North	GPS West
1	Leeward	11°18.767	060°33.404
2	Leeward	11°18.762	060°33.465
3	Leeward	11°18.736	060°33.975
4	Leeward	11°18.670	060°34.065
5	Leeward	11°18.876	060°34.115
6	Leeward	11°18.826	060°34.280
7	Leeward	11°18.784	060°34.478
8	Leeward	11°18.847	060°34.506
9	Leeward	11°19.186	060°34.661
10	Leeward	11°19.145	060°35.297
11	Leeward	11°18.870	060°35.967
12	Leeward	11°18.471	060°37.662
13	Leeward	11°18.026	060°37.926
14	Leeward	11°17.486	060°38.015
15	Leeward	11°17.769	060°39.269
16	Leeward	11°17.386	060°40.162
17	Leeward	11°17.276	060°40.320
18	Leeward	11°17.221	060°40.353
19	Leeward	11°17.203	060°40.850
20	Windward	11°18.862	060°32.292
21	Windward	11°18.538	060°32.250
22	Windward	11°17.665	060°32.425
23	Windward	11°17.054	060°32.595
24	Windward	11°16.401	060°32.628
25	Windward	11°14.395	060°35.699
26	Windward	11°13.959	060°35.870
27	Windward	11°13.951	060°36.410
28	Forest	11°15.759	060°34.969
29	Forest	11°16.478	060°35.198
30	Forest	11°16.616	060°35.068
31	Forest	11°16.742	060°35.111
32	Forest	11°17.067	060°35.468
33	Forest	11°17.221	060°35.676
34	Forest	11°17.163	060°36.217
35	Forest	11°16.949	060°37.406

Table 2: Site GPS coordinates and road name for 2012 informal surveys.

Informal Site #	Road	GPS North	GPS West
1	Leeward	11°17.172	60º41.012
2	Leeward	11°17.104	60º41.076
3	Leeward	11°16.993	60º41.409
4	Leeward	11°16.933	60º41.582
5	Leeward	11°16.212	60º42.021
6	Leeward	11°16.253	60º42.091