**Temporal changes in species diversity of fishes in the center of marine biodiversity**

# **Introduction**

The Coral Triangle has long been considered the area of highest marine biodiversity ​(Bellwood and Wainwright 2002; Mora et al. 2003)​. The Philippines has been identified as the global epicenter of marine biodiversity and fish diversity ​(Carpenter and Springer 2005; Allen 2008; Sanciangco et al. 2013)​ with peaks in the central Philippines, which is known as the Visayas Region (Fig. 1). Hypotheses attribute this peak of species richness within the Coral Triangle to processes that occur at geological time scales. However, changes in biodiversity may occur on ecological time scales, allowing for the assessment of the potential impact of anthropogenic activities. The Philippines also ranks highest globally for threats to marine biodiversity and coral reefs ​(Bryant et al. 1998; Roberts et al. 2002)​. Therefore, identifying the impacts of human activity, such as overexploitation and habitat degradation, on the diversity of fishes in the Philippines is of growing concern.

Within the Philippines, the Visayas Region is the center of marine conservation adversity ​(Nañola et al. 2011)​. Coastal development, pollution, and overexploitation of marine resources have caused widespread and severe habitat degradation and ecological impacts over recent centuries (Halpern et al. 2008, 2015; McCauley et al. 2015). Overexploitation is the leading threat to marine fishes ​(Collette et al. 2011; Dulvy et al. 2014)​ with exploitation related species richness losses and local extirpations recorded in the Visayas Region ​(Lavides et al. 2009, 2016; Nañola et al. 2011)​. Furthermore, the Philippines is the top supplier of marine ornamental fishes to the global aquarium trade resulting in intense overharvest ​(Rhyne et al. 2017)​, and destructive collection methods (e.g. cyanide, overturning coral heads, dynamite) indirectly affect fish populations through habitat degradation ​(Mamauag 2004; Ochavillo et al. 2004; Pratchett et al. 2008)​. Additional studies are needed to better understand the extent of diversity loss in the most marine biodiverse and anthropogenically threatened region on Earth.

The Smithsonian Institution fish biodiversity collection was a survey of Philippine nearshore fishes from the Visayas Region in 1978 and 1979 (Fig. 2.A) These two collections provide a historical baseline of species diversity and genetic diversity that will be compared to contemporary collections to evaluate the effect of fishing pressure and habitat degradation on changes in nearshore fish diversity in the Visayas Region of the Philippines.

Thus, to explore the potential impacts of overfishing and habitat degradation on fish populations in the Visayas Region, I will identify spatiotemporal decadal changes in species diversity of fishes from an ichthyocide study.

Q1: To what extent has reef fish biodiversity changed over decades of human impacts in the Visayas Region?

H1: Biodiversity indices will decrease over time (Lavides et al. 2009, 2016; Nañola et al. 2011).

# **Methods**

Historical and contemporary fish biodiversity datasets will be compared to determine the impact of human activity on fish biodiversity over four decades in the Visayas Region. We can take advantage of a collection from the Smithsonian Institution (SI survey) that surveyed fish biodiversity in 1978 and 1979, which will serve as a baseline of historical fish biodiversity in the Visayas. The Smithsonian survey collected fishes using the ichthyocide rotenone from 71 stations (Fig. 2). Rotenone targets smaller, cryptic species compared to other types of biodiversity surveys such as fish visual census (FVC), which are more biased towards highly visible reef fishes that swim over and above the reef ​(Labrosse et al. 2002)​. The crew of the 1970s Smithsonian collection included members of the present Philippines PIRE Project (NSF Award #1743711) that is supporting the proposed research. The Smithsonian crew recorded important metadata such as station coordinates, depth, distance from shore, and habitat, which allows these sites to be accurately duplicated.

The contemporary dataset consists of two surveys: 1. The California Academy of Sciences conducted a rotenone survey in 2016 (NSF Award #1257632), which sampled 13 stations in the Central Visayas (CAS survey). 2. The present study has duplicated 24 of the rotenone stations from the historical SI survey in collaboration with Silliman University (SU survey, Fig. 2). Three of the SI rotenone stations were sampled in 2019 and 21 in 2022. Twenty-two of these stations are coral reef habitats and will be the focus of the present study.

The CAS and SU surveys will be analyzed as both a combined dataset and independent datasets to compare to the historical SI survey. Sample- and individual-based abundance data will be analyzed from the rotenone surveys to create species accumulation curves to test for biodiversity changes between surveys. Tests for differences between historical and contemporary biodiversity samples will use rarefaction and extrapolation analytical methods that have proven robust in comparisons of biodiversity using large samples sizes, while assuming data is not standardized in terms of collection methods and area sampled ​(Beck and Kitching 2007; Colwell et al. 2012; Carvalheiro et al. 2013)​. The data will be treated as non-random, and nonparametric methods will be applied using a biodiversity assessment approach comparing historical versus contemporary collections ​(e.g., Zuur et al. 2007; Williams et al. 2010)​.

The statistical package PRIMER ​(Clarke and Gorley 2018)​ and the vegan package ​(Oksanen et al. 2020)​ in R will be used to generate (1) multivariate analyses on changes in fish community structure, (2) similarity matrices to test for statistically significant clustering using similarity profiles (SIMPROF), and (3) the similarity percentage routine (SIMPER) will be used to identify species that contribute the most to the differentiation between clusters. Associations of fish assemblage and habitat, as well as other geographical patterns, will be identified by analyzing rotenone stations with non-metric multidimensional-scaling (MDS) ordination based on the Bray-Curtis similarity matrix of incidence data. Principal components analysis (PCA) and discriminant analysis of principal components (DAPC) will be used to further understand which species assemblages are responsible for differences among stations. These analyses will be conducted on both the historical and contemporary datasets to compare differences in both spatial and temporal patterns in species communities.

# **Preliminary Results**

To estimate the extent to which species richness has changed over approximately four decades the historical dataset (n=71) was compared to the contemporary dataset (n=16), which included the CAS survey (n=13) and the SU survey stations that were sampled in 2019 (n=3; Fig. 2.A).  Interpolation, rarefaction, and extrapolation of the datasets created sample- and individual-based species accumulation curves for the historical and contemporary surveys. Across the 16 rotenone stations (samples) from the contemporary survey 4,995 individuals were encountered, which consisted of 360 unique species. This dataset was extrapolated three times to 48 samples (Fig. 3.A) and 14,985 individuals (Fig. 3.B). When comparing the two sample-based species accumulation curves (Fig. 3.A), a total of 31.8% reduction in species richness was observed, which equates to a reduction of 8.3% per decade. The individual-based species accumulation curve (Fig. 3.B) still observed the same reduction in species richness, however the two datasets would not be considered significantly different on a per individual basis after extrapolation of the contemporary survey. Nevertheless, the extrapolation suggests that if more stations were sampled and more individuals were encountered, then the curves would diverge further, supporting a significant decrease in species richness in the Visayas Region between the two surveys.

The observed 8.3% decrease in species richness per decade in the Visayas Region is over four times higher than the 2% per decade reported in a previous study ​(Nañola et al. 2011)​. Yet, given that the authors did not have a historical baseline of species richness to compare to contemporary surveys, they used space as a proxy for time when comparing the species richness of the Visayas Region to the adjacent Sulu Sea, the region with the highest species richness. It is important to note that Nañola et al. (2011) used data from FVC surveys, which are biased towards the highly visible reef fishes that swim over and above the reef, instead of the smaller, cryptic species, which are better represented in rotenone surveys. Therefore, due to differences in sampling methodology, the current study can’t be directly compared to the results from Nañola *et al*. (2011), but our preliminary results provide further evidence of biodiversity loss in a global biodiversity hotspot and unless threats such as overfishing and habitat degradation are reduced, further declines can be expected.

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