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Examination and Application of Body Condition Methods in Cetaceans

By

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Submitted in Partial Fulfillment of the Requirements For the Degree of Master of Earth and Environmental Resource Management in

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Abstract

Body condition assessments are a valuable tool for evaluating the relative health of a population through various metrics, indexes, or proxies. Long-term data collection can be used to examine the relationship between fluctuations in body condition and natural or anthropogenic drivers. Application of this information is vital for monitoring the success of the conservation management decisions for a species or population. Cetaceans have a variety of methods available to assess body condition, including invasive methods like biopsies and necropsies or observational methods such as photogrammetry. Exploration of the application of these methods in the literature revealed an emphasis on necropsies for providing data on an individual's body condition. With the continual improvement in technological capabilities, non-invasive methodologies to study living individuals will increase to offer a better perspective on the condition of a population. For a species of cetacean, the gray whale (*Eschrichtius robustus*), a new method to assess body condition was developed. Utilizing boat-based photographs from a 30-year long dataset, the method presents a more

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sensitive tool than previously developed for gray whales to track fluctuations in body condition over a season. Application of this method to the larger gray whale population could help reveal underlying drivers of the population's health, particularly in response to the recent Unusual Mortality Event.

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Chapter 1

Assessing the Assessments: A Metaanalysis of Body Condition Methods

Across Cetacean Taxa

¹Telford, K. A., and E. L. Meyer-Gutbrod. To be submitted to *Aquatic Mammals*.

Abstract

Cetaceans experience a variety of natural and anthropogenic stressors, including prey fluctuations from climate change and overfishing, or exposure to vessels, fishing gear, contaminants, and noise. Many cetacean species are closely monitored to support marine mammal conservation policies, and cetacean population statuses can serve as sentinel species representing a key indicator of the health of lower trophic levels within the ecosystem. Monitoring changes to body condition can help to inform conservation decisions. An assortment of methods exists across species to assess the body condition of an individual, and new methods will continue to arise as technological improvements for drones, satellites, and infrared sensors offer new observing opportunities. However, while these methods have potential to aid conservation efforts, there appears to be little focus in the literature on their application. With the use of a meta-analysis, we examined literature coverage of cetacean body condition assessments and how they have been applied in management practices to identify gaps and provide insight on potential use. Here we present the results of that meta-analysis, including the taxa,

geographic location, and body condition assessment methodologies that are most commonly covered in the literature. The three species most prevalent include two ubiquitous species, the common bottlenose dolphin and harbor porpoise, followed by the North Atlantic right whale, a critically endangered species. The majority of studies were conducted on species or populations in the Northern hemisphere, 80%, with 47% of the total located along the eastern or western continental shelf of the North Atlantic. Necropsies were the most common method for assessing cetaceans, followed by pathology. Of 200 articles examining body condition, only 10% also considered the potential application of the method or data collected to support conservation monitoring or management decisions. Assessing the focal areas and gaps in cetacean body condition research is useful for guiding future research directions and prioritizing the development of novel assessments that will address these gaps and support conservation needs.

Key words: meta-analysis, literature review, cetacean, body condition, conservation management

Introduction

Across animal taxa, body condition assessments have been developed to establish expected baselines for the species and evaluate for deviations (Ndjaula, et al., 2013; Smith, et al., 2023; Wells, 2009). Understanding the health of a population and how it fluctuates over time can be instrumental in creating effective conservation measures (Cabezas, et al., 2010; Fernando, et al., 2009). Individuals will experience a variety of natural and anthropogenic stressors that will influence their health and body condition over time. For cetaceans, some of those drivers include prey fluctuations from climate change and overfishing, or exposure to vessels, fishing gear, contaminants, and noise (Kebke, et al., 2022; Oldach, et al., 2022). Body condition assessments include a variety of methods depending on the species being studied, but primarily vary as a result of the different aspects of individual health that can be examined (Bolger and Connolly, 1989; Labocha and Hayes, 2012; MacCracken and Stebbings, 2012). These assessments examine a range of biological factors to serve as an indicator or proxy of health for an individual or species and are useful for studying a wide range of stressors.

In cetaceans alone, a broad variety of methods are employed to examine the body condition of most families and species (Castrillon and Nash, 2020; Hooker, et al., 2019; Hunt, et al., 2015). Most often, the goal is for an objective, empirical assessment that may test one or multiple physiological parameters of health. However, marine mammals come with unique challenges of being difficult to find, present very little of their total anatomy when surfacing, and direct access to their bodies is challenging and often highly regulated (Hunt, et al., 2015). For these reasons, methods to evaluate cetacean body condition often focus on data collection that is remote, minimally invasive, or performed on dead specimens.

Body condition assessments have been developed for a large number of species and are used to detect visual or quantitative changes, such as, respectively, categorizing levels of emaciation or accessing changes in chemical concentrations, in an individual's body over time. Repeated measurements help to develop a database of information to track these changes, which can be utilized to create expected baselines for a species. For example, blubber thickness and lipid content are often considered the most significant parameters to measure when assessing the body condition of a cetacean. This tissue layer, composed primarily of lipids, serves as long-term storage of energy

(Iverson and Koopman, 2018; Koopman, et al., 2002). Thus, any assessment that can measure the blubber layer will act as a proxy for an individual's foraging success and blubber reserve accumulation (McDonald, et al., 2008).

Many assessments exist to estimate the blubber layer, ranging from direct measurements to indexes (Bradford, et al., 2012; Dannenberger, et al., 2020; Guo, et al., 2022; Krahn, et al., 2004; Pettis, et al., 2004; Telford, unpublished). Historically, the only way to measure the blubber tissue was through a lipid analysis from a biopsy, alive or dead, or indexes that create approximations of body condition based on apparent thickness of the blubber layer. As technological capabilities improve, new methods will continue to arise for drones, satellites, and infrared sensors offering new observing opportunities (e.g., Green, et al., 2023; Guazzo, et al., 2019; Nowacek, et al., 2016). Most methods in photogrammetry are using top-down drone images to develop body area indexes to track apparent changes in overall girth from changes in the blubber layer (Bierlich, et al., 2021; Burnett, et al., 2018; Burnett, et al., 2019; Christiansen, et al., 2016; Christiansen, et al., 2020; Christie, et al., 2022). However, improvements in technology are leading to further developments in 3D photogrammetry that would provide the most accurate, noninvasive, body condition assessment thus far (Bräger, et

al., 1999; Irschick, et al., 2020; Irschick, et al., 2021). Body condition of a live individual most often is determined through these assessments or proxies that either measure only one component of overall body condition or utilize an index that provides a relative comparison of the population's condition. These methods test or evaluate one or more parameters relating to an individual animal's health and the results are used to determine the relative health of the population.

Estimating blubber thickness or composition is not the only method to studying health. Tissue collection is common across taxa and can be used to test for biomarkers or chemical pollutants (Borrell, et al., 1999; Dannenberger, et al., 2020; Gallo-Reynoso, et al., 2015; Stockin, et al., 2021). There are other proxies to determining body condition that can provide important information but are not as representative of the individual's health, often by tracking the results of behaviors associated with worse health or condition, i.e., increased rake marks or evidence of anthropogenic interactions. Reviewing stomach contents or fecal matter, for example, can provide data on the individual's diet, but does not provide any direct evidence of overall body condition. Each method has its own unique strengths and weaknesses and can be used to answer different questions. Understanding all methods available

when attempting to conduct a body condition assessment on a cetacean population will allow the researchers to select the most appropriate method(s) for their focus. For example, conservation managers are more likely to use biopsies to track the concentration of a chemical pollutant in a population or lipid concentration. However, researchers and managers alike should consider the use of multiple methods to examine the different facets of a population's health. No single method will be able to capture all pertinent information, and by utilizing different methods to target different aspects of body condition, scientists can get a clearer picture of the population.

Many countries have dedicated stranding and necropsy teams to respond to dolphin and whale strandings and as a result, these necropsies of stranded animals are one of the most common methods for collecting information on cetacean body condition. Data collection methodologies during necropsy are relatively consistent between researchers and stranding networks, making them a useful tool to evaluate health across space and time. Typical necropsy procedures include taking body parameter measurements, i.e., girth and length, with many also taking more detailed measurements specific to that species' anatomy. Most necropsies also note any apparent pathologies, such as skin diseases or

trauma. Often, various tissue samples are collected, including skin, blubber, teeth / baleen, and internal organs, for later biochemical examination, and blubber layer thickness is measured. However, this information can vary based on the team responding and the decomposition state of the animal.

Since necropsies are performed in response to random stranding events, and the ability to conduct a necropsy depends on access, timing and immediate availability of researchers and funds, necropsy data are not consistently collected over time and sample sizes tend to be small. Poor body condition preceding the animal's death and/or extensive decay prior to necropsy can bias the sample when trying to consider or create body condition baselines from a necropsy. Stranded individuals often represent poorer body condition than the living population, especially when disease, injury or entanglement contributed to the cause of death. However, some data are collected on whales that have been harvested either by sustenance hunters or whalers who intentionally harvest cetaceans; these practices may target animals in better perceived body condition for larger yields. Information from these studies would provide better data for establishing health baselines as opposed to necropsies (Breton-Honeyman, et al., 2016; Christiansen, et al., 2021). Thus, another important factor when

considering the use of a method, or its data, is if and how necropsies contributed to their creation.

Unlike necropsies, animals held in captivity, whether permanently or temporarily, provide a unique opportunity for more thorough body condition assessments over time (Blanchet, et al., 2008; Lockyer, et al., 2003; Mazzaro, et al., 2011). Their habitat and diet are often controlled, which would allow for a clearer examination of trends and drivers. Additionally, these individuals tend to be trained in husbandry behaviors, which allows for better opportunities for body handling and more invasive data collection. This creates easy access to most of the anatomy and serves as an effective method of establishing baseline health parameters, particularly when their environment is held consistent. However, the body condition of animals in captivity may not be representative of the condition of animals in the wild population, since they have different diets and behaviors, and are exposed to different threats and diseases.

Application of these assessments to populations can be vital tools in a manager's toolbox, particularly for threatened or endangered species (Bradford, et al., 2012; Hunt, et al., 2015). Kershaw et al., (2018) examined the effectiveness of using blubber biopsies as a representation of body condition and found that the lipid content captured from shallow-depth biopsies may not be a robust representation. Identifying the weaknesses of a methodology can allow a manager to select several, appropriate, assessments for a more holistic approach. Conservation management should have the goal of long-term monitoring with minimal disturbance to a population, thus invasive methods like biopsies may not be viable, particularly for threatened or endangered species like the North Atlantic right whale (Hunt, et al., 2015).

Figure 1.1. The PRISMA flow diagram of the meta-analysis. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

A unique exception to avoiding disturbing wild animals is the 50+ year, multi-generational, study on a resident population of bottlenose dolphins in Sarasota Bay, FL, USA, operated by the Chicago Zoological Society. They collect both observational and direct measurements of the population, frequently rounding up the wild individuals and collecting valuable measurements (Wells, 2009). While animals in captivity can serve as a surrogate for long-term body condition assessments, such intimate knowledge of a wild population can provide novel insights. Additionally, the data collected from this long-term study served to support the development of a mobile application to estimate the body mass of captive or wild individuals from simple body measurements (Hart, et al., 2017).

Pathologies, natural or anthropogenic such as trauma or pollutants, are also considered by conservation managers. Injuries and scars resulting from anthropogenic interactions are often a main topic of focus because of their direct link to fishery and vessel management (Marks, et al., 2020; Minton, et al., 2020; Raverty, et al., 2020). Many studies examine the trend of chemical pollutants found in various cetacean species, another pressing concern for conservation managers (Balmer, et al., 2018; Durante, et al., 2016; Williams, et al, 2021).

By tracking fluctuations in the body condition of a cetacean population, we can not only assess the status of the population, but also the degree of success of conservation management practices. Additionally, many cetacean populations can serve as sentinel species representing a key indicator of the health of lower trophic levels within the ecosystem and drive conservation policies (Hazen et al. 2019, Williamson et al. 2021).

Figure 1.2. Total number of articles published each year that were selected for inclusion in the meta-analysis. The final year (2023) is incomplete, and only shows studies published by the time each database was queried.

This study presents the results of a meta-analysis of peer-reviewed research articles focused on cetacean body condition assessments and examines the use of these assessments in the literature across species, location, and method employed. This work examines the distribution of body condition assessments across species and geography to identify potential gaps in the literature that can help

drive research forward. Identification of knowledge gaps and an understanding of method application, along with their potential strengths and weaknesses, can help create more informed conservation decisions. Proper body condition assessment of a population will likely require the use of multiple assessment methodologies to ensure a holistic understanding of health and prevent biases from one method type.

Methods

Literature Selection Protocol

The goal of a meta-analysis is to determine trends in the literature on a particular subject and this type of study should meet a series of recognized standards to ensure the most effective examination (PRISMA, 2020; Figure 1.1). These standards include careful selection of search terms, use of a publicly available literature database(s), and clear inclusion/exclusion criteria to ensure replicability. Rigorous search parameters will help capture all relevant literature. Here, we selected 4 topics that we considered relevant when searching for articles using a method to determine cetacean body condition: cetaceans, body condition, body condition assessments, and conservation management. These topics were used to develop a list of search terms to be included in the search string.

Within each topic there is a list of keywords such as, respectively, "odontoceti", "body length", "morphometr*", and "conserv*". A full list of terms is included in Appendix A. A total of 43 search terms were used, 3 of which are conservation management terms that were only used to identify the subset of articles that included some discussion of that topic. No exclusion terms were included to minimise the risk of missing relevant literature.

Search terms were run through 2 different databases: Aquatic Sciences and Fisheries Abstracts (ASFA) and Biological Science Collection (BSC). ASFA is focused on offering articles on the science, technology, management, and conservation, including legal and socio-economic aspects, of marine, brackish, and freshwater systems (ASFA, 2023). BSC is a database that provides more articles in microbiology and biochemistry while also ensuring a comprehensive selection of literature in zoology and ecology (BSC, 2023). BSC and ASFA specialize in the primary fields, biology and aquatic science, that pertain to the topic of cetacean body condition.

A total of 552 articles were returned from the search results: 230 from ASFA (April 2023) and 322 from BSC (June 2023) (Fig. 1.1). Each available article was downloaded for analysis. All articles that could be accessed based on the University of South Carolina's research library

subscriptions were downloaded. Articles that were not included were either behind a paywall, in a foreign language, or a duplicate. Even if an abstract was available, an article would not be considered for review without access to the entire article. A few articles published in a foreign language did not have an English translation readily available, and thus had to be excluded from consideration. The methods section of each article was particularly important in determining relevant information about the study. A total of 411 articles were downloaded and reviewed: 176 from ASFA and 235 from BSC. Some duplicates were missed in the initial exclusion phase, thus the article was downloaded twice and represented in the downloaded total, but one of the copies was excluded later as a duplicate. There were 122 articles, 51% of total duplicates, that were excluded because they appeared in both the ASFA and BSC databases. The remaining 49% were articles that appeared more than once within a single database (37 in ASFA and 79 in BSC).

The program Rayyan (Rayyan 2022) was used for organization and labelling of all downloaded articles. This software creates a list of the articles and allows the analyst to either "include" or "exclude" the article and apply user-defined labels. Use of this program simplifies

the sorting and categorization process and provides a dynamic list of labels and their relevant count total.

Following the assimilation of the literature, each article was manually reviewed, and the content's relevance was used to determine inclusion or exclusion. There were two exclusion criteria: the study species was not a cetacean or the focus of the article was not body condition assessments. Each article received a label that included the year, study species, study location, and body condition assessment method used. If an article had a specific focus on the body condition methodology, it would receive another label detailing whether it was introducing a new method, built considerably upon an established method, reviewed available methods for a species or family, or conducted an analysis on a method(s). The latter contained 4 articles that took an analytical approach to assessing the effectiveness or uncertainty associated with one or more methods.

The subset of articles that were returned from the conservation search were scanned for a description of how the assessment method could be incorporated into monitoring programs or a description of how the assessment could assist management decisions for a specific population. Many articles from the subset were excluded because the term "conservation" only appeared once or twice, providing no

more than a sentence or two containing a general comment on conservation.

A full list of labels, with descriptions, is included in Appendix B. Label counts were gathered and organized into appropriate categories for comparison. Visualizations of the data were conducted with R (R Core Team 2022).

Results

The combined search results included a total of 552 articles (Fig. 1.1). Ultimately, 352 articles were excluded from analysis because it was either a duplicate, behind a paywall, in a foreign language, or was not focused on cetaceans or body condition. This left a total of 200 articles that were selected for inclusion in the study and were reviewed for content.

To examine spatial and temporal trends of publications focused on body condition, articles were parsed by year and by geographic region. Cetacean body condition article publications have increased over the past three decades, and in recent years (since 2019) about 20 articles have been published each year (Fig. 1.2). Article study location was complex to aggregate. Many articles reported a specific country(s) for their location. However, there were some studies that covered a much larger area, such as the North

Atlantic, thus were labeled appropriately to represent the full study area.

All studies were categorized into larger geographic regions, which were demarcated by ocean basin and latitude range: the Arctic Circle, Tropic of Cancer, Topic of Capricorn, and Antarctic Circle (Fig. 1.3 and 1.4). About 80% of studies focused on species or populations in the Northern hemisphere, especially on either the North American or European shelves of the North Atlantic (Fig. 1.3 and 1.4). The North American coast of the North Pacific had considerably fewer studies than the North American coast of the North Atlantic (10 studies vs. 53 studies, appendix D). More studies focused on taxa in temperate and subpolar regions and in subtropical regions (Fig. 1.3). A full list of countries and regions is available in Appendix C.

The focal species or taxa from each study included in the meta-analysis were recorded to gauge the taxonomic distribution of cetacean body condition research. The family of oceanic dolphins (Delphinidae) comprise the majority of studies with 106 articles, with a third of those articles (38 out of 106) pertaining to the body condition of common bottlenose dolphins (*Tursiops truncatus*, 24 articles) or common dolphins (*Delphinus delphis*, 14 articles) (Fig. 1.4).

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Figure 1.3. Study regions and countries were grouped into larger regions for easier visualization. Symbols scaled relative to count with article counts provided for some regions as reference. World map created by Encyclopædia Britannica, Inc.

The next most common taxonomic grouping was rorquals (Balaenopteridae) with 52 articles, and the most common focal species in this taxon were humpback whales (*Megaptera novaeangliae*, 14 articles), fin whales (*Balaenoptera physalus*, 11 articles), and common minke whales (*Balaenoptera acutorostrata*, 10 articles) (Fig. 1.4). The next two most common taxonomic groupings found in the literature are Balaenidae, the family of bowhead and right whale species, and porpoises (Phocoenidae), both with 29 articles (Fig. 1.4). Harbor porpoises (*Phocoena phocoena*) were the second most common study species behind the bottlenose dolphin with 23 articles. While most articles focused on one or multiple specific study species, a few articles studied body condition at a higher taxonomic order: 6 studies focused on the infraorder of Cetacea, one study focused on the parvorder Mysticeti and one study focused on the parvorder Odontoceti (Fig. 1.4, top right).

Given the wide range of methodologies that can be used to measure cetacean body condition, articles included in the meta-analysis were reviewed and categorized by the type of method used in the study. There was a total of 14 identified method types to evaluate body condition, with some articles reporting using two or more methods (Fig. 1.5). These methods were then sorted into two categories: methods that take a direct or highly quantitative

measurement and methods that functioned as a proxy of body condition or created a categorical index. The most common methodology found in the literature was necropsy (94 studies), followed by pathology (63 studies). Pathology most commonly referred to the examination of pollutants, but also included disease, or trauma resulting from intraand interspecific or anthropogenic interactions. Invasive methodologies, those that require direct contact with the animal (live or dead), included studies that took biopsies or examined reproductive organs, teeth, or stomach contents. Biopsies are often used to collect tissue samples that are then used to analyze blubber tissue content or test for chemical pollutants.

The remaining methodologies found in the literature are less invasive, relying on photos instead, including techniques like photogrammetry or reviewing individuals for visual indicators (Fig. 1.5). Photogrammetry would be considered a direct measurement as it takes a sensitive, quantitative approach to evaluating body condition in cetaceans. Methods that categorize or score photos based on an established scale, including rake marks and anthropogenic interactions, would be an examination of visual indicators. Additionally, these serve as a proxy for body condition since they are tracking the results of behaviors associated with worse health or condition.

While most articles included in the meta-analysis applied an existing body condition metric to address questions in biology, ecology, or conservation, a subset of 42 articles focused on the development of the body condition assessment itself. The majority of articles that focused on the body condition assessment method provided considerable adjustments or expansions to established methods (20 articles, Fig. 1.6). The next most common article type in this subset were the 14 articles that presented new methods for examining the body condition of a cetacean (Fig. 1.6).

Finally, 46 articles were returned from the conservation search, but over half (26 articles) did not actually have any substantial focus on conservation (Fig. 1.7). Inclusion of the term "monitor*" returned many articles that simply utilized the word to discuss general monitoring and data collection on a population without any focus conservation or management efforts. Additionally, several articles were excluded because conservation applications were only briefly mentioned as a single or a couple sentences. Of the remaining articles that had a sufficient focus on conservation issues, the most common thematic subset focused on using body condition assessments as a tool for population monitoring to guide conservation policy (12 articles, Fig. 1.7).

Figure 1.4. Treemap showing the number of studies included in the meta-analysis, separated into the taxonomic groupings, and when possible, species of interest.

Many suggested continuing to monitor the population as has been done to collect the data thus far, with a focus on using that information to detect major changes in the population's health. Management included 5 articles that presented data to support either conservation or captive management decisions (i.e., Williams, et al., 2021, and Lauderdale, et al., 2019). There were 4 articles that were classified as a methods summary (Fig. 1.6) and 4 articles that were classified as a conservation summary (Fig. 1.7), and 3 of those articles were assigned to both of these categories. These summary articles provide a review of current methods available and also discuss their past or potential use in conservation.

Discussion

This meta-analysis examines body condition assessments across cetacean taxa with the goal of uncovering trends in method application and identifying potential gaps in the literature. The literature selection protocol employed in this study aimed to gather a comprehensive collection of relevant articles to discover the most common geographic regions, focal taxa, and methodologies represented in the cetacean body condition literature. The search revealed that the majority of body condition studies are most commonly conducted in waters adjacent to North America and Europe (Fig. 1.3), and the

majority of studies are focused on a small number of species, especially bottlenose dolphins and harbor porpoises (Fig. 1.4). Review of the type of body condition assessment methods reported in the literature shows that necropsies are the most common method (Fig. 1.5). An additional set of search terms were used to identify the subset of articles employed in conservation management.

Figure 1.5. Treemap showing the number of studies that received each label regarding a type(s) of body condition assessment methodology used in the article that were included in the meta-analysis. Methods were split into two groups: methods that take a direct or highly quantitative measurement and methods that functioned as a proxy of body condition or created a categorical index. Descriptions of each category are included in Appendix B.

A meta-analysis requires rigorous search parameters to capture all relevant literature (Crowther, et al., 2010). In this meta-analysis, to improve the replicability of the analysis, neither grey literature nor

Figure 1.6. Number of articles that had a specific focus on the methodology.

forwards/backwards citation chaining were included in the collection of literature, as the protocol for collecting these articles is less precise than for the published literature and research/document quality becomes extremely variable (Cribbin, 2011). Inclusion of grey literature may provide more information on various assessments of body condition, such as methodologies that were less successful or unpublished data on alterations or expansions to methods (Easterbrook, et al., 1991). However, the inclusion of citation chaining and grey literature in this type of study would most likely support the trends found within this narrower search.

This study only examined literature from two databases (ASFA and BSC), although other databases could have been queried. The selection of these databases and the approach to standardizing search terms and keywords across databases helped ensure consistency within the study and replicability for future studies.

BSC and ASFA collect literature across both of the broad fields, biology and aquatic science, relevant to the topic of cetacean body condition. ASFA is one of the primary databases that marine science librarians utilize likely due to their wide coverage of literature types, including grey literature and conference proceedings (FAO, 2020). Both databases aim to cover international publications as well, providing English translations when available, minimizing the potential source of bias by missing international studies. BSC claims 1/3 of their content is from foreign language articles and provide abstracts in English (ProQuest, 2020). It is important to know, however, that for this study we required a complete translation in order to conduct sufficient analysis. There are software programs that could enable the search for published articles across a broader range of databases, such as Google Scholar or Web of Science, but searching for literature through these programs has limitations. Google Scholar has limited "Advanced Search" capabilities to refine search criteria
relative to the other databases. As a result, there is a much higher proportion of articles whose theme and content fall outside of the goals for this meta-analysis. As with BSC and ASFA, pulling literature from multiple databases will result in duplicate articles returned. There is a large overlap between Web of Science's different databases, so each additional database yields diminishing returns. Although no database is perfect, the considerable difference in literature focus between ASFA and BSC should provide a comprehensive look at publication trends, and expansion to further databases will likely strengthen what has been found here.

While there was a diverse array of methodology types found in this meta-analysis, only 14 articles were found that introduced a new methodology and 20 articles presented an expansion on established methods. Several of the newly introduced methods are species-specific, but there is diversity in what the assessment is targeting. For example, one study created a body condition assessment only for beluga whales as it was derived from morphometric measurements of beluga carcasses (Larrat and Lair, 2020), while another article suggested mink (Mustelidae) as a surrogate model to determine marine mammalian PCB toxicity benchmarks (Folland, et al., 2016).

Necropsies were a common source of data for conducting body condition assessments, representing at least one data source in 47% of the total included articles (Fig. 1.5). Four of the articles that introduce new methods relied on measurements taken from necropsies. Body condition assessments established by necropsies can be extremely helpful for understanding the fate of that individual. However, stranded individuals often exhibit poor body condition in the period leading up to its death, and thus necropsies are not especially helpful for characterizing the health of the living portion of the population (Christiansen, et al., 2021). The portion of dead animals available for necropsy may also be a biased sample of recent mortalities. Some causes of death, such as the slow emaciation caused by severe entanglements, are more likely to lead to a sunken carcass, whereas an animal struck by a ship is more likely to float or strand on a beach (Moore, et al., 2020). The quality of necropsy data also depends on the carcass's decomposition state. The loss of oil soon after death may low-bias estimates of the blubber tissue thickness and composition (Krahn, et al., 2001), but other aspects of decomposition, like gas production, can also impact the accuracy of tissue thickness and composition measurements (Bernaldo de Quirós, et al., 2013). However, as is the case with the beaked whale family (Ziphiidae), low detection rates can limit opportunities to develop

assessments based on live animals, and necropsies can be one of the only ways to collect data on individual and population health (Hooker, et al., 2019).

Pathology studies can be valuable for assessing a population's status since they often rely on determinate, quantitative criteria, like measuring levels of pollutants in tissue. Many of the articles found in this meta-analysis examined trends in chemical pollutants in an individual's blubber or muscle tissue, using samples typically obtained from necropsies of stranded or harvested animals (e.g., Peck, et al., 2008 or Hoekstra, et al., 2002). However, several articles used biopsies from live animals in addition to necropsy data (e.g., Gallo-Reynoso, et al., 2015). Lipid loss has been observed in necropsies from decomposition, but Ryan and Kershaw (2022) found that the lipid content of biopsies is significantly lower than the actual layer, highlighting the need to continue developing and expanding on new, non-invasive, methods. Pathologies such as parasites or disease are predominantly determined through a necropsy but can occasionally be detected visually on live animals (e.g., Hamilton and Marx, 2005).

Unfortunately, there were few studies found in the meta-analysis that apply or examine the application of body condition assessments to conservation monitoring or management decisions. The majority of articles that

included methods for collecting pollutant concentrations during necropsies focused on establishing baselines or biomarkers (e.g., Andvik, et al., 2023 or Garcia-Cegarra, et al., 2020). There has been less follow up in the literature about the application of these methods, in particular for monitoring the health of a population or species, or the efficacy of conservation policy.

Out of the 200 articles included in this analysis, only 46 were returned in a restrictive search focused on conservation (Fig. 1.7). However, after reviewing this subset, 26 did not include a considerable focus on conservation. This left 20 cetacean body condition articles (10% of all articles included in this study) that address conservation in some capacity. The North Atlantic right whale was the most common focal species in the conservation subset, accounting for 4 of the 20 conservation articles. The remainder of the conservation articles were distributed evenly between various species of mysticetes and odontocetes. Several conservation articles identified the drivers of poor body condition in a population or species, namely anthropogenic interactions, and suggested that management decisions take this information into consideration (e.g., Neilson, et al., 2009 or Raverty, et al., 2020). Schick et al. (2013) modeled movement, health, and survival for the North Atlantic right whale to help

managers identify vulnerable periods throughout their annual cycle. This type of comprehensive model has potential for application to other species.

While there is a diverse array of methodologies available to estimate the body condition of cetaceans, the application of these methods to conservation outcomes is lacking. Conservation management relies on consistent and accurate data to help inform decisions, and body condition assessments can be a valuable addition to that data $(e,q,$ Hoare, et al., 2006; Sun, et al., 2021). However, when utilizing an assessment or data collected from a previous assessment, it is important to know the source of data collection. Necropsies were a common source of body condition information but are typically representative of poor body condition. Many studies compared data collected from both necropsies and live animals as a part of their assessment, thus careful consideration should be given when determining how to monitor a population. Notably, management agencies may be using body condition assessments in the conservation decision making process without reporting it in the literature, which may justify an extension of this type of analysis beyond peer-reviewed publications. Feedback from managers who see the impact of employing body condition assessments into management frameworks is valuable for improving conservation outcomes.

The long-term data collection from the wild bottlenose dolphin population in Sarasota Bay, Florida has been collated into a comprehensive database on morphometric data (Wells, 2009) and utilized by Lauderdale et al. (2019) to create a program that allows agencies that manage bottlenose dolphins to generate an estimated mass. Their body size measurements are inputted into an algorithm that uses the collected data from the wild population to produce the estimated mass. This mobile application allows facilities of captive animals to better evaluate dolphin body condition when it is not possible to weigh the individual. While this is a unique and valuable application for management, this type of program has not been developed for any cetacean populations that are threatened or endangered.

Body condition assessments have the potential to provide sensitive and objective metrics to examine the outcomes of management practices. Particularly, body condition assessments are useful for tracking changes in health before, during, and after conservation efforts. More thorough investigation of the results of management decisions will allow scientists and managers to identify the least and most effective policies, and better prioritize conservation decision-making in the future. Increased research on the link between conservation policy

and changes in cetacean body condition could inform the development of species- or location-specific conservation agendas.

This examination of cetacean body condition assessments published in the literature has provided valuable insights. There is a robust body of literature on cetacean body condition, however, research is not evenly distributed across locations or taxa. Due to the challenges associated with collecting data on living cetaceans, the most common methods are employed during necropsies. Although these data are extremely valuable for determining cause of death, contaminant concentrations, and previous life history information, necropsies are not necessarily representative of the living, healthy population. This underscores the need to continue to develop non-invasive methodologies that are useful for assessing the living population with minimal disturbance. Only a small fraction of articles included in this meta-analysis addressed conservation issues (10%), suggesting that the literature should be expanded to use body condition to evaluate conservation status of a population and measure the impacts of disturbances and the efficacy of mitigation efforts utilizing a variety of appropriate methods. This study shows that cetacean body condition research has clear

avenues for future growth with the potential to improve coastal and marine ecosystem management.

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Chapter 2

How Robust is *Eschrichtius robustus*? A Novel Photographic Index of Body Condition from boat-based photographs of Gray Whales*

*Telford, K. A., J. Calambokidis, K. Flynn, J. Witt, A. Perez, J. Durban, H. Fernbach, and E. L. Meyer-Gutbrod. To be submitted to *Frontiers in Marine Science*.

Abstract

Access to 30 years of photographic data of a unique gray whale (*Eschrichtius robustus*) feeding aggregation has provided an opportunity to develop and test a new methodology for assessing body condition. At the time of this study, the aggregation that regularly enters the Puget Sound, Washington, USA, to forage for ghost shrimp in the spring and early summer and contains just over 10 individuals, while the greater eastern North Pacific population has reached ~20,000. Around 35,000 images of the feeding aggregation in the Puget Sound (the "sounders") were examined and 729 were selected as suitable for photographically assessing their body condition using geometric tools in open-source image analysis software to indicate changes in blubber thickness as a proxy for body condition. This body condition angle, *°BC*, varied significantly with day of the year, with individuals increasing their body condition throughout a feeding season. This novel index of body condition illustrates the opportunity to develop more creative methods to quantitatively assess change on individual, seasonal, and annual scales. Expansion of

this method to the larger eastern North Pacific gray whale population may be effective for a more detailed examination of fluctuations in condition and the potential environmental and anthropogenic drivers. There is also potential for adaptation of the method to historic photo catalogues of other mysticete whale species, creating opportunities for enhanced data-driven management plans.

Key words: eastern North Pacific gray whales, photo-identification, body condition, Puget Sound, mixed effects models, photogrammetry

Introduction

Objective and quantitative indicators of body condition and health status are critical components of ecosystem-based management and conservation of protected species (Bourdaud et al., 2016; Pettis et al., 2017; Riisager-Simonsen et al., 2020, Stewart et al., 2021). To assess cumulative environmental conditions and threats throughout a species' range, a body condition index can prove valuable for tracking the relative success of a population's foraging season by monitoring health changes across seasons (Christiansen et al., 2020; Fearnbach et al., 2020; Torres et al., 2022). Health assessments may be an important tool to evaluate the impact of natural environmental drivers along with variations in human activity, such as vessel traffic and entanglements, on body condition of a cetacean population over seasonal, interannual, or interdecadal periods to better increase our understanding of ecosystem-wide processes. (Akmajian et al., 2021; Blair et al., 2016; Castellote et al., 2012; Ingman et al., 2021; McHuron et al., 2021; Pettis et al, 2017; Saez et al., 2021; Silber et al., 2021; Stewart et al., 2021).

Most large whale species are considered capital breeders, relying on an accumulation of fat stores during summer foraging in high latitude feeding grounds to supplement them throughout their migration and calving season to lower latitudes (Bengtson Nash et al., 2013; Eisenmann et al., 2016; Young, 1976). Without a successful foraging season, individuals may be at risk of emaciation, disease, or delayed reproduction (Pettis et al., 2017; Stimmelmayr et al., 2020). Individuals that are not able to build the necessary blubber reserves are associated with poor body condition, which can serve as a proxy for identifying ecological fitness and a possible indicator of the health of the ecosystem (Riisager-Simonsen et al., 2020).

As is typical of baleen whales, the gray whale (*Eschrichtius robustus*) is a filter feeder that spends their foraging season in high latitudes where ecosystem productivity is higher and returns to low latitudes during the winter to breed and calve. There are two distinct populations: western and eastern North Pacific gray whales (NOAA Marine Mammal Stock Assessment Report: Western North Pacific Stock and Eastern North Pacific Stock; 2020). The western gray whales exist along the coast of China and Russia and have not recovered from whaling; they are listed as endangered with a population size <300 (Bröker et al.,

2020). The eastern North Pacific gray whales, which have returned to their carrying capacity of ~20,000 individuals, migrate from their southern wintering grounds in Baja California, Mexico, to their northern feeding grounds in the Bering, Chukchi, and Beaufort seas (Durban et al. 2015; Stewart and Weller, 2021).

The main prey source for gray whales are amphipods and mysids, and previous studies have documented variation in gray whale health and demography connected with changes in prey abundance (Burnham and Duffus, 2022; Darling et al., 1998; Hildebrand et al., 2022; Lemos et al., 2020). Often prey is affected by ocean processes, such as the NE Pacific marine heatwave of 2014-2016, and can have a cascading effect on the food chain (Rogers-Bennett and Catton, 2019; von Biela et al., 2019). If recruitment is poor for prey, foraging success will be impacted resulting in worsening body condition. Ongoing limitations in prey will ultimately reduce a populations' ability to successfully reproduce or lead to starvation (e.g., Ward et al., 2009, Meyer-Gutbrod et al. 2015). Behavior or distribution shifts resulting from declines in prey availability have even led to Unusual Mortality Events (UME) in other baleen whale species (Meyer-Gutbrod et al. 2018). Such prey-driven declines may seriously impact vulnerable mysticete populations and increase threat of extinction (Meyer-Gutbrod et al. 2021).

Although feeding rates and success is difficult to measure, fluctuations in body condition can be used as a proxy of health since it is vital in energy storage and insulation, and it is useful as an indicator of foraging success (Miller et al., 2011). Previously developed methods to assess body condition of gray whales have focused on indicating changes in blubber thickness by describing notable physical features such as the scapula, spine, and the depression behind the post-cranial hump (Bradford et al., 2012). This body condition index (hereafter referred to as the "Bradford method") has been used as an effective tool to assess gray whale health at the individual and population level (Bradford et al., 2012).

The Laguna San Ignacio Ecosystem Science Program has been monitoring the body condition of the gray whales that winter in Laguna San Ignacio for several years using the Bradford method (Valerio-Conchas, 2022). They produce annual reports on the status of the population that they photograph. Additionally, the method has been used to study the intra- and interannual body condition changes in the PCFG population (Akmajian et al., 2021).

Although the Bradford method has been used effectively to measure body condition changes in gray whales, there are some limitations. The Bradford method uses a 3-point categorical scale, but since scores are assigned at the

researcher's discretion, there is some subjectivity to the scoring. To better understand the full variation in health for gray whales, we propose the development of a more sensitive and objective method that relies on a continuous standardized measurement index, named the body condition angle (hereafter referred to as *°BC*), rather than categories as an alternative or a complement to other body condition index methods.

The development of Unmanned Aircraft Systems (UASs), specifically multicopter drones, has created the opportunity for a new technological approach to marine mammal health research (Durban et al., 2015; Durban et al., 2016; Christiansen et al., 2016). Drones provide a vertical aerial perspective of individuals, which allows for photogrammetric analysis of body shape. This has proven to be an effective method of measuring small changes in body condition (Fearnbach et al., 2020) and their efficiency enables measurements of large portions of populations (Stewart et al., 2021).

However, many photographic sighting datasets exist that were collected from boats many years, even decades, prior to the use of drones. To accommodate limitations in operating capacity and high costs that some researchers face, most photographic sighting data is still collected on standard DSLR cameras rather than drones. To accommodate

the large amount of boat-based photographic data, objective photographic measurements are needed when access to a drone is not possible, as well as to provide historic context that includes photographic data collected prior to drone use.

In this study, a boat-based photographic technique was developed to objectively and quantitatively indicate body condition in gray whales, using a metric we term the body condition angle (*°BC*). This technique was developed and tested on a small group of 11 whales known as the "Sounders" that gather annually in Puget Sound between February and June to feed on ghost shrimp (*Callianassa californiensis*). Due to consistent sightings of the same group of 2-10 individuals in each year over a 31-year monitoring period, the Sounders aggregation serves as a valuable test group to evaluate individual and temporal effects using our novel body condition index. Most of these individuals have been sighted since monitoring began in 1990 and return almost every year (Calambokidis et al., 2002; Calambokidis et al., 2010). Occasionally, new individuals are observed within the Sound, but individuals are not designated as a member of the Sounders aggregation unless they have been observed in the Sound during two separate years. After demonstrating the efficacy of this photographic health assessment tool in tracking individual and temporal variation in body

condition, and with further development this method may improve in sensitivity. Additionally, this method could be used in future studies to compare the health of individuals in the Sounders aggregation to the health of other gray whales that do not enter the Puget Sound or serve as a complement to other body condition assessments.

Methods

Whale sighting data

From 1990 to 2020, annual boat photo-identification surveys of the Puget Sound were conducted, collecting photos of gray whales during sightings (Table 1). Sightings ranged from the months of February to June with the majority in April followed by March. Photos were reviewed over the 31 years, but these were filtered for measurement quality. Only photos where the whale was visible from at least the blowhole to behind the post-cranial hump were selected (Fig. 2.1); the individual should be parallel to the boat and in their most elongated (flat parallel to the water surface) position of their breathing sequence to minimize the effect of body flection.

Year ID	21	22	44	49	53	56	185	356	383	531	723	Total
2021	6	\circ	3	$\mathsf{O}\xspace$	5	$\mathbf{2}$	\circ	$\mathbf 1$	8	$\overline{2}$	4	31
2020	\mathcal{S}	13	5	6	6	$\overline{2}$	$\overline{0}$	$\mathbf 1$	6	4	3	49
2019	$\overline{4}$	15	O	8	9	$\overline{4}$	5	$\overline{2}$	12	$\mathbf{2}$	0	61
2018	9	8	5	14	\mathcal{G}	4	O	O	12	$8\,$	$\overline{7}$	76
2017	$\overline{4}$	14	$\overline{0}$	5	5	$\mathbf 1$	\circ	O	10	\circ	3	42
2016	\mathcal{S}	$\overline{0}$	$\overline{2}$	$\mathbf{2}$	O	$\mathbf 1$	$\overline{0}$	$\overline{0}$	8	6	13	35
2015	$\overline{4}$	12	5	3	$\overline{4}$	$\mathbf 1$	$\overline{0}$	O	8	\sqrt{a}	4	41
2014	O	\circ	$1\,$	3	3	$\overline{2}$	$\overline{0}$	3	5	$\mathbf 0$	6	23
2013	5	8	$\overline{0}$	$\mathbf 1$	7	7	\circ	$\overline{0}$	4	$\mathbf{1}$	4	37
2012	3	4	$\overline{0}$	$\mathbf 1$	4	$\overline{0}$	$\overline{0}$	$\overline{0}$	3	\circ	3	18
2011	\circ	$\overline{0}$	$\mathbf 1$	$\mathbf 1$	12	$\overline{4}$	\circ	$\mathsf{O}\xspace$	$\overline{2}$	$\mathbf{1}$	6	27
2010	$\overline{2}$	9	$1\,$	5	3	O	O	$\overline{4}$	\mathfrak{Z}	$\mathbf{2}$	\overline{O}	29
2009	5	$\mathbf 1$	3	6	$\mathbf{2}$	O	\circ	O	5	$\overline{0}$	$\overline{2}$	24
2008	$\overline{2}$	3	O	\mathfrak{Z}	O	$\mathbf 1$	\circ	O	O	$\mathbf{1}$	0	10
2007	$\mathbf{1}$	Ω	O	O	$\overline{0}$	\circ	\circ	O	$\mathbf 1$	$\mathbf{1}$	0	\mathcal{S}
2006	\mathfrak{Z}	10	O	O	$\overline{4}$	5	$\overline{0}$	$\overline{0}$	$\mathbf{2}$	$\mathbf{1}$	$\overline{4}$	29
2005	\circ	$\mathbf{1}$	O	O	$\mathbf 1$	O	\circ	$\mathsf{O}\xspace$	$\mathsf{O}\xspace$	O	0	2
2004	$\mathbf 1$	$\overline{0}$	$\overline{2}$	O	O	O	$\mathsf{O}\xspace$	$\mathbf 0$	$\mathbf{2}$	\circ	$\overline{4}$	9
2003	\circ	$\mathsf{O}\xspace$	O	O	O	O	$\mathsf{O}\xspace$	O	O	\circledcirc	0	\circledcirc
2002	\circ	$\overline{0}$	O	$\mathbf 1$	O	O	$\mathsf{O}\xspace$	$\mathsf O$	O	\circledcirc	0	$\ensuremath{\mathbbm{1}}$
2000	\circ	$\overline{0}$	O	O	O	$\mathbf 1$	$\mathsf{O}\xspace$	O	O	$\overline{0}$	0	$\mathbf 1$
1998	O	$\mathbf 1$	O	$1\,$	O	O	$\mathsf{O}\xspace$	$\overline{0}$	$\overline{0}$	$\mathbf 0$	0	$\sqrt{2}$
1992	$\mathbf 1$	$\mathsf{O}\xspace$	$\overline{2}$	O	O	O	\circ	$\mathsf{O}\xspace$	O	O	0	\mathcal{S}
1991	$\overline{2}$	$\overline{0}$	$\overline{0}$	$\overline{0}$	$\mathbf 1$	0	$\overline{0}$	0	O	\sqrt{a}	0	3

Table 2.1. Counts of measurements used in the assessment of eastern gray whale body condition by year that the photo was collected (row) and individual gray whale ID (column).

Finally, photos should have a high standard of image quality (i.e., low image noise, distance to the individual, lighting) for use where the line of the whale's body is evident. The best body condition index will be made from high resolution images with minimal distance between the photographer and the animal.

As of 2020, the Sounders were comprised of 11 individuals, all of which had been sighted in 14-27 years since 1990 (Table 1). The Sounder population consists of 8 males and 2 females, with 1 individual of unknown sex. Exact age of individuals is unknown, but photogrammetry measurements have shown all to be adult size (Segre et al. 2022; Durban and Fearnbach, unpublished data). Neither female has been sighted with a calf in the Puget Sound. Individual gray whales are identified by unique pigmentation or scarring on the flanks and tail, or their knuckle count and shape. The head of an individual was identified through sequence shots where they could be connected to traditional identification. A database of images for the left and right side of each individual was developed for images that could not be identified through a sequence.

Body condition angle (*°BC*)

To create a more sensitive and objective gray whale body condition index, we developed a new technique to indicate

blubber thickness at the post-cranial hump from boat-based photographs. While gray whales are typically identified from photographs by pigmentation patterns along their dorsal flanks (Darling 1984, Weller 1999), variation in blubber thickness in this region is less obvious.

Bradford et al. (2012) used the flanks as part of their assessment, but also focused on the post-cranial region and assessed apparent blubber thickness behind the post-cranial hump and around the scapula. Depletion of the subcutaneous fat in this region results in a post-cranial depression, which is considered to be representative of a cetacean in poor body condition with less-than-optimal blubber reserves (Bradford et al. 2012; Joblon et al. 2014; Pettis et al. 2004). We therefore focused measurements on the depth of the depression behind the post-cranial hump as it shows evident changes in blubber thickness, is a relatively easy and common shot in photo series. The postcranial hump may seem obscurred as whale's body condition increases, however the shape will still be apparent.

Figure 2.1. Example image depicting the standardizing measurement. In yellow, L represents the measurement from the blowhole to the top of the post-cranial hump. In red, 2L represents the doubled length of L to obtain the location of the anchor point for the angle measurement, B. Offset angle of this image was scored as a 1.

Figure 2.2. Example image depicting the angle measurement, °*BC*. Point 1 (B) is the previously found anchor point along the back. Point 2 (PCH) is the top of the post-cranial hump. Point 3 (D) is the lowest point of the depression. Following placement of the points, an angle measurement can be taken.

We examined digital and film photos from sightings of the Sounders and selected images where the individual's head was out of the water, but parallel to the water line. The photographs were not collected specifically for this study, and most images taken during a sighting were of flanks or flukes, thus the number of images including the full head anatomy was considerably smaller. The aim was to reduce bias from the flexion of the body during its breath cycle. Additionally, photos were sorted based on an index of estimated offset angle, which consists of 10° bins representing the estimated angle of the individual from the boat. This index ranged from 0 for individuals parallel to the boat $(-5^{\circ}$ to $5^{\circ})$ to 9 for individuals perpendicular to the boat $(85^{\circ}$ to $95^{\circ})$. Images with a higher offset angle index (3 and above) were excluded from analysis, as measurement precision is reduced at higher offset angles (Table 2).

We used ImageJ, an open access photo-editing software (Schneider et al. 2012), to implement a novel method to measure the post-cranial depression. This method uses the "angle tool" to create a new measurement called the body condition angle (*°BC*). An initial measurement (*L*) used to standardize between photos was taken using the "straightline tool". The *L* line measures from the top of the blowhole to the forwardmost top point of the post-cranial hump. This

measurement is then doubled (*2L*) to find a reference point along the back (*B*) that will be used to measure the *°BC* (Fig. 2.1). The anchor point along the back (*B*) will be standardized to the same position, with little change from body fluctuation during the breath cycle, on the individual's skeleton as a result of two consistent body reference points: the blowhole and post-cranial hump.

Starting at the anchor point, the °*BC* can be taken as the degree of depression behind the post-cranial hump. The mid-point of the angle tool is placed at the top of the post-cranial hump (*PCH*) and the final point, which is the only variable point of the angle measurement, is placed at the lowest point of the depression (*D*) (Fig. 2.2).

ImageJ then allows us to take a measurement, up to three decimal places, of the °*BC*. This is where fine-scale changes can be captured and allow for the more detailed comparisons between measurements on subsequent photos. A larger °*BC* indicates a deeper depression behind the postcranial hump; thus, a large °*BC* corresponds to a thinner blubber layer and worse body condition. A °*BC* on a gray whale with a thick blubber layer would be close to 1 or smaller, sometimes even negative.

Statistical analysis – The R environment for statistical computing (R Core Team 2022), implemented within the RStudio software, was used to conduct all

analyses. A linear mixed model was used to determine the strength of the relationship between °*BC,* day-of-year, and random effects for year, individual identity, and offset angle of the photograph. Random effects were included in the model (as categorical variables). The linear mixed model was fit using the lme4 package in R (Bates et al., 2015). Model selection was based on the residual maximum likelihood (REML) with the most efficient model having the smallest REML.

Analyses were run with two separate data sets to determine and reduce the effect of offset angle on °BC. The first data set included all angle measurements. The second data set contained only offset angles < 3 (reduction from 729 to 559 photo measurements). A third data set was used to examine the relationship between time spent in the Puget Sound and body condition. In lieu of day-of-the-year and °BC, the difference between day-of-the-year and °*BC* from the first to last sighting were used for analysis.

Table 2.2. Results of the mixed effects models to test the effect of Julian day (fixed) and random effects on the °*BC* using two, progressively more restrictive, data sets. The third data set examined the relationship between time spent in the Puget Sound and how much an individual's °*BC* changed from first to last sighting.

Another analyst conducted measurements on the same set of photos to test the inter-researcher correlation. Additionally, the methodology was validated against two alternative methods for indicating body condition. Firstly, each measurement photo also received a Bradford score of the post-cranial hump for comparison, where a Bradford score of 1, 2, or 3 indicates poor, average, and aboveaverage body condition, respectively (Bradford et al. 2012). An alternative body condition index was also calculated from vertical drone images collected during the same March-June time period as boat-based images in 2020. Aerial images were collected using an octocopter drone at a typical altitude of 40-60m, using methods described in Durban et al. (2022), except the vertically gimballed camera was a full frame Sony Alpha A7R camera (7360 x 4912 pixels). The camera was equipped with a 55mm F1.8 Sony lens to ensure no wide-angle distortion of images. The drone BCI was calculated as the body width in pixels at a point 50% of the total length (TL) of the whale, represented as a proportion of the TL in pixels (e.g., Durban et al. 2016, although width at 40% of TL was used in that study of blue whales). Width at 50% of TL has previously been used to quantify body condition for gray whales (Stewart et al. 2023). The two measurement types were tested with a Spearman's Rank Correlation (unpublished data, Durban and Fernbach). To be considered for the comparison, there had

to be a °*BC* measurement within +/- 7 days from the date of the drone BCI score.

Figure 2.3. Distribution of all °*BC* measurements for each individual over the entire sighting period. Minimum and maximum are represented by the ends of the whiskers, lower and upper quartiles (creating the interquartile range; IQR) are represented by the ends of the box plots, the median is represented by the vertical line in the box plot, and outliers are represented by the dots on either side of the whiskers. Outliers calculated by Q1-1.5*IQR and Q3+1.5*IQR.

Results

For the 31-year study period, 1990-2020, from an average of 35,000 images reviewed, a total of 729 boatbased photographs were selected to be measured for *°BC* and 559 were included in analyses with 170 removed due to offset angle (Table 2). Photos with measurements above an offset of 2 begin to lose precision because *°BC* is most visible and accurate when the animal is parallel with the boat. Six

of the individuals were sighted frequently, with >44 photos collected for each of those individuals. The remaining five individuals were less commonly sighted, with the number of measurements ranging from 6 to 41 measurements. Sighting effort increased over the years with research and public interest in the Sounders' population expanded, and this is reflected in the total measurements per year. Sightings were spread between the months of February (10 measurements) to June (7), with the majority of sightings in March (236) and April (332) followed by May (144). °*BC* was relatively consistent between individuals, with an overall mean °*BC* of 2.071 (sd = 1.112) and a median °*BC* of 1.941 across all data. The majority of °*BC* outliers consisted of measurements that reflect extremely poor body conditions (e,q, e^b) $BC > 4^{\circ}$) (Fig. 2.3).

The results of the mixed effects analysis showed significant, negative, relationships between *°BC* and dayof-year, indicating that body condition generally improves as the season progresses (Table 2). The most parsimonious model for the mixed effects analysis for both data sets included all three random effects: year, ID, and offset angle, thus was used for analyses. Variance among the random effects in data set 1 was primarily explained by offset and ID. For data set 2, which removed measurements where the animal had an offset >2 , variance explained by

the offset angle decreased significantly, and most of the variance among the random effects in this model was explained by individual ID. In this model, the correlation between °BC and day-of-year was stronger (Table 2).

Figure 2.4. Mixed effects model prediction plot of °*BC* measurements across years. The data points are a collection of all measurements across years and IDs but filtered to remove any measurements with an offset of 3 or greater.

A third mixed effects model examined the relationship between days spent in the Puget Sound and difference in °*BC* (Table 2). As in the previous models, the days spent in the Sound had a strong, negative relationship with °*BC* (correlation of -0.64), indicating that the more time an animal spent in the Sound, the greater the improvement in body condition. Offset angle was not included as a random effect in this model because the difference in °*BC* was calculated from two different photographs: the first and last photograph of that individual within a given year.

Figure 2.5. Mixed effects model prediction plot of °*BC* measurements. The data points are a collection of all measurements across years and IDs but filtered to remove any measurements with an offset of 3 or greater.

After plotting seasonal trends using all *°BC* measurements, most years show a decline in *°BC* measurements with day-of-the-year, an indicator of individuals improving in body condition throughout their foraging season in the Puget Sound (Fig. 2.4). Across all years, individuals, and offset angles 0-2 included in this study, there is a clear decreasing trend in *°BC* across the season, again an indicator of successful feeding within the Sound (Fig. 2.5). Individual #22 had the most measurements of any individual in this study, and shows a strong, consistent trend in improving body condition throughout each season (Fig. 2.6).

The relationship between observed time spent foraging in the Puget Sound and change in *°BC* tends to follow the previous trend (Fig. 2.7). Most individuals show a greater decrease in *°BC* (thus a greater improvement in body condition) when more time is spent in the Puget Sound, with some years as an exception. Figure 2.7 shows the difference between the last and first *°BC* measurement of a season as a function of the number of days between those measurements.

The entire photo set was measured by two separate analysts and the correlation in *°BC* between analyst measurements was 0.712 with a p-value < 2.2E-16. To validate the methodology, measurements were compared to both the post-cranial hump measurement of the Bradford scoring system (Bradford et al. 2012, Fig. 2.8) and the drone BCI method (Durban et al. 2016, Fig. 2.9). The majority of *°BC* measurements were classified as a 2 under the Bradford method, but with good agreement between the

lower *°BC* measurements and 3s, indicating that the methods agree in classifying a whale with average vs. good body condition (Fig. 2.8). Very few photos were assigned a Bradford index of 1 (indicative of poor body condition), indicating that *°BC* measurements can show a wider range of variation than the 3-point Bradford scale.

A total of 11 BCI scores were available for comparison, with only 2 of the BCI scores measured on a different day than the °*BC*, but within +/- 7 days. The Spearman's rank correlation ρ for the comparison of drone BCI to *°BC* is -0.664 with a p-value of 0.0309. As an individual's body condition improves, drone BCI increases, and *°BC* decreases, thus the negative relationship in these indexes is appropriate. With only 11 drone BCI measurements available for this analysis, the statistical power in the correlation analysis is low, thus with outliers considered, this relationship indicates a strong agreement between the measurements in these two methodologies.

Figure 2.6. Mixed effects model prediction plot of °*BC* measurements for individual #22. The data points are a collection of all measurements for #22 across years but filtered to remove any measurements with an offset of 3 or greater. Missing years did not have any sighting data or only had a single measurement.

Discussion

Photogrammetry with drones has recently, and increasingly, been shown to offer a powerful approach to quantifying body condition of whales from aerial photographs (Christiansen et al. 2020; Durban et al. 2016; Durban et al. 2021; Fearnbach et al. 2020), particularly when condition over time can be monitored for the same individuals (Stewart et al. 2021). However, boat-based photographic records exist for a number of whale species, that extend for decades before the developments in drone

photogrammetry (e.g., Pirotta et al. 2023). To assess longterm changes in condition, and relate this to changes in the environment, there is value in gleaning objective and quantitative data on body condition from such valuable datasets.

Gray whales in Puget Sound have been studied using boat based photo-identification from 1990 to 2021 (Calambokidis et al., 2002; Calambokidis et al., 2010). In this study, we utilized this large dataset of photographs collected over 31 years to present a novel approach to assessing gray whale body condition from boat based photographs. This method offers an innovative way to gather quantitative body condition information and creates opportunities for future improvements and developments.

Specifically this method was created by the use of an angle tool with standardized points corresponding to an individual's anatomy, applied to digital images to generate quantitative measurements across time and uniquely identified individuals to find *°BC*. By measuring body condition as a continous variable, this method is sensitive enough to demonstrate variations within and between seasons that may have been obscured with a more coarse, categorical body condition index (e.g. Bradford).

Figure 2.7. Relationship between number of days spent in the Puget Sound and change in *°BC*, displayed using a mixed effects model prediction plot. The data points are a collection of all measurements across years and IDs but filtered to remove any measurements with an offset of 3 or greater. Years with less than 4 individuals were removed.

Through the use of this assessment, we were able to display utility in tracking changes in an individual's foraging season (Figs. 2.4 and 2.6). The animal with ID 22 is the most photographed Sounder and with those repeated sightings we were able to see clear trends in changes in body condition over 2 to 3 months. The benefit of being the most photographed individual in our sample results in a larger selection of images for measurement. Requiring photographs of high image quality and with the animal being parallel to the boat and the water, along with a clear outline, was the limiting factor in this study, thus her high sighting history lended more potential images for measurement.

As has been found with previous cetacean research, consistent access to feeding grounds and valuable prey sources can increase the body condition of individuals in a population (e.g. Lockyer 1987; Rice and Wolman 1971). The time spent in the Puget Sound by the Sounders reflects the significance of visiting this key foraging ground for this aggregation of gray whales to build their energy reserves (e.g., Fig. 2.6). The Sounders travel approximately 200km off their usual migration route to forage in the Puget Sound. Consistently devoting the extra energy and time over the last 31 years to travel to this foraging ground indicates good foraging opportunities.

By examining the relationship between time spent at the foraging ground and their changes in *°BC* (Fig. 2.7), we mostly identified years where the whales displayed expected results; more time in Puget Sound resulted in a larger improvement in body condition. However, we see evidence of potentially poor foraging success in 2018 where the trend was reversed and several whales' body condition

Figure 2.8. Comparison of all °*BC* measurements against the Bradford scoring system. Each photograph was given a score as described in the Bradford et al. (2012) paper and plotted against the °*BC* measurement the same image received. Bradford scores: 1=significant postcranial depression and hump, 2=moderate or slight postcranial depression, 3=flat or rounded back.

worsen with time in Puget Sound. This is the year preceeding the start of the most recent eastern North Pacific gray whale UME with the majority of associated strandings having poor body conditon suggesting poor foraging opportunities leading to emaciation in many individuals (Christiansen, et al., 2021). The Eastern population likely reached the carrying capacity of their foraging grounds when their population peaked at nearly 27,000 in 2016, but has since

fallen to 14,500 in the 2022/23 winter season (Eguchi, et al., 2023; Perryman, et al., 2020). Additionally, changes in prey availability can affect foraging opportunities at a once successful feeding ground (Meyer-Gutbroad et al., 2021).

Ghost shrimp, the primary prey in this region, is an energetically valuable food source that provides 2-15 times higher biomass than any other prey for the eastern North Pacific gray whale population (Weitkamp 1992). Gray whales take advantage of high tide to feed along the shallow mudflats where ghost shrimp burrow. In a method called suction feeding, the whales lay along their side and suck in a large mass of mud and biomass which they strain through their baleen plates (Johnston and Berta, 2011). The *°BC* provides evidence of success in this unique foraging strategy, as demonstrated by individual trends in increasing blubber thickness with increased time in the Puget Sound (Fig. 2.7). However, not every individual experiences success every season, perhaps as a result of low available prey biomass or competition with other ghost shrimp predators. Further research to characterize ghost shrimp availability in this habitat may be useful to explain the patterns in individual and seasonal blubber accumulation measured with this body condition method.

It should be noted that this novel body condition method has only been tested on a small sample of 11 individual gray whales. Additionally, the observation location was constrained to Puget Sound, which is a unique foraging location for gray whales. Expansion of this body condition assessment to more individuals and locations along the migration corridor could provide more detailed explorations of fluctuations in the general population's health. By applying this new body condition measurement to exising datasets for the larger eastern North gray whale population, researchers may gain more insight into the fine-scaled variations in body condition between different structural groups, such as feeding aggregations. There also exists large historic photo databases for other species of mysticetes lending potential for application of this methodology with only small, or no, changes to examine a population's body condition over longer periods. Many threaten species in particular, like the North Atlatic right whale, have a long history of monitoring with urgency to assess the status of the current population.

Figure 2.9. Comparison of °*BC* measurements against the Body Condition Index method. A linear smoother was applied. Only 11 pairs of measurements, one from each method, were available for comparison. All measurements were taken during the 2020 foraging season and the °*BC* measurements used were from the data set with offsets 3 or greater removed.

While this method provides a new development on previous boat-based body condition assessments, the complex nature of the measurement comes with challenges. There is some variation in *°BC* that cannot be fully accounted for due to changes in the angle between the observer and the animal, a factor we called the offset angle. Developing a quantitative method, such as a standaridized angle measurement, to determine the offset would likely reduce the noise in the data and allow for a more objective analysis. Other factors such as the position and flexion of the animal, and variations in environmental condition, such as sea state, may also impact the success of the *°BC*. With these factors in mind, there is room for improvement

as this method shows consistent trends in seasonal increases in *°BC* in distinct years but noise in measurements could be reduced through more rigorous image selection parameters (e.g., Fig. 2.6).

A true measurement relies on accurate placement of the points which can be obscured by a number of factors. Photo quality is important; in particular, the size of the individual in the image combined with the resolution of the image. In lower quality photos, the edges of the whale become more pixelated, which makes it difficult to place precise anchor points and lines. Weather conditions also can alter the perception of the outline of the individual. Cloudy conditions may be preferable because they reduce or eliminate the sun's reflection along the animal's back, which can blur the edge. However, in cloudy conditions the whale blends in more with the color of the water, which can make it difficult to identify an edge. While photo quality was not categorized, sessions spent measuring and comparing between researchers indicated the most disagreement between *°BC* resulted from photos of poorer quality.

The use of proper photographic techniques during image collection could reduce some of the variation and difficulty encountered during measuring. Furthermore, even though the points are considered standardized, they still rely on precise identification of an anatomical area, i.e.

the blow hole and the top of the post-cranial hump. While small differences in the placement of the points will alter the resulting measurement, the consistency among seasonal trends, along with good agreement in *°BC* measurements between researchers, indicate that changes in *°BC* result from meaningful changes in the body condition of the animal. With further development of this methodology, sensitivity would increase and can serve as a strong complement to aerial photogrammetry body condition assessments.

This study introduced the development of a novel body condition method that can quantitatively assess fluctuations in individual blubber thickness across time. With more development or modifications to this methodology, there is potential to create new, quantitative tools to detect changes in cetecean body condition through boatbased photographs. With more precise, objective monitoring of individual and population health, we can create more effective conservation monitoring and management practices.

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Chapter 1

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Appendix A:Search Terms

Cetaceans

- Cetacea, Cetacean*
	- o Odontoceti, Odontocetes
	- o Mysticeti, Mysticetes
- Whale*
- Dolphin*
- Porpoise*
- Narwhal*

Body Conditions

- Body condition(s)
- Body length
- Body mass
- Body volume
- Body growth
- Body size
- Body girth
- Body density

Body Condition Assessments

- Photogrammetry, photo*
- Drone*, uncrewed aerial system(s), unoccupied aircraft system(s), UAS, unmanned aerial vehicle(s)
- Blubber
- Body composition analysis
- Isotope dilution
- Morphometr*
- Glide method
- Lipid*
- Biopsy
- Lice
- Scar*
- Rake mark(s)

Conservation Management

- Conserve*
- Manage*
- Monitor*
Example string for the Biological Science Collection database: ((MAINSUBJECT.EXACT("Cetacea") OR MAINSUBJECT.EXACT("Odontoceti") OR MAINSUBJECT.EXACT("Mysticeti")) OR abstract((cetacea OR cetacean* OR odontoceti OR odontocetes OR mysticeti OR mysticetes OR whale* OR dolphin* OR porpoise* OR narwhal*)) OR title((cetacea OR cetacean* OR odontoceti OR odontocetes OR mysticeti OR mysticetes OR whale* OR dolphin* OR porpoise* OR narwhal*))) AND ((MAINSUBJECT.EXACT("Body size") OR MAINSUBJECT.EXACT("Body length") OR MAINSUBJECT.EXACT("Body density")) OR abstract(("body condition" OR "body conditions" OR "body length" OR "body mass" OR "body volume" OR "body growth" OR "body size" OR "body girth" OR "body density")) OR title(("body condition" OR "body conditions" OR "body length" OR "body mass" OR "body volume" OR "body growth" OR "body size" OR "body girth" OR "body density"))) AND ((MAINSUBJECT.EXACT("Drones") OR MAINSUBJECT.EXACT("Morphometry")) OR abstract((photo* OR drone* OR "uncrewed aerial system" OR "uncrewed aerial systems" OR "unoccupied aircraft system" OR "unoccupied aircraft systems" OR "unmanned aerial vehicle" OR "unmanned aerial vehicles" OR UAS OR blubber OR morphometr* OR "glide method" OR lipid* OR biopsy OR lice OR scar* OR "body composition analysis" OR "isotope dilution" OR "rake mark" OR "rake marks")) OR title((photo* OR drone* OR "uncrewed aerial system" OR "uncrewed aerial systems" OR "unoccupied aircraft system" OR "unoccupied aircraft systems" OR "unmanned aerial vehicle" OR "unmanned aerial vehicles" OR UAS OR blubber OR morphometr* OR "glide method" OR lipid* OR biopsy OR lice OR scar* OR "body composition analysis" OR "isotope dilution" OR "rake mark" OR "rake marks"))) AND (MAINSUBJECT.EXACT("Conservation") OR abstract((conserv* OR manage* OR monitor*)) OR title((conserv* OR manage* OR monitor*)))

The final section in green is the conservation management portion of the string that is only added to determine the relevant subset.

Appendix B: Description of Method Terms

- Anthropogenic interactions
	- o Evidence of harmful interactions such as entanglements or boat strikes.
- Biomarker
	- o Any natural chemical found in the body that can be used as an indicator. Concentrations were examined and used to assess the health of individuals.
- Blubber
	- o Only included methods that directly measure the blubber content; lipid analysis or measuring the thickness of the blubber layer.
- Body parameters
	- o Direct measurement of anatomical parts such as length, girth, fluke width, etc.
- Fecal
	- o Chemical analysis on fecal matter as a proxy for assessing diet.
- Necropsy
	- o Included only the basic necropsy practice of measuring body parameters. Any necropsy that conducted further analysis including more sophisticated methods, such as pathology assessments, received the appropriate labels. This label also served to illustrate part of the data source for body condition assessments. Data collection was fairly evenly mixed between strandings and sustenance hunting (whaling and native hunting).
- Pathology
	- o Majority focused on chemical pollutants, but also included "natural" pathologies; predation, parasites, injury, or disease.
- Photogrammetry
	- o Usually 2D, top-down measurements, but also included 3D scanning techniques.
- Rake marks
	- o Rake marks are not included in pathology as they are not considered a major injury, but rather evidence of inter- or intraspecific interactions.
- Reproductive organs
	- o Studies that measured the testes or ovarian structures as a direct reflection of body

conditions or in conjunction with behavioural traits as an indicator of condition. The number of papers were evenly split between testes and ovarian.

- Scoring system
	- o Any system, index, or assessment that categorized changes in body condition as a proxy for a direct measurement.
- Stomach contents
	- o Analysis of contents to assess potential diet and connections to body condition.
- Tags
	- o Evaluation of whale swimming and diving patterns to extrapolate body condition estimates through buoyance/body density. One paper examined changes in body condition related to changes in foraging behaviour.
- Teeth
	- o Biochemical and mineralization analysis of teeth in conjunction with changes in the body.

Appendix C: List of Study Locations

Figure D. 1. Treemap showing the number of studies included in the meta-analysis, separated into the geographic region corresponding to the study species. Regions visually represented by figure 1.3 in the text.