

Title: Protecting Mississippi Sound Dolphins: Understanding the Role of Disease and Parasites in Dolphin Strandings

Project Narrative

Bottlenose dolphins (*Tursiops truncatus*) inhabiting the Mississippi Sound serve as an important sentinel species for coastal ecosystem health, reflecting both environmental conditions and emerging disease threats in the northern Gulf (Wells et al., 2004; Reif et al., 2017). Because Mississippi Sound bottlenose dolphins are a public-trust natural resource and sentinel of coastal ecosystem condition, improving disease diagnosis and surveillance directly supports the Mississippi Department of Marine Resources (MDMR) in mitigating damage to wildlife and guiding conservation of Mississippi marine resources. Over the past decade, increasing evidence has identified *Brucella ceti* as a significant contributor to morbidity and reproductive failure in this population, particularly among perinatal animals (McFee et al., 2020; Bloodgood et al., 2023). Numerically, the proportion of *Brucella*-positive dolphins has remained comparable across all years analyzed, suggesting that *B. ceti* is stable within the Mississippi Sound population. Despite repeated detection of this pathogen in stranded dolphins, critical questions remain regarding its transmission pathways, tissue tropism, interaction with co-infections, and potential environmental persistence. Importantly, *B. ceti* also represents a zoonotic concern, with sporadic human infections and documented potential for transmission through direct contact with infected marine mammals or environmental exposure, raising public health implications for coastal communities and stranding response personnel. Recent mortality events and ongoing health concerns in northern Gulf bottlenose dolphins underscore the need for improved diagnostic capacity and coordinated disease surveillance in the Mississippi Sound. This project also leverages existing datasets, partnerships, and infrastructure developed through our prior MDMR GOMESA-funded work and long-term collaboration with the Institute for Marine Mammal Studies (IMMS), ensuring cost-effective use of resources and rapid translation of findings into management-relevant outputs.

Much of what is currently known about *Brucella* in dolphins is derived from opportunistic investigations of stranded individuals. While these studies provide valuable snapshots of disease occurrence, they often lack the integrated framework needed to connect pathological findings, pathogen characteristics, and ecological drivers of exposure. In addition, the role of co-infections (particularly parasitic infections that may influence immune status or disease severity) remains poorly understood. Additionally, *B. ceti*-positive cases most commonly occur between February and April, suggesting a potential seasonal component to exposure or disease expression that warrants further investigation. These knowledge gaps limit our ability to interpret stranding trends, assess population-level impacts, and develop proactive management strategies. Addressing these gaps will improve consistency in cause-of-death determinations, support identification of seasonal and demographic risk patterns, and strengthen MDMR's ability to prioritize monitoring and stranding response efforts. This project complements existing dolphin health efforts in the Mississippi Sound by focusing specifically on *Brucella* tissue localization, parasite diagnostics, co-infection interpretation, and integration of environmental DNA surveillance, which are not currently addressed within a single coordinated framework.

This project builds on an established foundation of dolphin health research and stranding response in the Mississippi Sound. Since 2018, we have maintained a comprehensive epidemiological database of stranded dolphins in collaboration with IMMS, providing longitudinal health, pathology, and environmental data that directly support the proposed analyses. In addition, our team has led multiple MDMR GOMESA-funded projects examining population health, habitat use, and environmental impacts associated with the Bonnet Carré Spillway, resulting in peer-reviewed publications on genetic diversity, contaminant exposure, and freshwater-associated pathology in this population. These efforts provide an

existing infrastructure of archived samples, standardized workflows, and analytical capacity, allowing this project to efficiently expand into targeted investigation of *Brucella*, parasitism, and co-infection dynamics. This work complements, rather than duplicates, prior studies by focusing specifically on pathogen localization, parasite diagnostics, and integrated disease surveillance within a unified framework. Prior work includes studies on genetic diversity, contaminant burdens, and freshwater exposure impacts in Mississippi Sound dolphins (Arick et al., 2025; Landrau-Giovannetti et al., 2024, 2025; McNulty et al., 2026).

This project addresses these limitations through a comprehensive, multi-level investigation centered on systematic necropsy of stranded dolphins in the Mississippi Sound. Through the ongoing partnership with the Institute for Marine Mammal Studies (IMMS), we will perform full necropsy examinations on dolphins that strand within this region to establish baseline health data, identify lesions and causes of death, and collect high-quality tissue samples for downstream analyses. These efforts will leverage existing archived samples and longitudinal datasets generated through ongoing collaboration with IMMS since 2018. These efforts will support standardized diagnostic workflows and repeatable cause-of-death classifications that can be applied across future stranding events and incorporated into MDMR response protocols. Preliminary findings further indicate that lung tissue may represent a particularly reliable tissue for detecting *B. ceti*, emphasizing the importance of consistent sampling of respiratory tissues during necropsy. Necropsy-based investigation provides the critical foundation for understanding disease processes, allowing for direct correlation between clinical pathology, infectious agents, and contributing biological factors (Pugliares et al., 2007; Gulland et al., 2024).

We will integrate targeted analyses of *Brucella ceti* to characterize its role in dolphin health in the Mississippi Sound. This includes isolating and sequencing bacterial strains to evaluate diversity and transmission dynamics, allowing us to determine whether strandings reflect persistent local strains, seasonal exposure patterns, or recurring infection patterns relevant to monitoring and response priorities, as well as applying in situ hybridization (ISH) techniques to determine pathogen localization within host tissues. Linking pathogen presence to lesion distribution will improve interpretation of whether *Brucella* infection is incidental or contributing to mortality in stranded dolphins. Current evidence indicates that the proportion of sequence type 27 (ST27)-positive dolphins is also comparable across years, suggesting that this strain is similarly stable within the population. These approaches will clarify how infection manifests across organ systems, especially nervous, respiratory, lymphoid, and reproductive tissues, and whether any patterns are predictable by age class or disease state. These findings highlight the continued need for appropriate personal protective equipment (PPE) during handling, treatment, and necropsy of dolphins from the Mississippi Sound.

Recognizing disease dynamics extends beyond the individual and must also consider the hosts' environment. This project will also examine these pathogens in a One Health perspective by incorporating an environmental component through the use of environmental DNA (eDNA). Detection of *Brucella* DNA alongside dolphin DNA in water and sediment samples will allow us to explore whether pathogen signals are present within shared habitats, providing insight into potential environmental reservoirs or exposure pathways. We will interpret detections as environmental DNA signals indicative of exposure rather than confirmed reservoirs, and will incorporate field blanks, extraction controls, replicate sampling, and confirmatory sequencing to ensure data quality. This approach bridges the gap between individual-level infection and ecosystem-level processes (Patin and Goodwin, 2023; Sawh et al., 2025).

The project will also determine the role parasitism plays in strandings of dolphins in the Mississippi Sound, especially those already implicated by previous and ongoing research. *Nasitrema* are parasitic flukes that occur in the sinuses and ear-associated tissues of dolphins and other cetaceans. Infections in

the Mississippi Sound population of bottlenose dolphins are common and have been associated with neuropathology possibly contributing to strandings in severe cases. Nematode lungworm infections, typically caused by *Halocercus* spp., have also been documented in the Mississippi Sound, with severe cases of verminous pneumonia being associated with heavy infections (Colegrove et al., 2016). Understanding the impacts parasites play in strandings is hindered by the limited information we have regarding their species identification. Current literature is often limited to taxonomic reports predating availability of molecular identification tools or case reports from only a few dolphins. The proposed work will provide an unprecedented sampling and integrated identification of parasite samples supporting more robust analyses. These data will improve differentiation between incidental parasite presence and parasite burdens likely to contribute to disease or stranding risk, strengthening diagnostic interpretation and response prioritization.

The initial work to characterize these parasites in dolphins from both a combined morphological and molecular standpoint is paramount to strengthen the interpretation of cause of death or disease susceptibility in stranding necropsies. Importantly, perinates and calves appear to be at increased relative risk of testing positive for *B. ceti* compared to older animals, and infection is commonly confirmed in cases of suspected abortion and neonatal mortality. While coinfection dynamics between parasites and *Brucella* are beginning to be investigated, analyses can be hindered by lack of precise identification or targeted collection of the parasite species involved (Shields, 2025). Species identification is required for thorough parasitological study (Lamothe-Argumedo, 1981).

This project will produce specific management and decision-support products for MDMR, including standardized necropsy and diagnostic SOPs, annual dolphin health summaries, validated diagnostic assays, spatial and temporal eDNA detection maps, identification of risk indicators by age class and season, and a final stranding interpretation and management guidance framework. Together, these components form a cohesive and integrated framework that progresses from animal-level investigation to pathogen biology and environmental context. By anchoring the study in systematic necropsy and expanding outward to include molecular, histological, and ecological analyses, this project will generate a comprehensive understanding of *Brucella ceti* dynamics in the Mississippi Sound. The results will improve interpretation of stranding events, identify factors contributing to disease risk, and support MDMR in improving stranding interpretation, identifying disease-related risks, prioritizing monitoring efforts, and guiding conservation and mitigation actions for Mississippi Sound dolphin populations.

List Project Goal/Objectives:

1. Cause of Death Investigation: We will conduct systematic necropsies on stranded dolphins in the Mississippi Sound to establish baseline health data and support integrated disease investigations. To address this, the objectives include 1a) Perform full necropsy examinations following established marine mammal protocols, 1b) Record morphometrics, body condition, and decomposition state, 1c) Document gross lesions and collect representative tissues from major organ systems, including central nervous system, lung, lymph nodes, and reproductive tract, and 1d) Test and archive tissues for bacteriology, molecular diagnostics, histopathology, and parasitology. Outcomes will include standardized cause-of-death classifications, an archived tissue resource, and annual stranding health summaries for MDMR. These activities build on an established necropsy and sample archiving program developed through prior MDMR-funded work and ongoing collaboration with IMMS.

2. *Brucella* Characterization and Environmental Detection: We will investigate *Brucella* across host tissues and the environment to better understand strain diversity, tissue tropism, and potential transmission pathways. Objectives include: 2a) Strain characterization and sequencing through culture of

Brucella spp. from tissues collected during necropsy, confirmation of isolates using PCR, performing whole genome sequencing and phylogenetic analysis, and comparison of strain diversity across individuals, age classes, and time periods. 2b) Tissue localization: Apply RNA/DNA in situ hybridization (ISH) to detect *Brucella* within tissues, evaluate localization in nervous tissue, lung, lymphatic tissue, and reproductive tract, and assess relationships between localization, lesion distribution, age class, and co-infections and 2c) Environmental DNA (eDNA) surveillance: Collect water and sediment samples from dolphin-use habitats in the MSS, extract and analyze eDNA for dolphin DNA and *Brucella* spp. DNA, and evaluate spatial and temporal detection patterns to assess environmental exposure and potential reservoirs. These efforts will provide surveillance tools, improve interpretation of infection significance, and identify spatial and temporal exposure patterns relevant to monitoring and response.

3. Parasitological Assessment and Co-Infection Analysis: We will characterize parasite communities in stranded dolphins and evaluate their role in disease processes. Objectives include: 3a) Morphological, histopathological, and molecular characterization of adult *Nasitrema* spp. and lungworms (*Halocercus* spp.) using classic parasitology techniques and next-generation sequencing. Molecular characterization will support accurate species identification and improve interpretation of parasite-associated disease. 3b) Development of a multiplex quantitative PCR assay for distinguishing and quantifying the burden of *Nasitrema* spp. This assay will be used to test archived blowhole swabs and fecal samples to investigate overall prevalence in both antemortem and postmortem cases. Environmental DNA extracts from Objective 2c will also be tested using this multiplex qPCR assay to infer temporal and spatial trends of *Nasitrema* spp.-DNA in the Mississippi Sound. These data will inform possible areas of endemicity and guide sampling efforts to investigate the life cycle of these *Nasitrema* spp. through collection of intermediate hosts. 3c) Intermediate hosts likely consist of prey fish species of bottlenose dolphins in the Mississippi Sound. Fish will be collected opportunistically throughout the year and in conjunction with Mississippi Department of Wildlife, Fisheries, and Parks collection events. Preliminary data on the dietary composition of bottlenose dolphins in the Mississippi Sound has been generated and will be used to guide fish species to be prioritized for examination of parasites. Fish necropsies will be performed and larval trematode metacercariae will be sequenced as described previously, and any sharing high sequence identity with DNA generated from adult *Nasitrema* will be considered a linkage in the trophic level transmission of these parasites. These objectives utilize data collected from stranding events to influence future efforts to monitor these parasites and may offer insight into ways to limit exposure through long-term monitoring. These data will support identification of parasite-associated risk factors and improve differentiation between incidental infections and those contributing to morbidity or mortality.

Category B: Mitigation of damage to fish, wildlife or natural resources (650 characters)

Bottlenose dolphins in the Mississippi Sound are sentinel indicators of coastal ecosystem health. This project mitigates damage to natural resources by improving diagnosis and management of diseases impacting this population, particularly *Brucella ceti* and parasitic co-infections. Through systematic necropsies, pathogen characterization, and environmental DNA surveillance, we will evaluate potential transmission pathways, tissue impacts, and environmental exposure patterns. These data will strengthen interpretation of stranding events, inform targeted monitoring, and guide management actions that reduce disease risk, support population resilience, protect the broader coastal ecosystem, and reduce zoonotic disease risk.

Project Timetable/Milestones:

Year 1: Project Initiation and Baseline Data Collection

- Initiate systematic necropsy program for stranded dolphins in the Mississippi Sound

- Establish standardized sampling, archiving, and diagnostic workflows
 - Begin bacteriological, molecular, histopathological, and parasitological analyses
 - Initiate *Brucella* isolation, PCR confirmation, and sequencing efforts
 - Begin preliminary parasite collection and morphological characterization
 - Launch eDNA sampling of water and sediment at key dolphin-use sites
- Milestones: Established necropsy pipeline; initial dataset of dolphin health metrics; first *Brucella* isolates sequenced; initial eDNA detection data
- MDMR Deliverable: Deliver initial briefing to MDMR summarizing baseline findings

Year 2: Integrated Pathogen and Co-Infection Analysis

- Expand necropsy dataset across seasons and age classes
 - Conduct in situ hybridization (ISH) to define *Brucella* tissue tropism
 - Complete comparative genomic and phylogenetic analyses of *Brucella* strains
 - Advance molecular characterization of parasites (NGS-based sequencing)
 - Develop and validate multiplex qPCR assay for *Nasitrema* spp.
 - Continue and refine eDNA surveillance and spatial-temporal analyses
- Milestones: Defined *Brucella* localization patterns; validated parasite diagnostics; integrated datasets linking pathology, infection, and co-infection
- MDMR Deliverable: Provide interim risk-factor analysis and management briefing to MDMR

Year 3: Synthesis, Environmental Linkages, and Management Translation

- Complete integrated analysis of necropsy, pathogen, parasite, and eDNA datasets
 - Identify associations among *Brucella*, parasitism, lesion patterns, and environmental detection
 - Evaluate potential environmental reservoirs and transmission pathways
 - Develop risk-based interpretation framework for stranding events
 - Disseminate findings through reports, publications, and stakeholder outreach
- Milestones: Comprehensive dataset and synthesis; management-relevant recommendations; final reports and publications; guidance for long-term monitoring and mitigation strategies
- MDMR Deliverable: Deliver final management guidance document and decision-support framework to MDMR

In what way does this project meet the goals and objectives of the Department of Marine Resources, which includes enhancing, protecting and conserving the marine interest of Mississippi for present and future generations?

This project directly supports the Mississippi Department of Marine Resources' mission to enhance, protect, and conserve marine resources by addressing key threats to the health of bottlenose dolphins, an important sentinel species in the Mississippi Sound. By improving understanding of disease processes—particularly *Brucella ceti* infection and parasitic co-infections—this work strengthens the scientific foundation needed to interpret stranding events and assess population health.

Through systematic necropsies, pathogen characterization, and environmental DNA monitoring, the project generates actionable data on disease drivers, transmission pathways, and environmental exposure. These findings will inform more effective monitoring, early detection of emerging health threats, and science-based management strategies. By identifying and mitigating disease risks that impact dolphin populations and their habitats, the project contributes to maintaining ecosystem balance and resilience.

Ultimately, this research enhances the long-term stewardship of Mississippi's marine resources, supporting sustainable conservation efforts that benefit both current and future generations.

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Date 9/1/2027-8/31/2030

Salaries and Wages			YEAR 1	YEAR 2	YEAR 3	TOTAL
	Base Salary	Level of Effort				
Dr. Beth Peterman, PI	\$78,381	39.62%	\$31,051	\$31,982	\$32,942	\$95,974
Dr. Kaylin McNulty, CoPI	\$147,865	10.00%	\$14,787	\$15,230	\$15,687	\$45,704
Dr. Ethan Woodyard, CoPI	\$ 87,550	10.00%	\$8,755	\$9,018	\$9,288	\$27,061
Dr. Graham Rosser, CoPI	\$ 117,560	10.00%	\$11,756	\$12,109	\$12,472	\$36,337
Dr. Lifang Yan, CoPI	\$145,674	5.44%	\$7,930	\$8,168	\$8,413	\$24,511
Dr. Stephen Reichley, CoPI	\$180,188	2.00%	\$3,604	\$3,712	\$3,823	\$11,139
Dr. Mark Lawrence, Co, PI	\$249,274	2.00%	\$4,985	\$5,135	\$5,289	\$15,410
GCAHL Veterinarian - Alexandra Emelianchik	\$78,780	100.00%	\$78,780	\$81,143	\$83,578	\$243,501
Research Associate - Starkville - Jill Hudnall	\$48,720	46.92%	\$22,860	\$23,546	\$24,253	\$70,659
Research Associate - Gulfport - Adithya Bindhu Sreekumar	\$46,093	50.00%	\$23,047	\$23,738	\$24,450	\$71,234
Research Associate - Gulfport - to be determined	\$48,888	50.00%	\$24,444	\$25,177	\$25,933	\$75,554
Laboratory Technician - MVRDL - Jay Thornton	\$68,106	6.48%	\$4,415	\$4,547	\$4,683	\$13,645
Graduate student - histopathology/ISH (Amy Bodine, DVM)	\$33,000	100.00%	\$33,000	\$33,000	\$33,000	\$99,000
Graduate student - parasitology	\$28,000	100.00%	\$28,000	\$28,000	\$28,000	\$84,000
Student worker	\$7,800	100.00%	\$7,800	\$7,800	\$7,800	\$23,400
Intermittent worker	\$15,840	100.00%	\$15,840	\$15,840	\$15,840	\$47,520
Salary Total			\$321,054	\$328,145	\$335,451	\$984,650
Fringe Benefits - 12 month		42.07%	\$99,459	\$102,443	\$105,516	\$307,418
Student Fringe Benefits		0.41%	\$282	\$282	\$282	\$846
intermittent worker Fringe benefits		8.13%	\$1,288	\$1,288	\$1,288	\$3,864
Fringe Benefits Total			\$101,029	\$104,013	\$107,086	\$312,127
Total Salaries, Wages, and Fringe Benefits			\$422,083	\$432,159	\$442,536	\$1,296,778
Travel						
CVM faculty/staff travel to pertinent conferences to present/represent GOMESA			\$ 4,000	\$ 4,000	\$ 4,000	\$ 12,000
Travel to and from MS Gulf coast for sample collection and coordination			\$ 5,000	\$ 5,000	\$ 5,000	\$ 15,000
Travel Total			\$ 9,000	\$ 9,000	\$ 9,000	\$ 27,000
Equipment						
-20C freezer for storage of dolphin tissue samples				\$ 8,000.00		\$ 8,000.00
Total Nonexpendable Equipment			\$ -	\$ 8,000.00	\$ -	\$ 8,000.00
Other						
Materials and Supplies						
Necropsy supplies			\$ 5,000	\$ 5,000	\$ 5,000	\$ 15,000
Molecular reagents and testing supplies (PCR, DNA extractions kits etc)			\$ 12,000	\$ 12,000	\$ 12,000	\$ 36,000
Culture media/bacteriology supplies			\$ 5,000	\$ 5,000	\$ 5,000	\$ 15,000
Histology & ISH reagents and supplies			\$ 5,000	\$ 5,000	\$ 5,000	\$ 15,000
bacterial isolate sequencing			\$ 3,000	\$ 3,000	\$ 3,000	\$ 9,000
Parasitology supplies			\$ 5,000	\$ 5,000	\$ 5,000	\$ 15,000
parasite sequencing			\$ 5,000	\$ 5,000	\$ 5,000	\$ 15,000
eDNA sampling supplies			\$ 3,000	\$ 3,000	\$ 3,000	\$ 9,000
eDNA sequencing and analysis			\$ 5,000	\$ 5,000	\$ 5,000	\$ 15,000
Laboratory consumable supplies			\$ 10,000	\$ 10,000	\$ 10,000	\$ 30,000
Total Materials and Supplies			\$ 58,000	\$ 58,000	\$ 58,000	\$ 174,000
Contractual Services						
Booth rental at GulfCon or All Hands Meetings for dissemination of information			\$ 5,000	\$ 5,000	\$ 5,000	\$ 15,000
Conference registrations fees. one conference per year up to 5 people at \$600/person			\$ 3,000	\$ 3,000	\$ 3,000	\$ 9,000
Publication costs				\$ 4,000	\$ 4,000	\$ 8,000
Total Contractual Services			\$ 8,000	\$ 12,000	\$ 12,000	\$ 32,000
Additional Student costs						
Graduate student tuition	100.00%		\$ 31,000	\$ 32,548	\$ 34,172	\$ 97,720
* GRA Insurance			\$ 4,572	\$ 4,792	\$ 5,032	\$ 14,396
Total Additional student costs			\$ 35,572	\$ 37,340	\$ 39,204	\$ 112,116
Total Other Costs			\$ 101,572	\$ 107,340	\$ 109,204	\$ 318,116
Total Direct Costs			\$532,655	\$556,499	\$560,740	\$1,649,894
Modified Total Direct Costs (MTDC)			\$ 501,655	\$ 515,951	\$ 526,568	\$ 1,544,174
Total Indirect Costs	46.50%		\$ 233,269	\$ 239,917	\$ 244,854	\$ 718,040
Total Project Costs			\$ 765,924	\$ 796,416	\$ 805,594	\$2,367,934

Budget Justification

Title: Protecting Mississippi Sound Dolphins: Understanding the Role of Disease and Parasites in Dolphin Strandings

Mississippi State University Global Center for Aquatic Health and Food Security

SALARIES & WAGES: \$982,310

Funding is requested to support the following personnel responsible for implementing and managing all aspects of the project:

Dr. Beth Peterman (PI; 39.62% effort) will provide overall leadership and scientific direction for the project. She will coordinate all project components, including sample collection, laboratory workflows, data integration, and reporting. Dr. Peterman will oversee communication among all co-investigators, ensure adherence to project timelines and deliverables, and serve as the primary liaison with funding agencies and stakeholders. She will also directly participate in necropsy coordination, histopathological interpretation, and synthesis of multidisciplinary datasets (microbiology, parasitology, molecular, and environmental data) to evaluate drivers of dolphin health outcomes. The total salary requested is \$95,974.

Dr. Kaylin McNulty (Co-PI; 10% effort) will lead histopathological analyses and interpretation of dolphin tissues. She will develop and oversee histological workflows, including slide preparation, staining, and in situ hybridization (ISH) techniques to detect pathogens within tissues. Dr. McNulty will ensure quality control of histological data and integrate findings with microbiological and molecular datasets to support disease diagnostics and mechanistic understanding of lesions. The total salary requested is \$45,704.

Dr. Ethan Woodyard (Co-PI; 10% effort) will co-lead parasitological investigations and contribute to data integration and analysis. He will assist in parasite identification, quantification, and interpretation of parasitological findings in relation to dolphin health. Dr. Woodyard will also contribute to data management, statistical analyses, and integration of parasitology results with microbiological and histopathological datasets, as well as assist with reporting and manuscript preparation. The total salary requested is \$27,061.

Dr. Graham Rosser (Co-PI; 10% effort) will co-lead parasitological analyses and oversee parasite identification and associated molecular workflows. He will supervise parasite characterization, including sequencing efforts, and evaluate the role of parasitic infections in dolphin morbidity and mortality. Dr. Rosser will coordinate closely with Dr. Woodyard to integrate parasitology results with other diagnostic findings across the project. The total salary requested is \$36,337.

Dr. Lifang Yan (Co-PI; 5.44% effort) will support molecular and microbiological analyses, including DNA extraction, PCR assay development, and sequencing workflows. Dr. Yan will contribute to characterization of bacterial communities and pathogens associated with dolphin tissues and environmental samples, and assist with interpretation of molecular data in the context of disease processes. The total salary requested is \$24,511.

Dr. Stephen Reichley (Co-PI; 2% effort) will contribute to project coordination, data interpretation, and integration of biological findings across study components. He will assist in synthesizing microbiological, parasitological, and histopathological results to support evaluation of factors influencing dolphin health and mortality patterns, and will contribute to manuscript preparation and dissemination of results. The total salary requested is \$11,139.

Dr. Mark Lawrence (Co-PI; 2% effort) will provide oversight and guidance for the project bacteriological and microbiological analyses. He will assist with study design related to pathogen detection, advise on laboratory methodologies for bacterial culture and identification, and support interpretation of microbiological findings in dolphin tissues. Dr. Lawrence will also contribute to integration of microbiological results with other diagnostic datasets and to dissemination of project outcomes. The total salary requested is \$15,410.

Salary support is requested for a Gulf Coast Aquatic Health Lab (GCAHL) Veterinarian (Alexandra Emelianchik; 100% effort) who will lead all necropsy and diagnostic sampling efforts for stranded dolphins. She will perform comprehensive gross examinations and ensure consistent, high-quality collection of tissues for microbiological, molecular, histopathological, and parasitological analyses. The veterinarian will coordinate closely with stranding network partners and field personnel along the Mississippi Gulf Coast to facilitate timely recovery of carcasses and samples. She will also oversee proper sample handling, preservation, and documentation, including maintenance of chain-of-custody records and compliance with biosafety and regulatory standards. In addition, she will contribute to diagnostic interpretation and work collaboratively with project investigators to integrate findings across disciplines. The total salary requested is \$243,501.

Two research associates (Jill Hudnall in Starkville, MS 46.92% effort and Adithya Bindhu Sreekumar in Gautier, MS; 50% effort) and one additional research associate in Gautier (to be determined; 50% effort) will conduct sample collection, processing, laboratory analyses, and data management. The total salary requested for these positions is \$217,447.

A laboratory technician (Jay Thornton; 6.48% effort) will support diagnostic and laboratory workflows at the Mississippi Veterinary Research and Diagnostic Laboratory (MVRDL). Responsibilities will include performing and assisting with *Brucella* PCR diagnostics and bacteriological culture and identification, as well as supporting general microbiological testing of dolphin tissue samples. The technician will also assist with sample processing, data recording, quality control procedures, and maintenance of laboratory equipment to ensure accurate and timely diagnostic results. The total salary requested is \$13,645.

Salary/stipend support is requested for two graduate students (histopathology/ISH and parasitology; 100% effort each) will play key roles in conducting laboratory analyses and supporting data integration. The histopathology/ISH graduate student (DVM; \$33,000/year) will be responsible for tissue processing, slide preparation, staining, and implementation of in situ hybridization (ISH) assays to detect specific pathogens within dolphin tissues. With veterinary training, this student will assist with microscopic evaluation of lesions, interpretation of pathological findings, and correlation of histopathological results with gross necropsy observations and diagnostic data. This role will be critical for accurate disease characterization and integration of pathology findings with microbiological and molecular results. The parasitology graduate student (\$28,000/year) will assist with parasite identification, quantification, and sample processing, including preparation of specimens for both morphological and molecular analyses. Responsibilities will include supporting parasite sequencing efforts, maintaining detailed records of parasitological data, and contributing to interpretation of parasite-related findings in the context of dolphin health. Both students will participate in data analysis, preparation of reports and manuscripts, and presentation of research findings at scientific meetings, providing essential support for achieving project objectives while receiving advanced research training. Total salaries requested are \$99,000 and \$84,000, respectively.

A student worker (\$23,400 total) and intermittent worker (\$47,520 total) will assist with sample processing, data entry, and laboratory support tasks.

FRINGE BENEFITS: \$312,127

Fringe benefits are calculated at 42.07% for 12-month benefits eligible employees, 0.41% for students, and 8.13% for intermittent workers.

TOTAL SALARIES, WAGES AND FRINGE: \$1,296,778

TRAVEL: \$27,000

Travel funds are requested to support both field activities and dissemination of project results. Travel to and from the Mississippi Gulf Coast is essential for coordination with stranding network partners and for collection of dolphin tissue samples and associated environmental data. These trips will ensure timely response to strandings and proper sample acquisition, which is critical for meeting project objectives.

Funds are also requested to support travel for project personnel to attend and present findings at relevant scientific conferences. These meetings provide important opportunities to disseminate results to the scientific community, engage with stakeholders, and facilitate collaboration and knowledge exchange related to marine mammal health and Gulf ecosystem management.

TOTAL TRAVEL: \$27,000

EQUIPMENT: \$8,000

Funds are requested for the purchase of a laboratory freezer (-20 °C) for long-term storage of dolphin tissue samples. This equipment is essential to preserve sample integrity for microbiological, molecular, histopathological, and parasitological analyses and to ensure proper archiving of specimens for repeat and future testing as needed.

TOTAL EQUIPMENT: \$8,000

OTHER:

MATERIALS & SUPPLIES: \$174,000

Materials and supplies are requested to support laboratory analyses, field sampling, and diagnostic testing essential to project objectives. These costs include reagents and consumables required for microbiological, molecular, histopathological, parasitological, and environmental DNA (eDNA) analyses of dolphin tissues and associated samples. Necropsy supplies are requested to support tissue collection, preservation, and processing during dolphin examinations. Molecular reagents and supplies, including PCR reagents, DNA extraction kits, and sequencing-related materials, are required for pathogen detection, microbial characterization, and genetic analyses. Bacteriology supplies, including culture media and associated consumables, will support isolation and identification of bacterial pathogens. Histology and in situ hybridization (ISH) reagents are necessary for tissue processing, staining, and detection of pathogens within lesions. Parasitology supplies and sequencing costs will support parasite identification, quantification, and molecular characterization. Funds for eDNA sampling supplies and sequencing are requested to assess environmental microbial communities and potential pathogen presence in surrounding habitats. General laboratory consumables, including gloves, pipette tips, tubes, and other routine supplies, are required for daily laboratory operations and sample processing.

These materials are essential to ensure accurate, reliable, and timely analysis of samples and to support the multidisciplinary approach required to evaluate factors influencing dolphin health.

CONTRACTUAL SERVICES: \$32,000

Contractual funds are requested to support dissemination of project findings and participation in scientific meetings. Costs include booth rental at the Gulf of America Alliance (GOAA) GulfCon conference or All Hands meetings to facilitate outreach and communication of project results to stakeholders, resource managers, and the public.

Funds are also requested for conference registration fees to allow project personnel to attend and present findings at relevant scientific meetings, supporting knowledge exchange and collaboration within the marine mammal health and Gulf research communities.

Publication costs are included to support the preparation and open dissemination of project results in peer-reviewed scientific journals, ensuring broad accessibility of findings to the scientific community and stakeholders.

ADDITIONAL STUDENT COSTS: \$112,116

Tuition costs are requested for 2 graduate students \$31,000 for year 1, \$32,548 for year 2, and \$34,172 for year 3. That calculates to \$1,281 (FY28) per month for 10 months and \$1,345 (FY29) for 2 months for year 1 with a 5% increase each year thereafter. Total tuition costs requested are \$97,720.

Graduate Research Assistant insurance costs are requested for the 2 students. In year 1 at the rate of \$189 (FY28) per month for 10 months and \$198 (FY29) for 2 months for a total of \$4,572. In year 2, a total of \$4,792 and year 3 a total of \$5,032 is requested. A 5% increase has been added in years 2 and 3. Total insurance requested for the project is \$14,396.

TOTAL OTHER COSTS: \$318,116

Total Direct Costs: \$1,649,894

Modified Total Direct Costs: \$1,544,174

Total indirect costs: \$718,040

Indirect costs are calculated at 46.5% of the modified total direct costs: MTDC exclude tuition and equipment.

Total Project Costs: \$2,367,934