



Health Assessments of Common Bottlenose Dolphins (*Tursiops truncatus*): Past, Present, and Potential Conservation Applications

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The common bottlenose dolphin (*Tursiops truncatus*) is a global marine mammal species for which some populations, due to their coastal accessibility, have been monitored diligently by scientists for decades. Health assessment examinations have developed a comprehensive knowledge base of dolphin biology, population structure, and environmental or anthropogenic stressors affecting their dynamics. Bottlenose dolphin health assessments initially started as stock assessments prior to acquisition. Over the last four decades, health assessments have evolved into essential conservation management tools of free-ranging dolphin populations. Baseline data enable comparison of stressors between geographic locations and associated changes in individual and population health status. In addition, long-term monitoring provides opportunities for insights into population shifts over time, with retrospective application of novel diagnostic tests on archived samples. Expanding scientific knowledge enables effective long-term conservation management strategies by facilitating informed decision making and improving social understanding of the anthropogenic effects. The ability to use bottlenose dolphins as a model for studying marine mammal health has been pivotal in our understanding of anthropogenic effects on multiple marine mammal species. Future studies aim to build on current knowledge to influence management decisions and species conservation. This paper reviews the historical approaches to dolphin health assessments, present day achievements, and development of future conservation goals.

Keywords: dolphin, *Tursiops truncatus*, conservation, health assessment, veterinary medicine

INTRODUCTION

Assessment of marine mammal health is complex, both from an accessibility standpoint and from the diverse array of factors influencing both individual and population survival. Baseline data are particularly crucial to evaluate whether population, or in some cases species, health is deteriorating by providing points of comparison to assess trends in disease, mortality, and reproductive rates (1). From a veterinary perspective, a hands-on physical exam is the optimal approach to provide critical

information for a comprehensive understanding of both individual and population-level health status. Knowledge gained from physically examining smaller cetaceans can be extrapolated to larger, less-accessible cetaceans to improve understanding of the complexities of adverse health impacts on marine mammal conservation. The common bottlenose dolphin (*Tursiops truncatus*) is an effective model species for understanding both cetacean and marine ecosystem health.

The overarching aim of conservation is to actively preserve habitats and the diversity of species dwelling therein (2). Effective conservation benefits from a foundation of sound scientific understanding of species' biology, population dynamics, and stressors impacting the ecosystem (3). Marine ecosystem health is particularly challenging to assess due to immense biological diversity, as well as the vast scale and connectivity of the ocean environment. Multiple factors threaten marine mammal health (4); therefore, improving knowledge of the interplay of these factors and predicting their long-term effects are essential for successful conservation.

The emerging discipline of conservation physiology is particularly important in the marine environment, as it allows a mechanistic understanding of the drivers of conservation obstacles at an ecosystem level, in addition to species-specific challenges. Understanding how species physiologically respond to environmental alterations is important for successful tailored conservation strategies (5). The rate of expansion of the human population is exacerbating the challenges faced by wildlife with frequently detrimental consequences to their health (6). The unprecedented changes occurring in the environment on a global scale require novel mitigation strategies to ensure effective conservation actions and sustainable wildlife populations (7).

There is limited understanding of the scale of the negative anthropogenic impact on marine mammal biodiversity. Currently 29.2% of marine mammal species are classified as data deficient according to the International Union for the Conservation of Nature (8). As a result, shifts in population viability can be difficult to detect, as the basic natural history of the species has not been documented (9). Intrinsic traits of species can be more important predictors of risk than extrinsic environmental factors, as they provide a measure of the species' inherent susceptibility to human impacts and the ability of species to recover from them (10). The bottlenose dolphin is one of the most closely studied and widely distributed marine mammal species and provides an opportunity to extrapolate knowledge and understanding to other marine mammal species.

HISTORICAL PERSPECTIVE

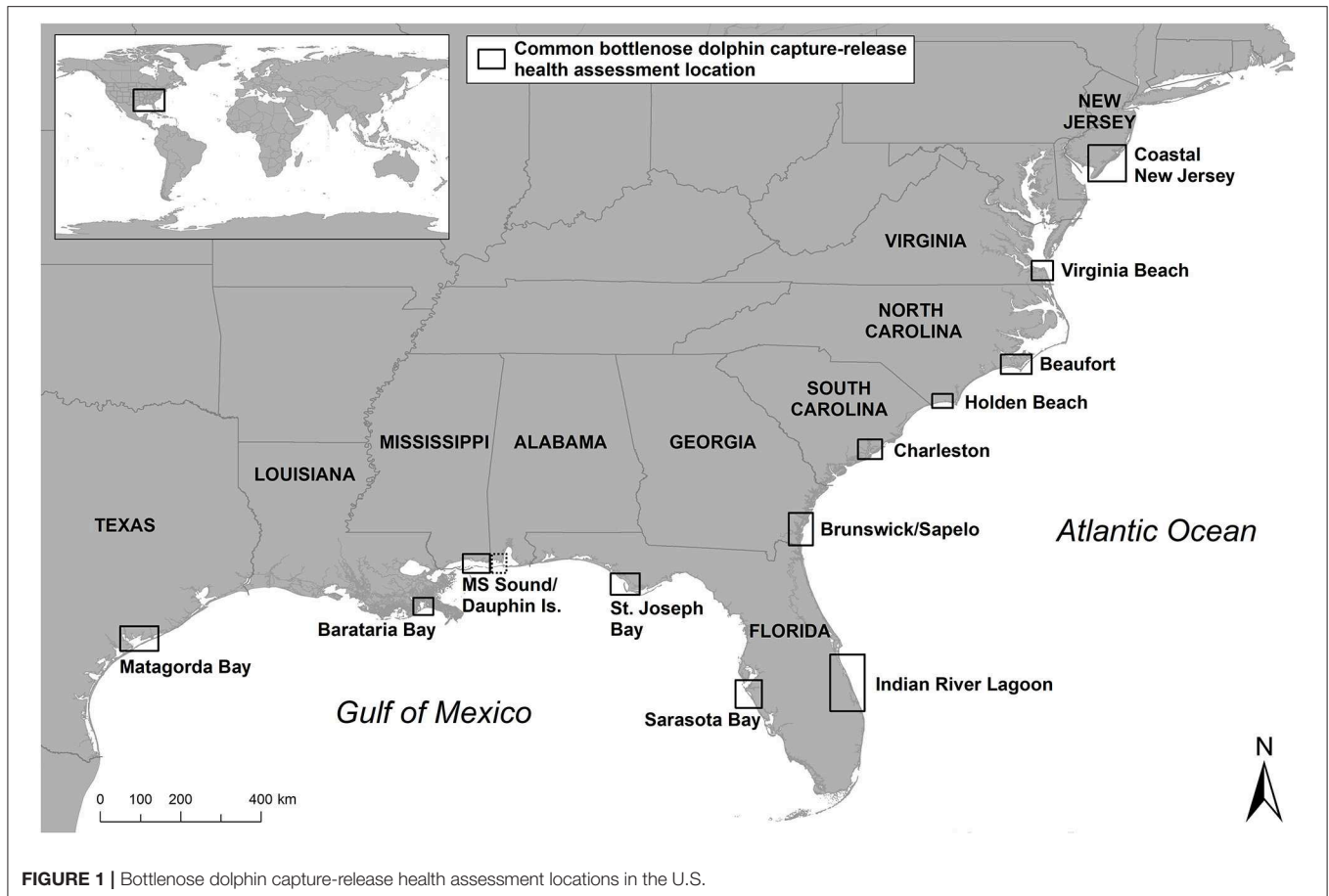
Initially the first common bottlenose dolphin captures occurred for acquisition for public display, research, or for stock assessment prior to collection. Over time health assessments of inshore dolphins have been utilized to better understand endemic disease, establish baseline physiological measures, and evaluate exposure to, and potential effects of, chemical, biological, and physical stressors. Historically, health assessments developed as additions to existing capture-release efforts for

other purposes, such as marking/tagging and population-level studies to understand movement patterns and site fidelity. Starting in 1979, Hubbs/SeaWorld Research Institute began collecting bottlenose dolphin health data in the Indian River Lagoon of east Florida during capture-release operations to assess potential population-level impacts in advance of upcoming zoologic collections (Figure 1) (11). Analyses from the samples and data collected included morphometrics, blood biochemistry, hematology and reproductive endocrinology, microbiology, genetics, and life history studies (12). Similar research was initiated by Marine Animal Productions in 1982 in Mississippi Sound, with the inclusion of skin, blubber, and liver tissue sampling in some cases, to establish a health baseline for dolphins inhabiting this area, again prior to collections and for comparison with managed animals (13).

The Sarasota Dolphin Research Program (SDRP) began incorporating additional biological samples and measurements in 1984 to their original capture-release program. This supported tagging and telemetry studies in Sarasota Bay, Florida, which were initiated in 1970. The inclusion of additional biological samples shifted the focus from studies of population range and social patterns to broader scientific investigations of life history, population dynamics, body condition and health, social structure, communication, reproductive success, and effects of human interactions. Samples and measurements included in-depth bloodwork analyses, genetic tests for population structure and paternity, ultrasonic measurement of blubber thickness, weight measurement, age determination (tooth growth layer group counts), further development of tags and tag attachments, and post-release population monitoring (14–19).

In 1992, the Marine Mammal Health and Stranding Response Program was formalized within the National Marine Fisheries Service (NMFS) through an amendment to the Marine Mammal Protection Act (MMPA), establishing the standards for sample collections and promoting collaboration and standardization of bottlenose dolphin health assessments (20). In 1995, NMFS conducted health assessments in response to the 1987–88 mortality event along the east coast of the U.S., in which over 600 bottlenose dolphins stranded as a result of a large-scale morbillivirus epizootic (21–25). Additional studies were also conducted to investigate unusual increases in dolphin strandings near Matagorda Bay, Texas in 1992 (26), and in the Florida Panhandle in 2005–2006 (27) (Figure 1) (Table 1).

Supplementary capture-release studies were initiated by the National Oceanic and Atmospheric Administration (NOAA) in support of stock assessment on the east coast, which was by that time a requirement for NOAA's NMFS (23, 24). Subsequent capture-release studies used telemetry to understand population structure along the Atlantic coast (39) following increased dolphin mortality (40) which was eventually determined to be associated with morbillivirus (22, 30). The studies found positive morbillivirus titers in some dolphins sampled in estuarine and coastal waters near Beaufort, NC, with no positive titers observed in dolphins sampled in estuaries near Charleston, SC (30). This provided crucial information to understanding dolphin population structure and interaction along the U.S. east coast, with a mosaic of migratory, non-migratory but coastal, and



small estuarine stocks rather than a single large population as initially presumed. In addition utilizing archived Sarasota Bay samples to retrospectively assess morbillivirus titers facilitated further understanding of virus exposure, seroconversion, and population naivety (41). Incorporating health assessments informed scientists that at least some small, estuarine stocks along the Atlantic coast were naïve to morbillivirus and therefore particularly vulnerable (38).

In 2003, the Health and Environmental Risk Assessment (HERA) population monitoring project was initiated for bottlenose dolphins in the Indian River Lagoon, Florida (IRL), and waters surrounding Charleston, South Carolina (CHS) (42). For both the IRL and CHS field sites, health assessments were conducted to establish baseline data and to compare morbidity temporally and across two geographic sites (34, 43). Higher concentrations of persistent organic pollutants (POPs) including legacy [e.g., dichlorodiphenyltrichloroethanes (DDTs), polychlorinated biphenyls (PCBs)] as well as “emerging” contaminants [polybrominated diphenyl ethers (PBDEs) and perfluorooctane sulfonate (PFOS) compounds] were detected in CHS dolphins as compared to IRL dolphins (43, 44). Mercury concentrations in the blood and skin of IRL dolphins were extremely high, approximately five times higher than those in CHS dolphins. Higher exposure to many pathogens (e.g.,

morbillivirus and lobomycosis) was also observed for the IRL dolphins (34).

The incorporation of health assessments into existing capture-release protocols provided the collection of baseline health data across multiple populations, thus allowing for investigation of geographic variability in health parameters (32, 42). The methodologies established and samples collected during these earlier projects formed the basis for developing a risk assessment framework to quantify the impacts for dolphins affected by anthropogenic threats (e.g., *Deepwater Horizon* oil spill) in geographical areas where baseline data were not available (36).

THREAT IDENTIFICATION AND ASSESSMENT

Dolphin capture-release projects provide a unique perspective to assess individual animal health and extrapolate to overall health of the surrounding population, species, and ecosystem. Over the past 40 years in the U.S., there have been numerous stressors that have impacted bottlenose dolphin populations, for which dolphin capture-release projects have been integral to threat identification and quantification of impacts from a given stressor (e.g., biotoxins, disease, environmental contaminants, oil spills, etc.).

TABLE 1 | Historical list of previous capture locations according to **Figure 1** including number of animals examined or health assessments (HA) performed, purpose of captures, and references.

Date	Location	Number of dolphins	Purpose	References
1979–1981	Indian River Lagoon FL	27 109 total HA	Population assessment	(11)
1982	Mississippi Sound MS	57	Commercial assessment	(13, 28)
1984–2019 (ongoing)	Sarasota FL	289 individuals 811 total HA	Biological sampling, technique development, reference population	(29)
1987	Virginia Beach VA	23	Mass mortality investigation	(21)
1992	Matagorda Bay TX	36	Mortality investigation	(26)
1995	Beaufort NC	31	HA post CeMV outbreak	(24)
1998	Virginia Beach VA	1	Stock assessment	NOAA unpublished data
1999	Charleston SC	14	Stock assessment	(30)
1999*	Beaufort NC	6	Stock assessment	(31)
2000*		11		(30)
2006*		19		(32)
2002–2003	Brigantine NJ	12	Stock assessment	(30)
2004	Holden Beach NC	10	Persistent organic pollutant assessment	(33)
2003–2018	Charleston SC Indian River Lagoon FL	118 246	Comparative health studies	(34)
2005–2006	St. Joseph Bay FL	30	UME investigation	(27)
2009	Brunswick GA	29	HA legacy environmental contamination	(35)
2011–2018	Barataria Bay LA	202	DWH investigation	(36)
2013	Mississippi Sound MS	20	DWH investigation	(37)
2015	Brunswick GA	19	UME investigation	(38)
2018	Dauphin Island AL	18	DWH investigation	

*The numbers provided are for those where samples were used and published it does not necessarily provide a list of the total numbers of animals handled in this location.

Unusual Mortality Events

An unusual mortality event (UME) is defined under the U.S. Marine Mammal Protection Act as “a stranding that is unexpected; involves a significant die-off of any marine mammal population; and demands immediate response.” The UME program was officially established under Title IV of the MMPA in 1992. Increased recognition of the occurrence of large scale dolphin mortality events in the late 1980s spurred the application of dolphin health assessments beyond population monitoring, to investigating causes and effects of such mortality events (21, 24).

Since 1999, a series of UMEs occurred along the northwestern Florida coastline (Florida Panhandle) including St. Joseph Bay (45). NOAA conducted two dolphin health assessments during 2005 and 2006 in response to these UMEs in which 30 dolphins were sampled and subsequently tagged (27) (Table 1). The initial mortalities were tentatively attributed to biotoxins from red tide algae (*Karenia brevis*) (46). Eosinophilia was observed in 23% of sampled dolphins, with associated increased neutrophil phagocytosis and T-lymphocyte proliferation (27). Chronic low-level exposure to another algal toxin, domoic acid, produced by the diatom *Pseudo-nitzschia* spp., previously linked to eosinophilia, was also identified (47). Prior to these UMEs, little was known about dolphin abundance, distribution, and

site fidelity in this region, and thus, it was unclear which population(s) of dolphins were impacted (48, 49). Post-health assessment tagging data suggested that the timing and spatial extent of biotoxin events and other potential stressors in the Florida Panhandle may greatly influence the severity of future UMEs (50). Cetacean post-mortem examinations can provide insight into identifying the underlying cause of a UME in addition to baseline data on disease presence and anthropogenic causes of mortality (51–53). Increased integration of live animal assessment with post-mortem findings encourages the transfer of information from the dead to the living, informing scientists of the underlying pathophysiological mechanisms faced within free-ranging populations (54).

Chemical Pollutants

The presence of PCBs and other lipophilic contaminants have been recorded to be accumulating in the tissues of bottlenose dolphins and other odontocetes for decades (31, 55–59). Many marine mammals, particularly piscivorous species, have a high potential to biomagnify pollutants (33), with increased levels resulting from the high trophic position and blubber acting as a reservoir for lipophilic contaminants (60). Both experimental and observational studies support the correlation between

increased PCB levels and endocrine dysfunction, compromised immunity, and/or reproductive failure (61–66). However, the common co-occurrence of similarly acting compounds and uncertainty regarding species-specific dose response functions makes assessment of the effects of these contaminants at a population level challenging (60, 67).

In addition to their applicability to investigating UMEs, dolphin health assessments have also been used to investigate health of populations at risk from environmental contaminants. For example, dolphins along the Georgia coast have been identified with some of the highest concentrations of PCBs in the world, and these levels are site-specific to a Superfund Site in Brunswick, Georgia (60, 68, 69). In 2009, NOAA conducted health assessments on 29 dolphins in the region and identified a high proportion (26%) of sampled individuals suffered from anemia (66). In addition, these dolphins had reduced thyroid hormone levels with total thyroxine, free thyroxine, and triiodothyronine negatively correlated with increased blubber PCB concentrations. T-lymphocyte proliferation and indices of innate immunity decreased with blubber PCB concentration, suggesting an increased susceptibility to infectious disease (66). As with previously described health assessments, telemetry and photo-ID data provided perspective on ranging patterns relative to exposure, subsequent reproductive success, and effects of long-term impacts from cumulative stressors (70). In 2015, another health assessment was conducted in Georgia to look at potential impacts associated with a recent morbillivirus-caused UME and extremely high levels of PCBs that were identified from previous studies. Dolphin morbillivirus titers differed between dolphins sampled in coastal and estuarine waters, and tagging data identified some degree of overlap between these individuals. This study suggested that estuarine dolphins in this region may be highly susceptible to future morbillivirus infections as a result of elevated PCB levels and spatial overlap between coastal and estuarine dolphins that would facilitate disease transmission (50).

Petroleum Toxicity

In 2010, the largest marine oil spill in the history of the U.S., the *Deepwater Horizon* oil spill (DWH), occurred in the northern Gulf of Mexico (GoM). Subsequently a multidisciplinary approach for evaluating the impacts upon cetaceans was undertaken (71, 72). Bottlenose dolphins were the focal cetacean species examined due to the accessibility of dolphins in shallow coastal and estuarine waters, and the heavy oiling in some of those same nearshore areas. Health assessments in heavily oiled areas, particularly Barataria Bay, Louisiana, were initiated as part of NOAA's Natural Resource Damage Assessment (NRDA). Veterinary clinical assessment of dolphins living within the oil spill footprint found significant lung pathology, impaired stress responses, high reproductive failure, and altered functional immunity, as compared to findings from un-oiled reference populations (35, 73–75). After the DWH oil spill, an abnormally high prevalence of lung and adrenal gland pathologies were documented in post-mortem examinations (73, 76). Identifying the baseline incidence of disease in stranded dolphins and diagnosing pathology in live dolphins through the application of enhanced health assessment protocols was required to determine

if these findings were correlated with the recent environmental exposure (75, 77, 78).

Determining a causal link for the multiple pathologies observed post DWH oil exposure has been via a diagnosis of exclusion; concluding the toxic effects of the oil spill as the primary differential to both the observed pathologies and the increased dolphin mortality (71, 75). Other differential diagnoses that were the potential causes of previous GoM UMEs were also ruled out including biotoxins (79), POPs (68), and infectious disease (45, 73, 80, 81). Successive health assessments during 2016–2018 have provided additional insight into the chronic health effects. Longitudinal photo-ID surveys allowed estimation of post-spill survival rate (0.80–0.85) and reproductive success (20%), the latter was very low for this population (82, 83). Long-term consequences from oil contamination are difficult to assess but have been suggested in other marine mammals such as the killer whale (*Orcinus orca*) and the sea otter (*Enhydra lutris nereis*) (84–87). Future research efforts will aim to improve understanding of the transgenerational or *in utero* exposure effects in addition to the direct exposure of those animals alive at the time of the oil spill.

Currently there is limited knowledge of the pathophysiology of reproductive failure in bottlenose dolphins. Improved understanding of the normal physiological changes occurring during gestation in successful pregnancies will aim to elucidate possible mechanisms of reproductive failure and identify abnormalities occurring during failed pregnancies. This knowledge will be essential to help understand reproductive challenges, not only for common bottlenose dolphins, but also for the future management of other cetaceans, some critically endangered, to help to understand the reproductive challenges faced in these species and improve future conservation management.

CAPTURE AND HANDLING METHODOLOGY

The standard approach to capture small (1–5) numbers of bottlenose dolphins in shallow waters is by encirclement with a seine net up to 500 m long and 7 m deep (88, 89). Shallow water (<1.5 m), minimal currents, and a solid seafloor are optimum for safe capture and restraint. The seine net is deployed from a specially designed boat at high speed around the target dolphin(s), creating a compass (**Figure 2A**), with well-trained handlers distributed around the circumference to provide aid and restraint when the dolphins contact the net (**Figure 2B**) (29). If capture occurs in deep water (>1.5 m), the net compass can be pulled into nearby shallow water, or the dolphins are handled from the side of response vessels and moved onto specially designed floating mats that are either towed to shallow water or directly onto a processing vessel for sample collection (**Figure 2C**). Capture of individual dolphins in waters exceeding the depth of seine nets has been performed via tail grabs or hoop-nets, which are placed over the head of bow-riding bottlenose dolphins or smaller cetacean species as they surface to

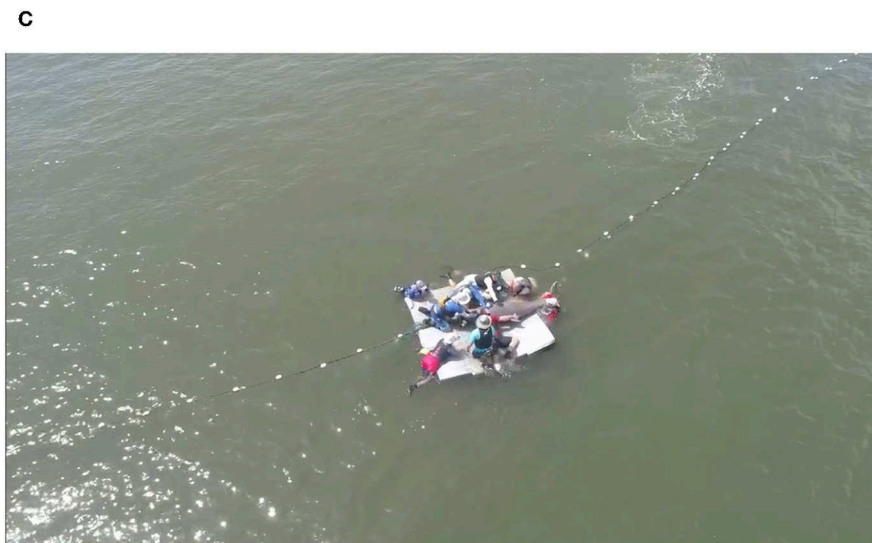


FIGURE 2 | Capture methodology with **(A)** seine net deployed from a specially designed boat creating a compass in the center of the image, with chase boats circling outside to help contain the animals before completion of the compass and to deliver handlers to the net (two dolphins are visible inside the compass on the left side). **(B)** Shallow water set, well-trained handlers distributed around the circumference of the compass to provide aid and restraint when the dolphins contact the net. **(C)** Deep water set, dolphin is placed onto a floating mat and disentangled from the net for transport to the processing vessel. All photos taken under NMFS MMPA/EAS permit No. 18786-03.

breathe (88–90). Once dolphins are safely restrained, veterinary examinations and sampling are performed.

VETERINARY PROCESSING

Current health assessment examinations require collaboration among scientists from multiple disciplines and veterinary specialties to obtain the maximum amount of information from a single snapshot in time while the animal is in-hand. The development of veterinary field techniques was enhanced by the successful management of dolphins in human care. Sampling varies according to research questions, but typically a suite of baseline data are collected to maximize knowledge obtained from a single veterinary exam (Table 2) (91). Ensuring a standardized approach to highlight the importance of inter-lab comparability and sharing information between multiple institutions has been paramount in the success of developing field collection methodologies and subsequent sample analysis procedures (32, 113). As described below, additional field procedures have been incorporated over the decades as technology, field techniques, and analytical assays have advanced, and as management needs have evolved.

Determination of reproductive status (e.g., pregnancy, ovarian activity, or testis size as an indicator of sexual maturity) using diagnostic ultrasound was first applied in Sarasota Bay in 1989 (26, 114). Full-body ultrasound has subsequently been proven to be an invaluable, real-time tool during dolphin health assessments, pioneered by the National Marine Mammal Foundation and the U.S. Navy Marine Mammal Program (Figure 3) (77, 97, 101–103). In addition, pregnancy may also be diagnosed remotely by using blubber biopsy hormone levels (115, 116). In some cases, post-release visual monitoring and photographic-identification (photo-ID) have allowed researchers to track the outcome of the pregnancy (that was determined by ultrasound or remotely) and determine whether a viable calf was produced (82, 116, 117).

A holistic approach of including dietary assessment into the health exam along with urinalysis, blood (Figure 4), and blubber sampling can aim to elucidate underlying causes of health abnormalities. In the field, urinary catheterization has enabled comparison with dolphins in managed care and further developed the understanding of the development of renal pathology in dolphins, especially when differences in diets have been considered (91, 97–99). Important ecological perspectives can be obtained from dietary assessments, along with information regarding prey availability and potential shifts in environmental pressure affecting the ecosystem (118–121). Further research is needed in this area to develop a more standardized nutritional status indicator which can integrate multiple measures. This could be used for example to improve understanding of the effects of prey instability or environmental stressors (122).

Electronic tagging technology to assess individual movement or habitat use has rapidly advanced over the past 40 years so that now small satellite-linked tags can be attached via a single-pin to the dorsal fin and have minimal to no long-term effects on the

tagged individual (70, 111). These tags provide fine-scale data on individual animal movements for several months, post-release, and can provide additional insight into the cause of health effects identified during the veterinary examination (37, 38, 117). The movement pattern data from these electronic tags can also be used to conduct follow-up monitoring to assess individual animal health, survival, reproductive success, habitat use, and exposure to threats (82).

MULTIDISCIPLINARY APPROACHES FOR ASSESSING POPULATION HEALTH

Utilizing a multidisciplinary approach to combine clinical veterinary knowledge with epidemiological analyses and population modeling enables long-term forecasting of population trajectories (36). In UMEs, modeling to estimate mortality based on the number of stranded carcasses can provide insight into the immediate losses to the population (71, 123–125). However, integration of available health information and veterinary interpretation of sublethal, chronic conditions, which are likely to influence long-term survival and reproductive potential, can provide a more accurate interpretation of the likely long-term impacts on the population (36). Aside from impacts from acute events such as oil spills, modeling has been used to simulate the likely population-level consequences from sublethal effects of chronic contaminant exposure (60, 67). Estimation of long-term population effects resulting from mortality or morbidity events is critical to inform restoration or recovery plans, and also to appreciate the magnitude of the impact of UMEs on population numbers or of environmental contaminants on the surrounding ecosystem. Advances in modeling application to marine mammal stock assessments will greatly shape the future of marine mammal conservation.

ADVANCES IN TECHNOLOGY AND CONSIDERATIONS FOR ANIMAL WELL-BEING

Dolphin health assessments provide fine-scale information on individual animals that can be extrapolated to evaluate overall population health. While much can be learned from hands-on health assessments, a major driver of current research is to develop techniques to obtain maximum health assessment information from remote sampling and observations. The methodology for safely handling, sampling, and releasing dolphins is continually evolving to minimize the risk to both the dolphins and researchers, as well as maximize the data collected. However, health assessments are still expensive, logistically challenging, have limited target populations, and there is an inherent risk when handling large animals (126, 127). The development of remote sampling technologies is essential to build upon the data collected during hands-on studies and to expand our ability to efficiently and comprehensively assess the health of dolphin populations beyond nearshore waters, as well as the health of larger, less tractable cetacean species.

TABLE 2 | List of veterinary processing sample collection from hands-on physical examinations during common bottlenose dolphin health assessments.

Procedure	Description	Use	References
Blood sample (Figure 4)	Obtained from the periarterial rete on the ventral aspect of the tail fluke	Biochemistry Hematology Blood gas analysis Endocrinology Immunology Serology Genetics	(23, 29, 30, 74, 91–93)
Surgical biopsy	Full thickness wedge biopsies of skin and blubber are routinely taken via an inverted “L” block under local anesthesia from the left lateral body wall caudal to the dorsal fin	Genetic population structure (skin) Foraging ecology (skin) Chemical contaminants (blubber) Hormone levels (blubber) Microbiome	(31, 33, 49, 57, 94–96)
Urinalysis	Bladder catheterization	Renal function assessment Dietary analysis	(91, 97–99)
Tooth extraction	Single tooth extracted under local anesthesia	Age determination	(17, 100)
Ultrasonography (Figure 3)	Thoracic and abdominal internal assessment	Lung pathology Reproductive Assessment Full abdominal exam including renal assessment Blubber thickness	(77, 97, 101–103)
Electrocardiography (Figure 6)	Adapted field use in and out of water	Cardiac assessment	(104)
Morphometrics (Figure 7)	Standardized full body measurements: lengths, girths, weight	Assess body condition and growth rates	(105)
Auditory evoked potential	Portable unit adapted for field assessment audiograms	Assess hearing range and sensitivity	(106)
Lesion biopsy	Sample of abnormal skin lesions e.g., pox or freshwater lesions	Histopathology	(57, 93)
Blow analysis (Figure 5)	Exhaled breath vapor	Pathogen and hormonal analysis Metabolites Respiratory function testing	(107, 108)
Microbiology	Swabs/culture plates from oral respiratory or genital orifices	Bacteriology Virology	(109)
Freeze brand	Dorsal Fin	Identification	(110)
Feces and urine collection	Swabs or catheter	Biotoxin analysis	(46)
Skin biopsy Electronic and/or roto tagging	Skin sample from biopsy or during dorsal fin tagging	Genetics, sex, stable isotopes identification Ranging patterns reproductive status Survival	(70, 111, 112)

Application of new technologies to improve remote sampling opportunities and maximize information obtained from cetacean health assessments is pushing the boundaries of current marine mammal science. Blow samples previously established when in-hand (Figure 5) can now be obtained remotely utilizing UAVs (unmanned aerial vehicles). Drones can be used to obtain aerial images to perform photogrammetry to assess health via body condition in large whales unable to be examined physically (107, 128). Drones can also be used in cetacean disentanglement approaches to provide accurate assessment of exact entanglement points and facilitate more informed decisions on disentanglement methodology. Thermography has been used to assess dolphin dorsal fin temperatures, as a measure of individual health status representing appropriate integumentary thermoregulation (129), and now thermal imaging from drones

is being developed to apply to large whale health assessments. Remote temperature assessment will be of increased value in the future when potential climate change impacts could result in cetaceans being exposed to higher or lower environmental temperatures (130–132).

Historically, the standard method of estimating the age in dolphin health assessments is via tooth extraction under local anesthesia and counting the growth layer groups present on longitudinal section (17). Dental radiography has been pioneered in an effort to replace the tooth extraction technique, and validation of the technique is ongoing. Bone density assessment has also been explored as a possible aging method, however correlation with age across the entire lifespan was limited (133). A promising new methodology is the use of pectoral flipper radiography to assess bone maturation (134).



MMPA/ESA Permit No 18786-03

FIGURE 3 | Ultrasound examination of a lymph node on board the veterinary examination and sampling vessel (MMPA ESA Permit No. 18786-03).



MMPA/ESA Permit No 18786-1

FIGURE 4 | Blood sample collection from the peri-arterial rete on the ventral aspect of the tail fluke (MMPA ESA Permit No. 18786-01).

The dolphin pectoral flipper displays both hyperphalangy and paedomorphosis enabling this method to be applied throughout the entire lifespan due to the predictable chronological osteogenic changes occurring to the metacarpal and phalangeal bones. This non-invasive technology could facilitate age estimation for older animals, replacing tooth extraction.

Additional biological information from remote biopsy dart sampling is expanding on the knowledge gained from each

individual sample (135). Currently, sex and population structure of the animal can be determined from genetic analyses of skin (112), and contaminant concentrations and stress and reproductive hormone levels can be measured from blubber biopsy (33, 116, 136). Present efforts are working toward using skin to assess the epigenetics of the individual to give an estimation of age (137). The NMMF are expanding on this even further in line with recent human advancements to provide an



MMPA/ESA Permit No 18786-03

FIGURE 5 | Exhaled breath sample collection for cytology (MMPA ESA Permit No. 18786-03).



FIGURE 6 | ECG leads attached during sampling and processing to closely monitor the dolphin's heart rate and assess cardiac function (MMPA ESA Permit No. 18786-03).



FIGURE 7 | Dolphin suspended in a stretcher for weight measurement via load cell on board the veterinary processing vessel. This image was published with permission of MMPA ESA Permit No. 18786-01 for the identifiable individuals in the image.

indication of biological age (138). This emerging technology could provide a means to assess increased environmental pressure or poor health status (139–141).

Remotely deployed suction cup satellite-linked tags can provide short-term (<24 h) data on bioenergetics, respiratory measures and cardiac data (142, 143). An additional remote

tool in development is the use of remotely attached single-pin satellite-linked tags. These techniques will be particularly useful in marine mammals where capture-release is impractical due to size, species intolerance to handling or cost restraints. Future research aims to combine different disciplines to expand scientific knowledge further and inter-species application of new technologies, for example, studying acoustic communication as a proxy to changes in health status (144, 145).

DISCUSSION

Health assessments with an epidemiological focus can aim to understand the pathophysiology of disease and interpret the demographic, anthropogenic, and ecological pressures contributing to individual disease susceptibility (146). Extrapolating from individual health assessments to accurately understand population health status, requires a strategic epidemiological approach (41, 147, 148). Integration of post-mortem examinations within the health assessment framework can provide additional projections from both diagnostic and scientific perspectives aiming to contribute to identifying the underlying cause of mortality and also predicting the future impacts on the population. Performing pro-active marine mammal health assessment examinations allows an opportunity to examine population health under natural environmental conditions, as opposed to during a mass stranding or UMEs. This baseline knowledge of population health status can aid understanding of post-mortem examinations during UMEs and ultimately aim to drive mitigation strategies for successful conservation and species management. Health assessments are facilitating a pro-active approach to marine mammal conservation in addition to a reactive response to UMEs.

The primary role of wildlife veterinarians is shifting from management of high mortality disease epidemics to preventative management and mitigation of anthropogenic causes of mortality (7). Unlike terrestrial species where mass mortalities garner a lot of public attention, marine species can die in large numbers, and the impact can go unnoticed (149). Sharing information regarding health and threats to local populations can facilitate public interest in coastal and estuarine bottlenose dolphin populations. Increased public awareness and reporting of marine mammals in distress can aid understanding of the current global changes and interactions between humans and wildlife, dictating the efforts required to conserve future marine populations globally.

Continuous monitoring of specific populations over time has the benefit of providing both cross-sectional analyses on an annual basis, as well as longitudinal analysis over several decades. Collecting health data consistently across multiple populations facilitates an understanding of geographic variability, can help to establish reference ranges that are generalizable across populations, and can provide a gradient of stressor exposures for cross-sectional or correlational studies. The combined approaches support a robust framework for epidemiological studies to investigate the causal factors for disease. The collection

of decades of archival samples from multiple populations facilitates retrospective studies to discern between sublethal pathogen levels, assess temporal and spatial trends, and elucidate the intricacies of disease susceptibility at a population health level. For example, dolphins in Florida are frequently exposed to various levels of *K. brevis* red tides (150, 151). Examining samples from 1994 to 2003 enabled knowledge of baseline levels of brevetoxin in the dolphin population and demonstrated that dolphin carcasses not associated with large scale mass mortality could also contain comparably high levels of brevetoxins (79). This information is invaluable for future research when the duration and intensity of red tides in Florida appears to be increasing (152, 153).

An additional benefit of long-term studies and sample archives generated by health assessments is the ability to apply new technology and diagnostic tests retrospectively enabling advanced monitoring of health changes over time. Identification of emerging infectious causes of mortality such as cetacean morbillivirus requires continued monitoring of levels of herd immunity over long periods of time (81). Sample archives can be used to establish normal levels and improve understanding of emergence, dynamics, and history of pathogens such as morbillivirus or retrospective analysis of brucella (23, 30, 73). Established baseline data can aid interpretation of normal or increased prevalence of positive antibody titers within the population.

Stress of Health Assessments (Alternative Perspective)

Prior to considering health assessments for a project, there should be an in-depth discussion of research priorities and if the short-term capturing of individual dolphins is truly the best tool to address the goals of the study. The value of the data obtained from health assessments of free-ranging dolphins needs to be balanced against the potential stress and risk to the individual from the capture, handling, and sampling process (154–157). The stress of capture often influences baseline data such as blood cortisol and aldosterone levels (158). If baseline hormone values are the focus of a study, remote biopsy sampling of blubber can give an accurate indication of baseline stress hormone levels without the elevation caused by the stress of capture (159). However, the adrenal response to a stimulus such as capture may be of interest (35), and capture-release studies enable the evaluation of an individual's hypothalamic pituitary axis and whether or not the animal is capable of mounting an appropriate stress response (75).

In general, the molecular physiological response to the stress of the veterinary examination is well-documented across species; it is transient and the valuable information obtained from the exam typically offsets any acute stress that may be caused. Based on ongoing population monitoring, long-term health consequences of repeated captures have not been found for individuals examined as many as 15 times or more (160). Ensuring capture and restraint are relatively brief and as calm as possible is important, as it has been shown that short holding times do not induce a significant neuroendocrine stress

response (154). An experienced team and ongoing training opportunities among organizations, in both managed and free-ranging dolphin populations, promote this high standard of assessment.

Globally, free-ranging dolphins are exposed to a wide range of anthropogenic stressors including environmental contaminants, acoustic shipping disturbances, fisheries interactions, habitat degradation or even loss altogether, exposure to biotoxins, climate disruption, and human interactions (161–168). Variability in the level of anthropogenic stress occurs across geographical locations. Health assessments provide a portal into the individual and population health status to facilitate understanding of ecosystem health, and drive conservation and management decisions. The cost of each individual health assessment is offset by the biological information obtained from each examination. Combining these data with additional stranding information, such as post-mortem examinations and field observations, helps to improve understanding and interpretation of biological health assessment data from a conservation perspective.

International Perspective

Biologists and veterinarians from around the world have had the opportunity to partake in dolphin health assessments across U.S. waters with the aim of facilitating capacity building for global cetacean conservation (92). Established dolphin health parameter reference ranges enable comparison between international locations in an effort to tease apart the health effects of different stressors. Throughout the majority of the world, health monitoring of dolphins primarily involves post-mortem exams of stranded cetaceans in Europe, physical exams during translocations such as out-of-habitat animals in Asia and South America such as the recent intervention in Bolivia, or during remote biopsy sampling in photo-identification studies in the Mediterranean (169–173).

Increased international collaboration is essential for mitigating conservation crises and aiming to reduce the number of marine mammals becoming extinct such as the recent loss of the Yangtze River dolphin, the baiji (*Lipotes vexillifer*), or the high-risk Mediterranean monk seal (*Monachus monachus*) and vaquita porpoise (*Phocoena sinus*) (4, 174). Knowledge of capture techniques gained from dolphin health assessments in the U.S. has been applied to alternative species conservation approaches, such as with the vaquita, in an effort to temporarily remove animals from a dangerous habitat and relocate them to a protected environment (175). Marine mammal stranding networks exist world-wide with varying capacity dependent on funding, degree of public interest, number of strandings per year, facilities available, and the extent of inter-agency cooperation (176). Sharing knowledge and organizational structure from locations with financial support can aid capacity building in areas where marine mammal stranding networks are currently limited.

From examining dolphin communication to understanding energetics, lung capacity, respiratory metabolomics, and the mechanisms involved in deep diving physiology, health assessments are contributing to advancing scientific knowledge

(108, 177–179). Scientists have improved our understanding of dolphin anatomy and physiology by observing natural behavior during health assessments and monitoring activity and behavior post handling. Collaboration among scientists, veterinarians, and biologists at different health assessments enables a synergistic approach to understanding marine mammal health.

Universally, comprehensive cetacean conservation benefits from an integration of all available cetacean assessment techniques; hands on veterinary health assessments, post-mortem examinations, photo-ID surveys, and field observer data. Ideally a collaborative approach among scientists, biologists, fishers, local community members, and government officials would achieve maximum success from a management perspective. Increased discussion will aim to improve future inter-disciplinary approaches and address anthropogenic impacts on marine mammals.

CONCLUSION

The advancement of common bottlenose dolphin health assessments, transitioning from initial population assessments to endangered species conservation applications has occurred over several decades, expanding knowledge of marine mammal medicine and science. As veterinary standards for dolphins in human care have evolved, so have the standard protocols for handling and monitoring free-ranging dolphins, and diagnostics such as clinicopathology, ultrasonography, radiography, electrocardiography, respirometry, microbiology, and morphometry (29, 32, 43, 77, 104, 108, 180, 181). Dolphin health assessments are a valuable tool to extrapolate from the individual to understanding both population and ecosystem health. Combining scientific investigation with longitudinal population monitoring over multiple dolphin populations provides information to facilitate informed decisions regarding conservation, regulations, and protection of marine mammals.

Social education regarding the presence, longevity, and residence of bottlenose dolphins also allows the public to engage with the species and appreciate their intrinsic value within the ecosystem; dolphins share the same habitat and are impacted by some of the same stressors as local human communities. Citizen science and public interest peaks during UMEs when multiple carcasses are observed on the beaches in a short time frame. This is an opportunity to engage with people to highlight the environmental pressures faced by these apex predators and explain the anthropogenic impacts on these charismatic species.

An interdisciplinary and interagency approach is needed to fully understand the complexities of the challenges faced by marine mammals. Shifts in the tide of social attitudes, interests, regulations, and funding will have consequences to marine mammal populations both free-ranging as well as managed. Understanding the current global challenges and increasing human and wildlife interactions will dictate the mitigation efforts required to conserve future marine populations. Remaining at the cutting edge of science and advancing the field will aim to facilitate conservation of marine mammal populations for future generations.

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All authors contributed to the past, present, or future development of dolphin health assessments. AB and RW completed the manuscript writing with significant contributions from LS and CS. All authors provided edits and contributed to the final submitted manuscript.

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Conflict of Interest: JS was employed by the company Dolphin Quest.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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