



GoMAMN STRATEGIC BIRD MONITORING GUIDELINES: AVIAN HEALTH

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INTRODUCTION

THE GULF OF MEXICO (GOM) HAS A RICH DIVERSITY of avian species, comprised of residents and migrants from a wide geographic range (Burger 2018). These birds have encountered substantial changes in the quality and availability of coastal, terrestrial, and marine habitats in the GoM, including anthropogenic and natural stressors. A primary concern for this region is environmental contamination associated with the high concentration of chemical/petrochemical industries (Inglis et al. 2014), and oil and gas operations with associated activities in Louisiana and Texas. Elevated levels of mercury are also commonly found in fish throughout much of the northern GoM, distinct from other environmental chemicals. Habitat quantity and quality is also being directly impacted by increasing rates of urbanization, red tides, and natural weather events such as hurricanes. For example, the full implications of Hurricanes Harvey and Maria are still emerging, especially for avian populations (Burger 2017, Ward 2017). Trends in infectious diseases that impact avian health are also changing as a result of warming and increased rainfall. Stressors such as contaminants and poor habitat quality worsen the impacts of disease on avian populations. Furthermore, runoff from agricultural, residential, and industrial areas often carries substantial concentrations of fertilizers and environmental chemicals, which may result in water quality degradation, toxic algal blooms, or otherwise increase the risk of exposure to both humans and wildlife to potentially toxic chemicals. As such, both migratory and resident birds are exposed to a suite of environmental challenges including complex chemical exposures and weather related events. Collectively, these natural and anthropogenic stressors set the context and provide the impetus for understanding implications to avian health across the northern GoM.

Across the northern GoM, significant restoration efforts are being implemented in the wake of the Deepwater Horizon oil spill by a myriad of conservation partners (DHNRDAT 2016, GCERC 2016, NFWF 2018). To ascertain the effectiveness of these restoration activities, it is imperative that land managers understand both population- and individual-level effects. To that end, avian health metrics can serve as

reliable indicators of long-term system restoration and success, separate from, or in conjunction with abundance and reproductive metrics. For example, restoration efforts may increase local bird abundance via immigration and/or increased reproductive success, while long-term, negative physiological outcomes to populations and overall poor ecosystem health may still persist. Short-term increases in abundance (e.g., bird abundance) alone may not be reflective of population health; it is important to have health and fitness metrics in order to make informed conclusions and decisions related to management effectiveness and overall restoration success. Thus, long-term comparisons of health and vitality of avian populations are warranted for land managers to accurately assess restoration activities across the northern GoM.

In this chapter, we review avian physiological adaptations related to migration, survival, and reproduction to provide a foundation upon which an avian health assessment can be conducted. Drawing upon these adaptations and work presented in the previous taxon-specific chapters (Chapters 3-9 herein), we present diagrams and a table to more precisely link physiological attributes to restoration actions to provide a guiding framework for the collection of avian health metrics. For the purpose of this document, we define health to include, but not be limited to selected measures that have been utilized in the field and/or otherwise shown to be indicative of stress-related responses. Further, we define health assessment in the context of birds and as such, encompass the concepts of fitness or condition within the consideration of health assessments. Other aspects of avian health, particularly those that are invasive or not stable measurements for field applications are also often part of health assessments, but are not treated here since they represent more complex approaches that may be beyond basic monitoring. The information and recommendations herein are intended to facilitate the ability of resource managers to establish avian health and fitness baselines. Moreover, these baselines will contribute to the conduct of future avian health assessments that document: 1) positive effects to avian populations afforded by restoration programs; and/or 2) physiological metrics providing an underpinning to assessing success of restoration efforts.

AVIAN LIFE HISTORY AND PHYSIOLOGICAL LINKAGES

Stressors for bird populations in the GoM can take many forms, such as hurricanes, droughts, pollution from industrial sources, pesticides and other pollutants in runoff from agriculture and urban areas, changes in food abundance, predation pressure, and infectious disease (Ottinger et al. 2009; Hooper et al. 2013; Bursian et al. 2017a). Moreover, habitat loss and fragmentation are among the most significant factors affecting avian populations (e.g., Fahrig 1997, 1998, 2001, 2003). Stressors are always present in the environment and as such can impact individuals separately; population-level effects are often the result of a particular suite of stressors experienced at a given time or in a cumulative fashion. The context encountering stressor(s) provides a context for response and potential adverse outcomes. Birds may be differentially impacted by stressors depending on their life history strategies. In short, life history traits reflect a series of events that govern a bird's life—birth, fledging, maturation, reproduction, and death. More specifically, timing of juvenile development, age of sexual maturity, number of offspring, level of parental investment, aging, and lifespan are dependent upon the physical and ecological system within which the bird lives (Lack 1968, Stark and Ricklefs 1998, Martin 2004). Additionally, understanding avian physiological adaptations can provide insight into the potential mechanisms that underlie avian responses to such environmental stressors. While all vertebrate species have similarities in physiological and developmental processes, as a group, birds have developed a suite of unique characteristics. These include specific adaptations to the reproductive, metabolic, immune, visual, and auditory systems, as well as general physiological adaptations including high body temperature, and lightweight bones with specialized microstructure (Sullivan et al. 2017). Below, we provide a high-level review of avian life history strategies and physiological processes important for understanding and assessing avian health and response to environmental stressors.

A centerpiece of life history theory is the trade-off between reproductive effort and survival of individuals (Williams 1966), which is widely supported by patterns of fecundity and survival in experimentally manipulated populations of wild and laboratory animals (Stearns 1992). Reproduction is inherently costly for both ecological and physiological reasons. Because reproduction requires extra nutrients, breeders risk predation (Magnhagen 1991), parasitism (Apanius and Schad 1994, Knowles et al. 2009), and other ecological consequences (e.g., competition) associated with increased foraging. In birds, increased foraging effort results in increased reproductive output, but decreased

parental body condition and survival (Daan et al. 1996, Golet and Irons 1999), in part due to elevated energy demands (Potti et al. 1999). It is presumed that parents reduce self-maintenance processes (e.g., immune function) and draw from body reserves, in order to fuel the additional physical activity. Hence, short-lived species are expected to invest more in current reproductive attempts and less in overall immune defense, because the reproductive value of their current brood is high relative to potential future broods. In contrast, the reproductive value of the current brood is low relative to potential future broods for long-lived birds because they have fewer natural extrinsic causes of adult mortality (Stearns 1992). Thus, long-lived species should have relatively higher allocations of resources to self-maintenance functions compared to that of short-lived species, particularly related to immune functions.

Looking more closely at avian ontogeny from the lens of physiological process and functional responses reveals two different strategies: 1) altricial chick development; and 2) precocial chick development, suggested by Starck and Ricklefs (1998) as endpoints along a spectrum. Altricial species (e.g., passerines) hatch in a relatively immature state and require parental care until at least fledging. At the other end of the spectrum, precocial species (e.g., waterfowl) are fairly well developed and mobile at hatching and require little parental care. Altricial and precocial birds appear to have differential risk from environmental chemical exposure because sexual differentiation of endocrine and behavioral components of the reproductive system develop later for altricial species compared to precocial species (Adkins-Regan et al. 1990). While exposure to environmental chemicals *in ovo*, especially to endocrine disrupting chemicals can be extremely damaging to individuals in both groups (Ottinger and Dean 2011), precocial birds are primarily impacted during embryonic development, while altricial birds remain vulnerable for an extended post-hatching period. Understanding these physiological processes and functional outcomes, especially related to the response of reproductive, immune, endocrine, and organ systems to environmental chemical exposures can provide invaluable insights into individual and population health status.

The thyroid system modulates and maintains metabolic homeostasis; it is critical for pre-migratory fattening and for migratory energy utilization (McNabb, 2007). Stressors activate adrenal hormones, which can act beneficially as a hormetic to stimulate homeostatic and immune responses. However, chronic stress ultimately impairs fitness through reduced physiological resilience and reproductive success (Calabrese et al. 2001).



Nesting Double-crested Cormorants (*Phalacrocorax auritus*). Photo credit: Donna A. Dewhurst

Birds have a relatively high metabolic rate and body temperature (40.6°C). A high metabolic rate and elevated body temperature may contribute to altered toxicokinetics with exposure to environmental chemicals. For example, raptors (e.g., Osprey [*Pandion haliaetus*] and Bald Eagles [*Haliaeetus leucocephalus*]) experience high rates of bioaccumulation of pollutants leading to weakening of eggshells (Grier 1982). Further, these bioaccumulated lipophilic compounds including environmental pollutants may be released from storage in fat cells during times of high energy utilization such as with migration. Hence, it is important to link environmental stressors to the health of individual birds and ultimately to avian populations.

Many birds also have unique physiological and endocrine characteristics that support long-distance migration and survival under highly variable and sometimes extreme conditions (Gill 2007, Ricklefs 2010). In addition to having lighter bones and shorter gastrointestinal tracts than mammals, birds have a highly efficient respiratory system in which the passage of air is aided by numerous air sacs (Gill 2007). Feather integrity is critical for flight, and can be compromised by oil exposure, which subsequently impairs flight and thermoregulation (Maggini et al. 2017). As mentioned above, the thyroid system promotes fat storage and modulates energy utilization during flight. Any compromise to these metabolic systems and/or feather integrity can inhibit flight performance or reduce individual body condition during migration, subsequently leading to compromised reproductive success or survival.

The link between environmental stressors and lifespan is a critical factor to assess with regard to fitness as it brings together health, productivity, and longevity of individuals

(Hausmann and Heidinger 2015). While it would be predicted that high body temperature and metabolic rate would result in short lifespans, surprisingly many birds, including hummingbirds, parrots, and seabirds exhibit remarkably long lifespans compared to mammals of equivalent body size (Ottinger et al. 1995, Nisbet et al. 1999, Holmes and Ottinger 2006, Ottinger and Lavoie 2007, Finch 2009). Long-lived birds have physiological and behavioral adaptations supporting long life, including resistance to oxidative damage (i.e., the ability to detoxify reactive compounds and repair the damage they cause; Ogburn et al. 2001, Ottinger, 2018) and the ability to prioritize adult survival over annual productivity (Drent and Daan 1980). That is, long-lived birds can forgo breeding in order to survive brief stressors and breed again when conditions improve. As such, long-lived bird species are better able to deal with exposures to pollutants (physiologically) than are short-lived species. However, they are not tolerant, and when long-lived species are affected in a way that increases adult mortality, it has a larger effect on their population stability because of their slow reproductive rate (e.g., Croxall and Rothery 1991). Thus, exposure to chemicals and other health consequences that increase adult mortality can have disproportionate impacts on long-lived species (Congdon et al. 1994).

Dramatic increases over the past century have occurred in the production and use of chemicals in industrial, agricultural, and residential settings that have resulted in a wide diversity of chemical pollutants in the coastal and marine systems of the northern GoM. Increasing exposure to pollutants heightens the risk of adverse effects (Cheek et al. 1995; Ottinger et al. 2009). The potential for adverse effects of these pollutants is a complex issue due to the range

of pollutants in our environment, the diversity of actions and potencies, bioavailability, life-cycle of compounds, and myriad of exposure scenarios. Nevertheless, a suite of recent publications has documented adverse consequences from oil exposure stemming from the Deepwater Horizon Oil Spill (DWH) (see Bursian et al. 2017 for review). Specifically, studies of Laughing Gulls (*Leucophaeus atricilla*) and Double-crested Cormorants (*Phalacrocorax auritus*) showed increased oxidative damage and deleterious effects on cardiac tissue, and mortality of some birds (Horak et al. 2017, Pritsos et al. 2017, Harr et al. 2017). Fallon et al. (2017) documented physiological damage to a range of species from even light levels of oiling. Homing Pigeons (*Columba livia domestica*) showed altered flight paths after light oiling, suggesting both impaired navigational capabilities and flight ability (Perez et al. 2017). Western Sandpipers (*Calidris mauri*) exposed to ingested oil showed reduced blood and liver related responses to contaminant exposure, and histological indicators of a stress related adrenal response (Bursian et al. 2017). Birds also had difficulties with takeoff and flight maintenance following feather oiling with small amounts of crude oil (Maggini et al. 2017). Seaside Sparrows (*Ammodramus maritimus*) living in areas exposed to Deepwater Horizon oil had radiocarbon signatures indicating that the oil entered the terrestrial food-web and demonstrated reduced reproductive success in oiled areas (Bonisoli-Alquati et al. 2016). Exposure to sub-lethal levels of contaminants has also been linked to increased susceptibility of avian species to infectious diseases as a result of immunosuppression (Grasman 2002, Fairbrother et al. 2004, Acevedo-Whitehouse et al. 2009). As such, it is imperative that both short-term toxicological studies and long-term cumulative assessments on overall fitness (recognizing difference in life history strategies) of individuals and the potential impact on avian populations be implemented to ascertain efficacy of restoration programs.

ECOSYSTEM RESTORATION AND MEASURES OF FITNESS AND HEALTH

The large-scale restoration underway in the northern Gulf of Mexico under the RESTORE Act, National Fish and Wildlife Foundation, and Natural Resource Damage Assessment Trustee Council presents an opportunity to increase wildlife populations and improve their habitats. Collectively, state and federal agencies in partnership with numerous conservation organizations and citizen groups are making substantial conservation investments along the coasts of Florida, Alabama, Mississippi, Louisiana, and Texas. This unprecedented investment in ecosystem restoration along the Gulf Coast requires accountability for the effectiveness of large-scale restoration efforts across a broad geographic area.

To that end, the millions of birds using the northern Gulf of Mexico (for all or part of their annual life-cycle) provide an unparalleled indicator of ecosystem health (Burger 2017, 2018). The Gulf ecosystem supports hundreds of avian species that occupy virtually all trophic levels within the northern GoM food web and are direct beneficiaries of most restoration projects, regardless of the resource for which the restoration project was designed. As such, the overall health and fitness of birds may offer an opportunity to assess the collective benefits of diverse and broad-scale restoration efforts. Unfortunately, more information is needed regarding which health metrics are most appropriate, most informative, or most cost-effective/convenient for practitioners to collect in the field.

Frequently used indicators of avian population health are estimates of adult/juvenile survival (e.g., Maness and Anderson 2013) and reproductive success, as articulated in the previous taxa-specific chapters (see Chapters 3-9). However, measuring survival and reproductive success is often logistically difficult and costly. Physiological health metrics provide potential (cheaper/easier) alternative measures of population health. Physiological health metrics can also illuminate mechanisms underlying changes in population health, in that the health of individuals determines their productivity and survival, which ultimately drives both short- and long-term population status. Although data are available about potential impacts of chemicals and other stressors to avian populations, gaps still exist in our ability to directly link life history traits (e.g., reproductive success) to specific physiological metrics (see Lamb et al. 2016), and subsequently, to potential adverse outcomes and risk for wild birds. Monitoring specific health metrics of avian populations in tandem with other monitoring programs (e.g., abundance) will provide essential information about the current status of individuals within a population (Mallory et al. 2010), and possible species-specific differential health effects related to a variety of environmental stressors.

As previously stated, limited data currently exist related to avian health assessments in the northern GoM. This situation is further complicated by the fact that the environmental stressors impacting the system are not mutually exclusive. In that, while we collectively work to restore the northern Gulf ecosystem in the wake of DWH oil spill, there are a variety of concurrent stressors influencing birds and their habitats to include: frequently occurring, relatively small oil spills (BOEM 2018); contaminant laden runoff from agricultural practices and urbanization (EPA 2018); extreme weather events (e.g., hurricanes, drought); and a wealth of complex ecological processes (e.g., predation, parasitism, infectious diseases, competition) being disrupted via loss and fragmentation of habitat. Hence, without very specific and targeted questions, it is and will continue to be, extremely

difficult to disentangle all the background noise associated with avian health assessments. Nevertheless, an understanding of the physiological outcomes and ramifications to overall fitness is critical for understanding ecosystem restoration. Towards that end, we present a suite of potentially useful health metrics, brief overview of available tests and procedures, and conclude with next steps for advancing our collective understanding of avian health in the northern GoM.

Given the complexity of the GoM ecosystem, the myriad of interactions associated with avian health assessments, and the vast number of stakeholders involved (e.g., varying objectives and needs), it is beyond the scope of this Chapter to provide specific, testable hypotheses, *per se*. Instead, it is our goal to provide a framework and means to identify the most pertinent and comprehensive health metrics associated with a suite of environmental stressors thought to be driving the system and bring the available data/information to Gulf Coast veterinary schools, rehabilitation facilities, agencies,

managers, and others. Below, we provide a brief overview of exposure routes and how each stressor is presumed to disrupt physiological processes, thereby manifesting itself through a demographic response at either the individual or population-level.

Stressors, Exposure Pathways and Physiological Impacts

EXPOSURE PATHWAYS: The detailed and encompassing influence diagram (Figure 10.1) articulates the various exposure routes and associated risks for birds following the DWH oil spill. For some environmental stressors (contaminants), exposure occurs both through external contact with feathers and skin and internally through ingestion (preening and feeding) and inhalation. Hence, the various exposure routes lead to both direct and long-term impacts, which may affect taxa or individuals differently due to variability in life history strategies (see above). For example, we

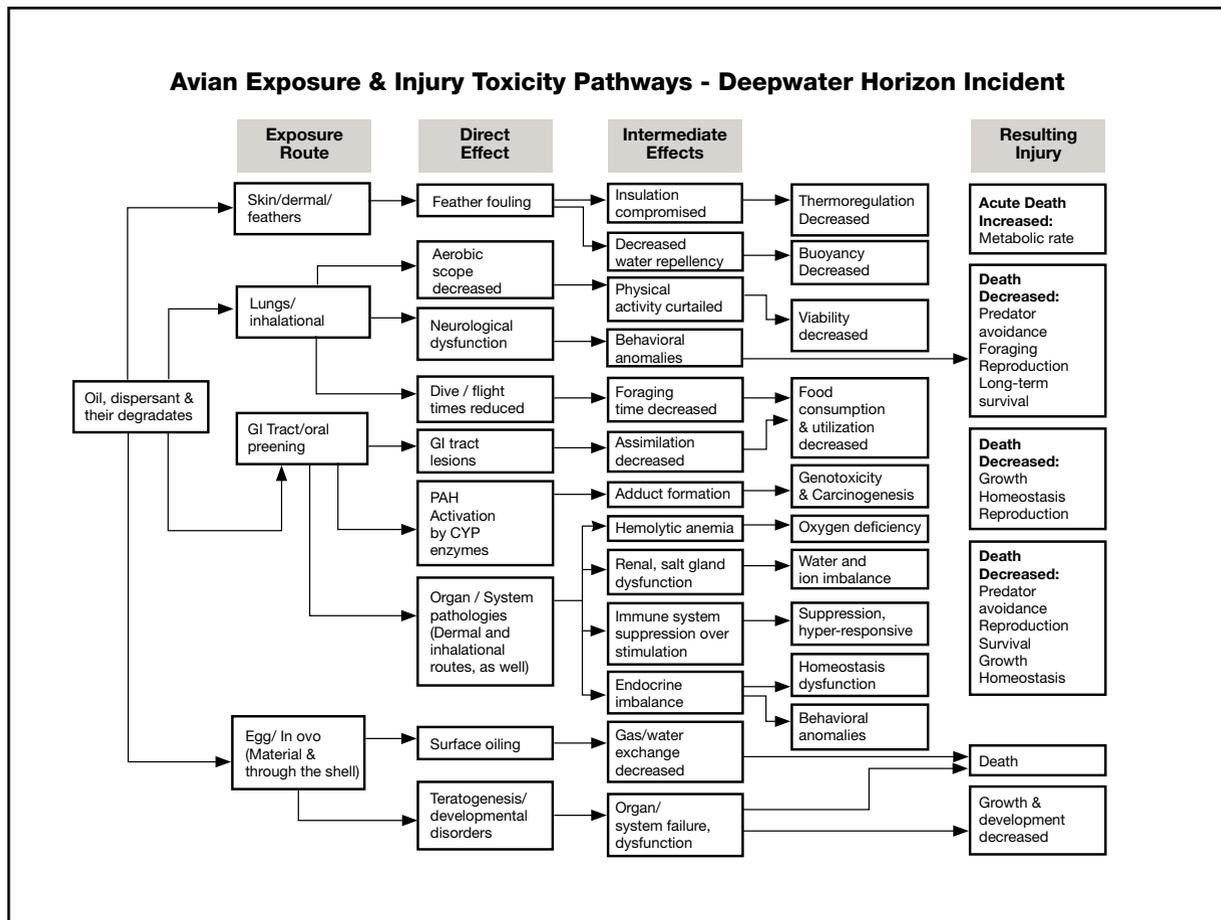


Figure 10.1. Influence Diagram showing potential routes of exposure, direct and intermediate effects, responses, and fate for exposure to toxicants associated with the Deepwater Horizon Oil Spill (Adapted from Milton et al. 2003 with modifications by Michael Hooper, U.S. Geological Survey).

know that females deposit both lipophilic and water-soluble compounds into the yolk and albumin, respectively, thereby exposing their embryos throughout development (Lin et al. 2004; Ottinger et al. 2000). Similarly, oils and dispersants on the exterior of the egg are readily absorbed through the eggshell matrix and pores, also exposing developing embryos. The direct and indirect effects of the specific exposure routes also range widely, including physical (e.g., feather fouling), physiological (e.g., neural dysfunction, liver enzyme activation resulting in higher detectable blood enzyme levels teratogenic effects), and functional/behavioral outcomes (e.g., impaired flight and navigation, organ system pathology) thereby impacting individuals through a myriad of mechanisms: all with negative consequences to growth and survival via lethal or sub-lethal adverse outcomes. Hence, the various exposure routes via which some stressors (e.g., contaminants) impact birds, as well as seasonality (e.g., breeding vs. wintering, vs migration), also warrant consideration. The exposure pathways emphasized here are relevant to potential health impacts associated with DWH; however we recognize that there are a variety of other environmental stressors and exposure pathways to also consider, for example sources of contaminants from agrichemicals, wastewater and pollutants allowed under

the National Pollution Discharge Elimination Permits, etc.

To facilitate our ability to articulate the physiological relationships, influences, and uncertainties associated with a variety of environmental stressors beyond the DWH Oil Spill, we developed an influence diagram (Figure 10.2) that elucidates the physiological impacts and associated responses for a variety of environmental stressors. In brief, each environmental stressor is associated with one or more physiological and functional responses at the individual-level, while noting the complex interactions and relationships among physiological and functional responses (e.g., disruption of metabolic function can have “trickle-down” consequences for immune function and vice-versa). Further, we link these functional responses with presumed demographic responses and provide a short list of potential monitoring metrics. Details of these metrics, including basic collection protocol, logistical constraints, financial costs and uncertainties associated with each metric are detailed in Table 10.1. Hereafter, we provide an overview of each of the environmental stressors including their impact on physiological processes, as well as an overview of each physiological process with implications to demographic responses. Although decision nodes are not delineated, it is important to consider these in the context of

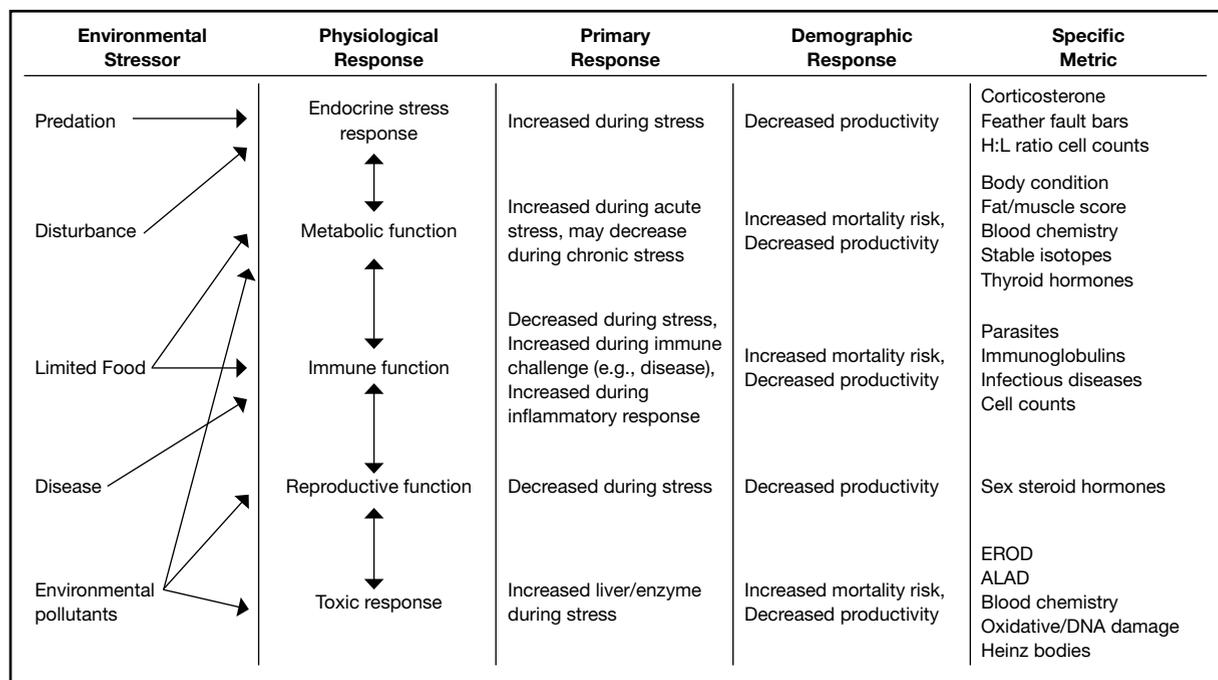


Figure 10.2. Diagram depicting the physiological responses of individual birds to environmental stressors, the primary and demographic responses associated with that physiological response, and the specific metrics that can be used to measure that physiological response. Double headed arrows indicate that physiological responses interact with each other.

restoration assessments involving health metrics. Further, the decision nodes will vary with species along with the metrics used to assess health.

Environmental Stressors

PREDATION: Pressure from predation is a biological stressor that can exert short- and long-term impacts (Clinchy et al. 2004), especially during the breeding season (e.g., Ghalambor and Martin 2002), on many avian taxa. Loss of protective foliage and other cover with loss of habitat, urbanization, and other anthropogenic development often contribute to greater vulnerability to predation. Further, climate or weather-related events can also disturb or modify habitat and protective cover, making nests and individuals more visible and vulnerable. Decreased food quality and/or increased energy demands may increase the amount of time spent foraging, when individuals are more vulnerable to predation. The response to heightened risk and frequency of predation includes an endocrine stress response and associated immune system effects. The endocrine stress response will increase during predation events, and can become chronically elevated if predation pressure continues. Management actions including predator control/removal (see all taxa chapters), habitat restoration (see all taxa chapters), provision of safe nesting sites (e.g., nesting islands; see Seabird Chapter 6), or regulation of shoreline development (see Chapters 4 and 7) could mitigate stress associated with predation risk.

DISTURBANCE: Here we define disturbance as any impact stemming from anthropogenic or natural events (e.g., human activities, hurricanes), which may subsequently result in negative effects. More specifically, disturbance leads to an endocrine stress response at the individual level, which subsequently results in negative effects to immune function (Nelson 2005, Burger et al. 2017). This increases indirect mortality risk (e.g., Grace et al. 2017), leading to population-level effects. Changes to the energetic demands and the physiological stress response both impact immune functions (Acevedo-Whitehouse & Duffus, 2009). Habitat restoration (see all taxa chapters), regulation of human activities (refer to Chapters 6 and 10) and shoreline development (see Chapters 4 and 7) are management actions that can decrease disturbance-related stress.

LIMITED FOOD RESOURCES: Birds respond to limited food in the short-term by increasing the endocrine stress response and metabolic function (i.e., energy mobilization). However, long-term food deprivation will decrease metabolic function and suppress immune function, with long-term negative effects on productivity and survival. Both food quantity (biomass) and quality (energy density and proximate composition) can strongly affect the reproductive success of avian taxa.

The nutritional stress hypothesis posits that food quantity provisioned to chicks affects growth, condition, and survival (Trites and Donnelly 2003), while the junk-food hypothesis posits that food quality is the primary driver (Jodice et al. 2006, Österblom et al. 2008, Lamb et al. 2017). These two hypotheses are not necessarily mutually exclusive within a species or systems and may operate differently depending upon the range of available food items in any given year. Changing climate conditions such as drought or excess rainfall events, as well as commercial fishing pressure can impact the quality and quantity of available food (Hooper et al. 2013). Adults and young are also affected by the quantity and quality of prey available during both the breeding and nonbreeding seasons. When food quantity or quality is insufficient, consequences can exist for birds at all stages of their life cycle, including impaired metabolic function, reduced immune system function, and greater vulnerability to disease and parasites. A similar concern of food quantity and quality during the breeding season is also pertinent for birds on staging and wintering grounds in cross-seasonal carryover effects, particularly for waterfowl. Reduction in food quality or quantity can also increase disease prevalence (Lochmiller et al. 1993, Birkhead et al. 1999, Hoi-Leitner et al. 2001, Strandin et al. 2018) through suppression of certain immune functions (e.g., immunoredistribution) when energy is limited (Martin et al. 2006, Bourgeon et al. 2010). The quality of food is also a critical factor in the availability of nutritional resources and this can be particularly important for proper development of young. Regulation of fisheries (see Seabird Chapter 6), removal of invasive species (see Marsh Bird Chapter 4), habitat restoration (refer to Chapters 3-9), prescribed fire (see Marsh Bird Chapter 4), and freshwater management (see Chapters 4, 7-9) are potential management actions that can improve food availability for birds.

DISEASE: Disease-induced mortality diseases and coincident decreases in productivity often occur due to exposure to chronic stressors (e.g., disturbance, predation, limited food, pollutants) that suppress immune functions. Furthermore, changes in global temperature are predicted to expand exposure to certain disease vectors (Harvell et al. 2002, Martin et al. 2010, Pigeon et al. 2013). Chemical contaminants entering the ecosystem via urbanization and agricultural practices can directly or indirectly modify the pathogens present in the environment and diminish the resilience of individuals to disease (Galloway and Handy 2003, Snoeijs et al. 2004, Kelly et al. 2007, Martin et al. 2010, Pigeon et al. 2013, Giraudeau et al. 2014, Lee et al. 2017). Management activities that reduce stress from predation, disturbance, limited food supply and exposure to contaminants should reduce the risk of disease in birds.

ENVIRONMENTAL POLLUTANTS: This environmental stressor may affect avian populations across a range of scenarios, including seasonal exposure for migratory species (breeding versus wintering grounds), spotty exposure for species near agricultural or residential areas (including golf courses) areas, chronic exposure for residential birds living near contaminated areas and waterways, and food chain associated exposure for predatory birds (Lazarus et al. 2016). Pollutants often have direct toxic effects. At higher concentrations, many pollutants may be lethal, while at lower concentration pollutants might compromise reproduction, immune function, predator avoidance, or otherwise reduce survival or overall fitness (Ottinger et al. 2009). Endocrine-disrupting chemicals often have more subtle, non-lethal effects on immune function, thermal resilience, energy balance, and homeostatic maintenance ability (Calabrese and Baldwin 2001; Ottinger and Dean 2011; Carro et al. 2018). Further, there is evidence for multi-generational carry-over effects through epigenetic alterations (Anway et al. 2005). Several management actions could reduce risk of damage to birds from pollutant exposure including promotion of sustainable agriculture (see Chapters 2 and 3), freshwater management (see Chapters 4 and 7), restoration of hydrology (see Chapters 5 and 8), and coastal habitat restoration (refer to Chapters 3-9).

Physiological Response

ENDOCRINE STRESS RESPONSE: Chronic stress can elevate (or in some cases, chronically depress, [e.g., Rich & Romero, 2005]) corticosterone levels, reduce sex steroid hormone production leading to impaired reproductive performance and reduced body condition and overall fitness (Acevedo-Whitehouse and Duffus 2009, Sapolsky et al. 2000), and shorten telomere length (Epel et al. 2004, Hau et al. 2015), which is associated with decreased life span (Heidinger et al. 2012). Immune function also becomes impaired with chronic stress (Sapolsky et al. 2000, Martin 2009) leading to increased vulnerability to disease and parasites, which contributes to diminished lifetime reproductive performance and survival. Measurements of the endocrine stress response include directly measuring corticosterone (feather, fecal, blood), evaluating heterophil/lymphocyte (H:L) ratios as part of a complete blood count (CBC), and counting feather fault bars, translucent bands in the plumage which occur when feathers are being grown under stressful conditions (King and Murphy 1984; Davis et al. 2008, Clark 2015). As such, an assessment of hematocrit and differential blood cell counts can provide critical insight into the health status of individuals.

METABOLIC FUNCTION: Environmental stressors will typically increase metabolic function in the short-term

to facilitate rapid response to stressors. However, chronic exposure to environmental stressors will impair metabolic function (Burger et al. 2017). Measures of metabolic function include body condition and fat/muscle score, selected blood chemistry analytes, and stable isotope analysis. Body condition provides a rough measure of available energy reserves and involves mass and body size measurements (Peig and Green 2009). Fat and muscle scoring also estimate body reserves and physical condition. Blood chemistry analyses can provide information about the nutritional status and general health of individuals (Fudge 2000, Campbell 2012, Maness and Anderson 2017). Stable isotope analysis provides information on the dietary sources available to individuals (e.g., Zimmo et al. 2012, Lazarus et al. 2016). Assay of thyroid hormones provides valuable insight into the metabolic status of an individual especially during periods of change, such as maturation and migration. The thyroid system is impacted adversely by exposure to PCBs and other environmental toxicants, resulting in reduced metabolism and impaired pre-migratory fattening; both are essential for survival during migration with cold stress and other environmental conditions (McNabb 2007, Ottinger et al. 2009).

IMMUNE FUNCTION: Several specific measures of immune function are available (e.g., Norris and Evans 2000) and some techniques that are amenable to field collection (Table 10.1) are described in detail. Differential measurements of circulating blood cells can provide insight into the health of individuals, as activation of the immune system and certain pathological states can alter hematocrit and blood cell counts. An increase in hematocrit (packed cell volume) could be due to dehydration (Thrall 2012). On the other hand, a decrease in hematocrit, or anemia, can be caused by blood loss, decreased red blood cell (RBC) production, or increased RBC destruction. Blood loss may be due to gastrointestinal parasites, gastrointestinal ulcers from toxin exposure or foreign bodies, or blood-sucking ectoparasites. Decreased production can result from bone marrow suppression from chronic illness or nutritional deficiencies (Fudge 2000). Increased destruction of blood cells can be due to hemoparasites, oxidative damage from exposure to certain toxins, or inappropriate immune responses (Fallon et al. 2017). An intermediate hematocrit has been associated with increased longevity and lifetime fitness in a migratory passerine (Bowers et al. 2014). The buffy coat is the fraction of whole blood containing white blood cells and thrombocytes. A large buffy coat may indicate infection, inflammation, or injury and is negatively associated with reproductive success in birds (Gustafsson et al. 1994).

REPRODUCTIVE FUNCTION: Reproductive function involves a number of components, including forming pairs and

Table 10.1. Hierarchical structure of sampling methodologies and avian health metrics with associated logistical considerations to guide decision making by resource managers.

| Invasive Sampling | Sample Collection | Sample | Health Metric | Collection / Preservation of Sample | Information Gained | Cost | Ease of Collection & Processing | Restrains on Sampling or Interpretation |
|-------------------|--------------------|------------------------|--------------------|---|--|--|---------------------------------|---|
| No | Capture & Handling | Direct Assessment | Body condition | Measure (wing, culmen, and/or tarsus and body weight) | Current condition (muscle and fat deposits combined) | \$ | High | High inter-observer variability for some measures |
| | | | Ecto-parasites | Count and/or collect visible ectoparasites. If collecting, brush bird and preserve ectoparasites in isopropyl alcohol | Parasite load (negatively correlated with health, and positively correlated with stress) | \$ | Moderate | Difficult to see and collect small ectoparasites; will vary with breeding status (e.g., increases during incubation); time intensive in the field |
| | | | Feather fault bars | Count fault bars on all or a consistent subset of feathers; measure distance between bars (from photograph) | Stress during feather development; feather growth rate | \$ | High | Difficult to see small fault bars or distinguish large fault bars from many small bars; time intensive in the field, but can be quantified from photographs |
| | | | Fat Score | Score fat deposits (clavicle, hips, abdomen) | Rough fat deposit | \$ | High | Will change with migration, breeding status |
| | | | Muscle score | Score keel muscle | Rough muscle condition | \$ | High | Will change with migration, breeding status |
| | | Feather | Cortico-sterone | In clean ziploc or envelope in dry, cool location (uncontaminated) | Stress during feather growth ^a | \$\$ | High | Must know when feather was grown for accurate assignment of stress causation |
| | | | Heavy metals | In clean ziploc or envelope in dry, cool location (uncontaminated) | Contamination | \$\$\$ | High | Must know when feather was grown for accurate information gain |
| | | | Infectious disease | In clean ziplock, refrigerated or frozen | Susceptibility to specific infectious diseases | \$\$ | High | Not all infectious diseases can be detected in feather pulp |
| | | | Stable Isotopes | In clean ziploc or envelope in dry, cool location (uncontaminated) | Nutrition sources during feather growth | \$\$-\$\$\$ | Low | Must know when feather was grown for accurate information gain |
| | | Environmental Sampling | Feces ^b | Infectious disease | Collect into tubes, refrigerate or freeze | Susceptibility to specific infectious diseases | \$\$ | High |
| | Cortico-sterone | | | Freeze (-20°C) | Stress during digestion | \$\$ | Moderate | Best if collected fresh; affected by circadian rhythm, activity, and recent behaviors |
| | Internal Parasites | | | Suspension in flotation solution, faecal smear on microscope slide | Presence of gut parasites & eggs | \$ | Moderate | Difficult to identify species |
| | Yes | Capture & Handling | Blood | Hematocrit | Spun within 30 minutes of sampling | Dehydration, anemia, white blood cell volume | \$ | High |

Table 10.1 (continued).

| Invasive Sampling | Sample Collection | Sample | Health Metric | Collection / Preservation of Sample | Information Gained | Cost | Ease of Collection & Processing | Restrains on Sampling or Interpretation |
|-------------------|--|---|--------------------|---|---|-----------|---------------------------------|---|
| Yes | Capture & Handling | Blood | Bleeding time test | Gently rock in glass tube containing diatomaceous earth until clotted, room temperature | Clotting ability | \$ | High | Normal clotting range unknown for many species |
| | | | Cell counts | Blood smear on microscope slide & fixed in methanol for 5-10min | Infection, inflammation, stress (heterophil: lymphocyte ratio), hemoparasites, monocytosis ^c | \$ | Moderate | Blood smear needs to be fixed immediately; morphology of cells not known in all species |
| | | | DNA | Preserve cellular fraction or whole blood in alcohol (e.g., 70% EtOH), buffer, or by freezing (-20°C) | Blood parasites | \$-\$\$\$ | Moderate | Easily contaminated by outside sources |
| | | | Corti-costerone | Freeze plasma/serum (-20 or -80°C) or preserve plasma/serum in a 1:2 ratio of 70% EtOH | Current baseline stress | \$\$ | Moderate | Sample must be collected within 3 minutes of disturbance; affected by circadian rhythm, activity, and recent behaviors / interactions |
| | | | Thyroid Hormones | Freeze plasma/serum (-20 or -80°C) or preserve plasma/serum in a 1:2 ratio of 70% EtOH | Metabolic status | \$\$\$ | High | Interpretation depends on detailed individual and population life history knowledge |
| | | | Sex Steroids | Freeze plasma/serum (-20 or -80°C) or preserve plasma/serum in a 1:2 ratio of 70% EtOH | Breeding investment and territorial behavior | \$\$ | Moderate | Interpretation depends on detailed individual and population life history knowledge |
| | | | Immuno-globulins | Preserve plasma/serum in 1:2 ratio of SDS buffer or freeze plasma/serum (-80°C) | Currently elevated or depressed immune response | \$\$ | Moderate | Normal reference values are not known for many species |
| | | | Micronuclei | Blood smear on microscope slide & fixed in methanol for 5-10min | Presence of DNA strand breaks | \$ | High | Baseline values not known for many species; can be modulated by genotype |
| | | | Heinz bodies | Blood smear on microscope slide & fixed in methanol for 5-10min | Presence of denatured hemoglobin in red blood cells | \$ | High | Can be difficult to detect with light microscopy |
| | | | Troponin | Freeze plasma/serum (-80°C) | Presence of heart damage | \$\$ | Moderate/Low | Baseline levels not known in most species |
| Hemoglobin | Measure with portable hemoglobinometer or estimate from packed cell volume | Oxygen carrying capacity of blood, positively correlated with measures of condition | \$ | Moderate/Low | Concentration is strongly affected by age, season and the process of moult; requires equipment (hemo-globinometer) or estimation which is less accurate | | | |

Table 10.1 (continued).

| Invasive Sampling | Sample Collection | Sample | Health Metric | Collection / Preservation of Sample | Information Gained | Cost | Ease of Collection & Processing | Restrains on Sampling or Interpretation |
|-------------------|--------------------|----------------|------------------------------|--|--|----------------|---------------------------------|--|
| Yes | Capture & Handling | Blood | Mercury | Freeze whole blood (-20 °C) | Degree of mercury exposure | \$\$ | Moderate/Low | Cannot distinguish methyl mercury; uptake varies by trophic-level |
| | | | Oxidative damage | Freeze plasma/serum (-80 °C) | Damage due to oxidative processes | \$\$ | Moderate/Low | Known age populations are best for this analysis, as oxidative damage typically increases with age |
| | | | Chemistry ^d | Freeze plasma/serum (-80 °C) | Nutritional status, liver function, kidney function, metabolism, pancreatic function, muscle injury, immune function | \$- \$\$\$ | Moderate/Low | Lipemic or hemolytic samples can interfere with assays; normal reference values are not known for many species (including age- or sex-specific values) |
| | | Liver | Oxidative damage | Freeze (-80 °C) | Damage due to oxidative processes | \$\$ | Moderate/Low | Known age populations are best for this analysis, as oxidative damage typically increases with age |
| | | | Heavy metals | Freeze biopsy (-20 °C), or lyophilize sample | Contamination | \$\$\$ | Low | Lethal sampling |
| | | Muscle | Stable Isotopes | Freeze biopsy (-20 °C) | Nutrition sources during muscle tissue growth (higher cell turnover than feathers) | \$- \$\$\$ | Low | Usually lethal sampling, but can be biopsied on live birds |
| | | Eggs | Egg shell thickness, quality | Collect and preserve at room temperature | Potential exposure to DDT and certain metals | \$ | Low | Requires species reference values or a control population for comparison |
| | | | Corti-costerone | Sample albumin, freeze | Stress during egg formation, and in ovo exposure | \$\$ | Low | Primarily reflects maternal deposition; sampling can cause embryonic death |
| | | | Heavy metals | Heat dried | Contamination and <i>in ovo</i> exposure | \$\$\$ | High | Reflects maternal deposition |
| | | Post Mortality | Necropsy | Full examination | Refrigerate of freeze fresh carcasses | Cause of death | \$ | High |

^aFew numbers of fault bars that are small in size reflect good health

^bCollection of feces requires capture and handling within some avian species.

^cCell counts include several different measures. For most of these, high or low values are indicative of poor population health and species reference values must be consulted to determine if values are within an acceptable range for good health. Blood smears can also be used to identify and count hemoparasites, for this measure a low number of parasites indicates good health.

^dAvian and Exotics advanced chemistry panel: Amylase, Aspartate Aminotransferase (AST), Blood Urea Nitrogen (BUN), Creatine Kinase, Calcium, Cholesterol, Chloride, Bicarbonate (CO2), Creatine Phosphokinase (CPK), Gamma Glutamyltransferase (GGT), Glucose, Lipase, Magnesium, Phosphorus, Potassium, Sodium, Total Protein, Albumin, Triglycerides, Uric Acid

associated pair-bond behavior, copulation and fertilization, nesting behavior, follicle development and egg production, nesting success, productivity, fledging success and parental care. Environmental stressors typically decrease reproductive function of individuals, potentially resulting in a risk for population level impacts both on reproduction and aging processes (Ottinger et al. 1995; Hau et al. 2015; Lamb et al 2016). All components of reproductive function are essential for the overall fitness of the population. Reproductive function is often measured physiologically with sex steroids (e.g., testosterone, estradiol). Testosterone increases in the pre-breeding and early breeding season in the male; estradiol and progesterone in the female are critical to producing sufficient number of eggs to ensure viable offspring and fledging chicks (Adkins-Regan 2005). Interpretation of sex steroid concentrations requires population reference values and a detailed understanding of individual and population life history.

TOXIC RESPONSE: Exposure to contaminants can be assessed by direct measurement of compounds in the tissues and/or eggs of birds. The primary route of exposure in birds is through the diet and secondarily through maternally deposited contaminants into the egg (Lin et al. 2004, Ottinger et al. 2000, 2009). As such, analysis of the egg shell, egg membrane, and egg contents following hatch provide information about the presence of contaminants and potential exposure of the chick. Samples from feathers and feces also provide information on contaminant exposure and cumulated load in the case of feather analyses; fecal analyses provide exposure information over the 24 hour period. Similarly, blood chemistry and analysis for contaminants provide a current dynamic view of exposures to the individual. Physiological responses to contaminants/toxins are measurable by aminolevulinic acid dehydratase (ALAD) to assess exposure to lead (e.g., Scheuhammer 1989); ethoxyresorufin-O-deethylase (EROD) provides a measure of the activation of liver enzymes in response to exposure to toxicants (e.g., Bohannon et al. 2018). Exposure to pollutants can damage DNA leading to negative health effects (Maness and Emslie 2002). Some types of DNA damage can be assessed by the presence of micronuclei in blood cells (e.g., Baesse et al. 2015). Micronuclei are small nuclei created by double strand breaks and chromosomal instability. Proteins exposed to oxidizing agents and pollutants can denature and precipitate inside cells. Denatured hemoglobin forms Heinz bodies in red blood cells which can be detected by light microscopy from blood smears (e.g., Harr et al. 2017).

SAMPLING METHODOLOGIES AND GUIDE FOR DECISION MAKING

In practice, choice of avian health monitoring metric will depend upon the species and question(s) being asked. That is, what information is needed and can the sample be collected from this species safely/ethically? Remember, there is no “silver bullet.” As previously discussed, assessing avian health is a complex and inter-twined endeavor given the various concurrent stressors and inter-relationships of physiological functions. Hence, researchers and resource managers will need to clearly articulate the questions, objectives, and data needs. Once the question is identified, a suite of additional issues (e.g., species and life history traits, feasibility of sample collection, validity of assay tests, costs, etc.) will need to be considered. To facilitate decision making, Table 10.1 provides additional information related to a variety of potential health metrics. To that end, we have organized the table in a hierarchical fashion grouped by sampling strategy (invasive vs. non-invasive), type of sample (blood vs. feather vs. tissue), and potential health metric(s) as a means to structure the information. It is our hope that information within Table 10.1 will provide: 1) a foundation to assist researchers and resource managers in identifying the most appropriate avian health metric given a specific question; 2) a means to evaluate trade-offs between costs, field application, value of specific-metric; and 3) a basis to initiate further discussions and coordination as we work collaboratively to unravel the complexities and interconnectedness of avian health issues across the northern GoM.

NEXT STEPS

This section provides suggested next steps that would facilitate the identification and use of health metrics by managers for assessing the success of restoration projects during the process of restoration and for proactively adjusting the project components.

- ★ Create an ad-hoc working group (aka Community of Practice) of scientists and land managers to develop adverse pathway models and further refine the list of appropriate physiological metrics with respect to specific, agreed upon objectives/data needs.
- ★ Conduct literature reviews of avian health assessments across the northern Gulf of Mexico to facilitate communication, coordination, and future collaborations. Laboratory and field studies have characterized physiological response to a range of environmental stressors. However, few regional reviews exist that draw

together published literature from the perspective of management and assessment of restoration effectiveness.

- ★ Link physiological metrics with reproductive success, as a means to further evaluate restoration success. Stressors and many identified health metrics ultimately relate to reproduction and successful fledging of chicks. However, it is often difficult to simultaneously monitor individual adult pairs, egg and nest fates, health and growth of nestlings, fledging success, and first year survival, at a spatial and temporal scale that matters. As such, establishing clear linkage of selected physiological metric(s) with reproductive success may provide an opportunity to more easily assess reproductive success and thereby population status. Also, identification of non-invasive and non-destructive biomarkers.
- ★ Develop standardized avian health measurement endpoints and protocols to promote the collection of consistent and comparable avian health data across the northern GoM.
- ★ Develop a data repository for the storage of samples and an online data portal for the collection and sharing of publications, diagnostic reports, etc., as a means of facilitating communication, coordination, and collaboration. Creation of a data repository that is available to researchers and resource managers will provide a dynamic record to assess and predict the efficacy of restoration and management projects.

- ★ Collect and maintain mortality data from wildlife disease diagnostic laboratories serving the GoM to detect trends in health impacts and cause of death in avian species. This information can be maintained within the online data portal referenced above.

- ★ Partner with groups in different regions, including stakeholders and Citizen Science, where appropriate.

CONCLUSION

In summary, avian health assessments represent a literal “Pandora’s Box,” given the myriad of non-mutually exclusive stressors, potential for multiple physiological processes to be disrupted, compounded by the complexities of different life history traits expressed across the avian community. Our goal here was to: 1) provide a high-level overview of the subject; and 2) put forth a suite of potential metrics and their associated collection costs and logistical considerations as a means to increase awareness and provide resource managers with a basis from which to start thinking about avian health assessments. As the conservation community works to restore the northern Gulf of Mexico, our ability to fully understand and evaluate holistic ecosystem restoration will be improved if we supplement other avian monitoring efforts targeted at abundance and reproductive success, with information to better capture consequences to avian fitness. 🌱

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