Budget and Justification (2 pages max)

Table 1. Proposed budget for this research proposal.

Line Item	Description		Cost
Personnel	PhD student salary, fringe benefits, and tuition for one semester	$	14,819
eDNA Collection Kit	Items that will be sent to collaborators to collect and filter seawater	$	2,932
Laboratory Supplies	Laboratory items and reagents needed to process samples, from eDNA extraction up to library preparation for sequencing	$	8,886
Contractual	Sequencing costs; shipment fees of items (e.g., collection kits, samples) to and from the Philippines	$	3,350
Total		$	29,987

The total budget requested by this project is **$29,987.** The line items and justifications are provided below and summarized in Table 1:

Personnel: $14,819

A PhD student will help to assemble the eDNA collections kits, carry out laboratory work and data analysis. We are requesting one semester (4.5 mo at 50% FTE) of support for the graduate student (4.5 mo * $2000 = $9,000). Fringe benefits for a student are 10.7% of their salary ($9000 * 0.107 = $963) and insurance is $562 per month (0.5 FTE * 4.5 mo * $562 = $1265), and tuition and fees is estimated to be $3,591 per semester.

Collection Kit: $2,932

The collection kit will be sent to the four collaborating institutions in the Philippines for the collection and filtration of seawater samples. Each kit contains the following: a 5-gallon bucket to hold the seawater prior to filtration ($23.04); 300 mL syringes to aspirate and administer the seawater to the filtration set up ($145.44); the serial filtration set-up, consisting of 80 µm Nitex Nylon Mesh Filter Sieve ($264.00), 0.45 µm Sterivex cartridges ($1,176.88), and 0.22 µm Sterivex cartridges ($1,174.44); 3 mL syringes to administer the preservative to the Sterivex cartridges ($13.99); dual male-female luer-lok caps to seal the Sterivex cartridges ($79.96); and 1 oz Whirl-Pak sterile bags to store the cartridges and prevent contamination or leaks during transport ($53.76). We will be sending 39 Sterivex cartridges per size fraction per site, summing up to 156 samples per size fraction for processing.

Laboratory Supplies: $8,886

The laboratory supplies will be used to process samples in TAMU-CC Genomics Core Laboratory. A PVC pipe cutter will be used to open the Sterivex cartridge ($25.98), and the filters will be placed and cut into smaller sections on sterile 90 mm disposable petri dishes ($214.00). eDNA from the 0.45 (n = 156) µm and 0.22 µm filters (n = 156) will be extracted using Qiagen Blood and Tissue Kit ($2,127.76) and Qiagen Plant Mini Kit ($1916.76), respectively. Metabarcoding markers will be amplified using custom oligos and PCR kits ($557.33), which will then be used to prepare the TaggiMatrix libraries for high

throughput sequencing (156 samples * 4 loci = 624 libraries @ $6.48/library = $4,044.38). Costs for the library preparation include PCR (2016 reactions including triplicates and controls: $947.01), agarose gel electrophoresis ($120.96), SPRI select ($1,233.12), ligation of iTru primers and clean-up ($383.93), and the consumables for all lab work including pipet tips, microtubes, gloves, bleach, ethanol, and sterile water ($1,359.36).

Contractual: $3,350

The contractual fees will cover the sequencing and shipping costs. The amplicon libraries will be sequenced using a NovaSeq 6000 PE 250 lane (400M read pairs); we will aim 130 M read pairs ($1,787.50). We will also ship memoranda of agreements ($328.24) and eDNA sample collection kits to the Philippines ($851.38), and eDNA samples back to TAMU-CC ($383.16).