Assessing cetacean body condition: Is total lipid content in blubber biopsies a useful monitoring tool?

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Assessing cetacean body condition: Is total lipid content in blubber biopsies a useful monitoring tool?

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ABSTRACT

1. Measuring the energy stores, or body condition, of cetaceans is vital for monitoring population health. Cetaceans are exposed to a range of anthropogenic stressors including for example, noise, contaminants, habitat degradation, reduced prey availability, ship strike and entanglement. Tools to assess body condition in these inaccessible and vulnerable animals are required to better understand the energetic consequences of anthropogenic stressors that can impact population health and ultimately conservation status.

2. Remotely obtained, dart biopsy samples are becoming an increasingly standard method of tissue collection from live, large cetaceans. Here, the potential applicability of using total lipid content in such biopsy samples to estimate overall body condition was assessed using full depth, dorsal blubber samples from stranded ziphiids (n = 8) and balaenopterids (n = 9). Firstly, variation in total lipid content through the blubber depth was investigated to assess the representativeness of shallow-depth dart biopsies taken from live animals. Secondly, how lipid content varies by species, cause of death, sex, age class, and morphometric indices of body condition was assessed to evaluate what information about individual energy stores can be gained from such analyses.

3. Total lipid content in dorsal, shallow-depth blubber biopsy samples from both cetacean families provides little information on overall body condition. Stratification of lipid content through the blubber layer in the balaenopterids means that superficial biopsy samples are not representative of the lipid stores available for mobilization through the rest of the tissue. A lack of variation in blubber lipid content, both within and between the ziphiid individuals, resulted in no ability to correlate these measures with morphometrics or other health, sex or age class covariates.

4. Other potential markers in the blubber from remote biopsy sampling should be explored in order to further develop robust tools for estimating the body condition of free-ranging cetaceans.

KEY WORDS

adipose tissue, blubber, body condition, diving physiology, lipids, ziphiids, balaenopterids

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35 **INTRODUCTION**

36 During the 20th century, the whaling industry's over-exploitation of large whales led to precipitous
37 declines in many populations (Tulloch, Plagányi, Matear, Brown, & Richardson, 2018; Whitehead
38 & Hooker, 2012). Many baleen whale populations are still listed as 'endangered' under the
39 International Union for Conservation of Nature's red list, and most of the 22 beaked whale species
40 are currently listed as 'data deficient'. While the majority of commercial whaling stopped after the
41 International Whaling Commission's moratorium in 1989, direct threats to these species now
42 include increased offshore infrastructure developments, fishing, and shipping traffic which result
43 in entanglement/entrapment in fishing gear and ship strikes (Thomas, Reeves, & Brownell, 2016).
44 For baleen whales specifically, that undertake long migrations between high latitude feeding
45 grounds and low latitude breeding grounds (Lockyer, 2007), entanglement in fishing gear is now
46 almost ubiquitous in some populations (Neilson, Straley, Gabriele, & Hills, 2009; Robbins,
47 Knowlton, & Landry, 2015; van der Hoop, Corkeron, & Moore, 2016). Baleen whales and beaked
48 whales are also subject to the other cumulative effects of anthropogenic disturbance such as
49 contaminant exposure and noise pollution (Thomas et al., 2016). For example, beaked whales have
50 been shown to perform dives that are, on average, the longest and deepest recorded of any marine
51 mammal (Baird et al., 2006; Tyack, Johnson, Aguilar de Soto, Sturlese, & Madsen, 2006), but
52 adaptations in their behaviour and/or their physiology that allow them to perform such extreme
53 dives appear to make them particularly susceptible to the harmful effects of anthropogenic noise.
54 In fact, certain species have been shown to mass strand in conjunction with naval sonar activities
55 (Balcomb & Claridge, 2001; Frantzis, 1998; Jepson et al., 2003; Simmonds & Lopez-Jurado,
56 1991). Finally, habitat quality degradation as a result of the short- and long-term effects of climate
57 change and ocean acidification on marine ecosystems are also significant threats to populations of
58 these species worldwide (Thomas et al., 2016).

59 There is therefore an urgent need to assess the consequences of anthropogenic disturbance in these
60 long-lived and particularly vulnerable cetaceans. Specifically, tools are required to estimate and
61 better understand the energetic consequences of anthropogenic disturbance through changes in
62 body condition that can impact population health and ultimately conservation status. Body
63 condition in mammals can be defined in various ways, but here the focus is on nutritive condition
64 quantified as the energy stores of an individual (Aguilar & Borrell, 1990; Pitt, Larivière, &
65 Messier, 2006). The size of these energy stores reflects an individual's foraging effort and success,
66 as well as their reproductive needs throughout their life cycle (Aguilar & Borrell, 1990). Animals
67 in poorer condition have smaller energy stores than those in better condition, and the size of these
68 energy stores will affect an individual's ability to resist infectious diseases, reproduce successfully,
69 and survive periods of food shortage (Møller, Christe, Erritzøe, & Mavarez, 1998; Verrier,
70 Groscolas, Guinet, & Arnould, 2011). Body condition affects an animal's behavioural patterns in
71 relation to foraging strategies, predator avoidance, and migratory movements (Bêty, Gauthier, &
72 Giroux, 2003; Friedlaender et al., 2016; Heithaus et al., 2007). Overall, body condition is a good
73 predictor of fitness by affecting both survival and reproductive success (Hall, McConnell, &

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74   Barker, 2001; Lockyer, 1986; Pitcher, Calkins, & Pendleton, 1998; Pomeroy, Fedak, Rothery, &
75   Anderson, 1999; Williams et al., 2013).

76   Fats (lipids), specifically triacylglycerols (esters made up of a glycerol and three fatty acids), are
77   more energy dense, and therefore take up less space than carbohydrates and proteins which makes
78   them the favoured energy storage form in mammals (Coleman & Lee, 2004; Young, 1976).
79   Adipose tissue lipid content has therefore been linked with total energy reserves and body
80   condition in a range of mammalian species (Beck, Smith, & Hammill, 1993; Shier & Schemmel,
81   1975; Stirling, Thiemann, & Richardson, 2008). When coupled with demographic and ecological
82   data, using lipid content as a body condition index can be a biologically relevant, relatively
83   inexpensive and rapidly assessed marker applicable on a broad scale (McKinney et al., 2014).
84   Cetaceans have evolved atypical and widespread deposits of lipids as a thick, subcutaneous
85   blubber layer (Pond, 1998). Blubber is the most important site of energy storage in cetaceans as
86   lipids are mobilized in times of energetic need and nutritional stress, and then deposited when food
87   is in excess (Iverson, 2009). As a result, the direct quantification of blubber lipid content in
88   cetacean carcasses has been used as a metric of overall energy stores and body condition in
89   mysticetes (Ackman, Hingley, Eaton, Logan, & Odense, 1975; Aguilar & Borrell, 1990; Konishi,
90   2006; Lockyer, 1986), and to a lesser extent, in odontocetes (Evans, Hindell, & Thiele, 2003;
91   Gómez-Campos, Borrell, & Aguilar, 2011; Montie et al., 2008; Read, 1990). The biochemical
92   characteristics of blubber lipids have also been extensively studied (Ackman, Eaton, & Jangaard,
93   1965; Litchfield et al., 1975; Lockyer, McConnell, & Waters, 1985; Varanasi & Malins, 1971),
94   and two main lipid classes, triacylglycerols and wax esters, have been identified. The relative
95   abundance of these two lipid classes varies between cetacean family groups, with wax esters
96   dominating the blubber tissue of deep-diving species (Koopman, 2007).

97   The collection of blubber and skin samples through dart biopsy sampling has become a standard
98   method of collecting tissue samples from live, large cetaceans since the 1990s (Hunt et al., 2013).
99   Shallow-depth, dart biopsy samples from the posterior dorsal area (visible above the water's
100   surface when the animals dive) are most often collected using either a crossbow or a rifle with
101   modified, hollow, stainless steel dart tips (Hunt et al., 2013). The collection of such biopsy samples
102   offers an opportunity to investigate markers of health and overall body condition to use as
103   important population monitoring tools. However, the validation of lipid content analyses in
104   cetacean blubber biopsy samples relative to accepted condition indices has not been fully
105   evaluated. It is therefore important to understand the potential variability in blubber lipid content
106   in order to correctly interpret the results from blubber samples collected by biopsy dart from live
107   animals.

108   Full depth, dorsal blubber samples were collected from dead-stranded individuals from two
109   cetacean families; ziphiids (beaked whales) and balaenopterids (one of the two families of baleen
110   whales). The aim was to assess if blubber lipid content obtained though biopsy sampling provides
111   a robust measure of overall body condition in these two groups of cetaceans with different life-
112   history strategies that shape the function, structure and chemical composition of their blubber.

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113 Firstly, changes in lipid content through the full blubber depth were investigated to estimate the
114 representativeness of shallow-depth biopsies. This is because remotely obtained biopsies are
115 comparable only with the superficial, outer layer of the samples taken here from stranded animals.
116 Secondly, the relationships between blubber lipid content and other individual covariates including
117 morphometric body condition indices, causes of death, sex, species and age class were investigated
118 to evaluate what information about individual energy stores can be gained from such analyses. The
119 use of total lipid content in shallow-depth blubber biopsy samples as a potential tool to measure
120 overall body condition in live animals from these two cetacean families was therefore evaluated.
121 The ability to monitor individual body condition in this way could ultimately offer vital insights
122 into population-level resilience to anthropogenic impacts and environmental change, and enhance
123 the ability to address critical population conservation issues.

METHODS

124 **METHODS**

125 **1. Sample Collection from Stranded Cetaceans**
126

127 Seventeen dead, stranded individuals, from two cetacean families, were sampled and are
128 summarised in Table 1. These were ziphiids (n = 8) - Cuvier's beaked whales (*Ziphius cavirostris*),
129 Sowerby's beaked whales *(Mesoplodon bidens)* and Northern bottlenose whales (*Hyperoodon*
130 *ampullatus*); and balaenopterids (n = 9) - minke whales (*Balaenoptera acutorostrata*) and
131 humpback whales (*Megaptera novaeangliae*). These were all freshly dead animals that showed
132 minimal signs of decomposition.

133 Of the 17 individuals, 15 were sampled by the Scottish Marine Animal Strandings Scheme
134 (SMASS) between 2013 and 2015. For these animals, the cause of death (COD) was determined
135 either by necropsy or based on visual observations of the carcass showing signs of trauma, disease
136 or emaciation. Two COD categories were generated. 'Acute' cases were individuals that died of
137 an acute trauma (bycatch and acute entanglement). 'Chronic' cases were individuals that died of
138 general debilitation and a prolonged decline in their health either though infectious disease
139 (parasitic, bacterial, viral, or mycotic infections), starvation (severely emaciated animals that died
140 of starvation/hypothermia) or chronic injuries (e.g. chronic entanglement). For the ziphiids, five
141 individuals were acute cases, and three were chronic cases. For the balaenopterids, five individuals
142 were acute COD cases, and two were chronic. Measurements of length, girth and blubber thickness
143 proximal to the dorsal fin along the dorsal axis were taken (Kuiken & Hartmann, 1991). Individuals
144 were classed as adults or juveniles using the lengths at sexual maturity for each species based on
145 published data (Hauksson, Víkingsson, Halldórsson, Ólafsdóttir, & Sigurjónsson, 2011; Heyning,
146 2002; Hooker & Baird, 1999; Mead, 1989; Whitehead, Gowans, Faucher, & McCarrey, 1997).

147 The final two individuals were an adult female minke whale and an adult female humpback whale
148 that dead stranded in the Gulf of St Lawrence, Canada, and were opportunistically sampled by the
149 Mingan Island Cetacean Study (MICS) during their 2013 and 2010 summer field seasons

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150 respectively. No cause of death was confirmed for these animals and morphometric measurements
151 were not taken.

152 Approximately 2.5 to 5cm^2 blocks of full depth blubber were collected from the dorsal area
153 immediately caudal to the dorsal fin. This area was chosen to emulate the standard biopsy site from
154 live animals. The entire blubber layer was sampled for all animals to include both the epidermis
155 and some underlying muscle so as to recognize the orientation of the sample. The samples were
156 wrapped in aluminium foil, placed individually in plastic containers, and frozen at -20^{0}C before
157 further analysis.

2. Comparative Morphometric Dataset from Stranded Cetaceans

159 To investigate where the study individuals lie in terms of their overall body condition in the
160 distribution of a larger sample size of animals, the potential range in individual morphometric body
161 condition estimates for both species groups was assessed using data collected by the SMASS
162 between 1991 and 2015. These data were from 51 balaenopterids from four species (minke,
163 humpback, fin (*Balaenoptera physalus*) and sei whales (*Balaenoptera borealis*)), and 42 ziphiids
164 from four species (Northern bottlenose whales, Sowerby's, Cuvier's and Blainville's beaked
165 whales (*Mesoplodon densirostris*)), where girth and length had been measured.

3. Lipid Extraction

167 The thickness of the fresh blubber samples was measured to the nearest millimetre as the length
168 from the interface between the skin and the blubber, to the interface between the blubber and the
169 muscle (Figure 1). Any freezer burnt edges with a dark yellowish colour were removed and
170 discarded. To reduce the loss of lipid while the samples were being prepared, the blubber was
171 subsampled while still partially frozen. A full depth subsample of the original block was cut, and
172 the epidermis and muscle tissue removed. While visible layering was apparent in the blubber of
173 some samples, others did not show visible differences through the blubber depth. For this reason,
174 the blubber was not divided according to visually discernible characteristics but was subdivided
175 into layers of approximately equal thickness. For samples over 30mm in depth, the blubber
176 thickness was sufficient to allow its subdivision into five layers of equal thickness (Figure 1). The
177 inner layer (adjacent to the muscle), the middle layer, and the outer layer (adjacent to the
178 epidermis) were used for lipid extraction while the two transitional layers were discarded (Figure
179 1). Samples less than 30mm in depth were not of sufficient thickness to follow this same protocol,
180 and were divided into thirds and each layer was used for lipid extraction. Duplicate subsamples,
181 each weighing between 0.15g and 0.3g were taken from the full blubber depth as well as the inner,
182 middle and outer layers, and lipid was independently extracted from each of the blubber samples
183 using a modified version of a previously published protocol (Folch, Lees, & Stanley, 1957).

4. Statistical Analysis

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185 The data from the ziphiids and balaenopterids were analysed separately. Two different statistical
186 approaches were used to firstly investigate differences in the distribution of total lipid content
187 through blubber depth, and secondly, to investigate what other factors may be affecting blubber
188 lipid content including blubber thickness, sex, species, age class, cause of death, and morphometric
189 body condition (girth/length).

190 All statistical analyses were performed using the statistical package, R, version 3.1.2 (R Core
191 Development Team, 2014). The main challenges for analysing these data were that multiple lipid
192 content measurements came from the same individuals, and these data did not have a normal
193 distribution. The modelling approach used here takes advantage of the statistical structure of
194 generalized linear mixed effect models (GLMMs). GLMMs combine the properties of two
195 statistical frameworks, linear mixed models, which are able to incorporate both fixed and random
196 effects, and generalized linear models which are typically used for non-normal data (Bolker et al.,
197 2008). Random effects can encompass variation among individuals when multiple responses are
198 measured per individual. For this reason, GLMMs were used here first, to investigate lipid content
199 through the blubber depth whilst accounting for the repeated measurements from the same
200 individuals. A GLMM (glmer function in the package *lme4*) with a gamma distribution, a log link
201 function and individual as a random effect was used to investigate the effect of blubber layer (full,
202 outer, middle, inner) on the lipid content of the samples from each species group.

203 Generalized Linear Models (GLMs) were then used to investigate the effects of cause of death
204 (COD), blubber thickness, morphometric body condition (girth/length), sex, species and age class
205 on the blubber lipid content of the full depth samples from each individual. GLMs with a gamma
206 distribution and a log link function were used to better model the non-normal distribution of the
207 lipid content data. All sampled individuals were used for the ziphiids (n = 8). However, for the
208 balaenopterids, the two individuals sampled by the MICS for which COD and morphometric data
209 were not available, were not included (n = 7).

210 For each species group, a GLM was generated to include the effects of all explanatory variables.
211 To ensure that the explanatory variables were not correlated, and thus to avoid multicollinearity in
212 the final model, variables were selected for inclusion based on variance inflation factors (VIFs).
213 VIFs measure how much the variance of an estimated regression coefficient increases if the
214 predictors are correlated. Starting with the full model, the VIF for each covariate in the model was
215 calculated (*car* package). The covariate with the highest VIF value was removed from the model
216 in a stepwise fashion until the VIF values for all covariates were below three (Zuur, Ieno, &
217 Elphick, 2010). These covariates were retained for further analyses. Then, the 'dredge' model
218 selection function (*MuMIn* package) was used to identify from this subset of uncorrelated
219 variables, which ones best explain the variation in blubber lipid content data and should be
220 included in the final model. The dredge function is a backwards selection tool that runs models
221 with all possible combinations of the explanatory variables including both the specified
222 interactions between variables and their respective main effects. The goodness of fit of each model
223 was assessed using the AICc (Second-order Akaike Information Criterion which uses a correction

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224   for small sample sizes). The models were ranked by their AICc to determine which combination
225   of variables best explains the relationships in the data. The model with the lowest AICc value was
226   used for further interpretation as this contained only the variables and/or interactions of
227   importance. Summary statistics of the model coefficients were used to assess the effect of each
228   covariate on blubber lipid content.

229   **RESULTS**

230   ### 1. GLMM for variation through blubber depth

231   The ziphiids showed very little variation in total lipid content with a factor of just 1.6 difference
232   between the highest and lowest measurements (full dataset range from $51.30 \pm 0.4\%$ to $84.06 \pm$
233   1.1%), and no differences between the outer layer and the other layers (Figure 2a). The
234   balaenopterids showed significant variation in blubber lipid content both within and between
235   individuals with a factor of 32.3 difference between the lowest and the highest measurements (full
236   dataset range from $2.40 \pm 0.3\%$ to $77.55 \pm 0.2\%$), and significant differences between all blubber
237   layers (Figure 2b). The outer layer had the highest lipid content and the inner layer had the lowest
238   (Figure 2b). The lipid content was significantly higher in the full depth sample compared to the
239   inner layer, but significantly lower than the outer layer (p values < 0.005). Interestingly, while
240   there was much higher variation in the lipid content in the balaenopterids, the range in
241   morphometric body condition estimates were similar between the two groups with a factor of 1.7
242   and 1.5 difference between the thinnest and fattest ziphiids and balaenopterids respectively (ziphiid
243   girth/length range $0.39 - 0.61$; balaenopterid girth/length range $0.29 - 0.49$;) (Figure 3e to h).

244   There was no overall increase or decrease in the extraction variability, quantified as the
245   measurement standard error between duplicate samples extracted in tandem, with increasing lipid
246   content (linear model: Adjusted $R^2 = 0.01$, $p = 0.15$), confirming that this lipid extraction method
247   is appropriate for the range of sample masses analysed here. There were also no differences in the
248   measurement error between the different blubber layers (ANOVA; $df = 3$, $F = 1.786$, $p = 0.154$).

249   ### 2. GLM for variation with other covariates

250   The lipid content in the full depth blubber samples was not correlated with blubber thickness for
251   either of the species groups (dorsal blubber layer mean thicknesses were 53.25 ± 7.56mm and 37.0
252   ± 5.57mm for the ziphiids and the balaenopterids respectively). No covariates were retained
253   following generalized linear model selection for the ziphiids, indicating that sex, age class, cause
254   of death, and morphometric body condition (girth/length) did not contribute to the explanation of
255   the variabity in the blubber lipid content of these full depth, dorsal samples. This lack of correlation
256   between lipid content measurements and other covariates of interest could have been a result of
257   the small sample size of individuals. It is possible that only a small number of animals in either
258   good or poor condition were sampled here. However, when compared to the larger morphometric
259   dataset, the individuals sampled for lipid extraction covered a large part of this range for the
260   ziphiids (Figure 3e and f). In fact, the full morphometric dataset collected by the SMASS showed

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261   that there was less than a factor of two difference between the thinnest animal in the poorest
262   condition, and the fattest animal in the best condition (girth/length range between 0.37 – 0.63),
263   indicating a very narrow range in apparent body condition in these species. Thus, the absence of
264   any correlations between blubber lipid content, morphometric condition and other covariates of
265   interest is probably not a result of the sample size, or a bias in the sampled individuals.

266   The best fitting GLM for the balaenopterid data included only COD as an important explanatory
267   variable with a significantly lower lipid content in the chronic cases compared to the acute cases
268   (p = 0.004) (GLM: df = 3, weight = 0.87, ΔAICc of 4.18 to the next best fitting model) (Table 2).
269   In fact, individuals that died as a result of an acute trauma had significantly higher blubber lipid
270   content across all layers than those with a chronic cause of death (ANOVA; F = 44.71, df = 1, p <
271   0.0001) (Figure 3c and d). The acute cases had higher girth/length estimates than the chronic cases
272   in the balaenopterids (Figure 3g and h) even though they covered a much smaller range in the
273   potential variation measured in the full morphometric dataset for these species collected by the
274   SMASS. In the full dataset, there was a ten-fold difference between the balaenopterids in the
275   thinnest and the fattest condition (girth/length range between 0.064 – 0.67).

276   The effect of morphometric body condition was not retained as an important explanatory variable
277   in the full depth lipid content models for either species group. The relationship between the lipid
278   content of each layer and morphometric body condition was then assessed separately. For the
279   balaenopterids only, there was a significant positive correlation between lipid content and
280   girth/length in the middle blubber layer alone (linear model; p = 0.035, Adjusted R^2 = 0.4) (Figure
281   4).

DISCUSSION

Lipid content stratification through blubber depth: Implications for biopsy sampling?

284   Stratification of lipid content through blubber depth was only seen in the balaenopterids. The
285   middle and inner layers had a lower lipid content than the outer layer closest to the skin, with the
286   inner layer showing the lowest lipid content overall. The stratification observed here is likely the
287   result of differential metabolism of fatty acids, and thus deposition and mobilization of lipid stores
288   through the blubber depth (Lockyer, McConnell, & Waters, 1984; Samuel & Worthy, 2004; Smith
289   & Worthy, 2006). Long-chain polyunsaturated fatty acids of dietary origin are present in higher
290   concentrations in the inner layer compared to the outer layer, and these are preferentially
291   metabolized due to their proximity to the body core (Koopman, Iverson, & Gaskin, 1996; Krahn
292   et al., 2004; Lockyer et al., 1984). This makes the inner and middle layers more metabolically
293   active in terms of lipolysis and lipogenesis compared to the outermost layer which is made up
294   primarily of short chain monosaturated fatty acids (Olsen & Grahl-Nielsen, 2003; Ruchonnet,
295   Boutoute, Guinet, & Mayzaud, 2006; Samuel & Worthy, 2004). The outer layer therefore likely
296   has a more structural, and possibly thermoregulatory role with a more stable composition over
297   time as it is more metabolically inert (Koopman, Pabst, McLellan, Dillaman, & Read, 2002).

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298 Therefore, had shallow biopsy samples been taken from the animals sampled here, they would
299 provide inflated estimates of blubber lipid content as the outermost layer did not reflect the lipid
300 available for mobilization in the middle and inner layers. Total lipid content in shallow depth
301 biopsies from balaenopterids is therefore not representative of an individual's lipid energy stores
302 through the full blubber depth.

303 In contrast, the ziphiids showed no variation in blubber lipid content between the different layers.
304 Blubber lipids in ziphiids are dominated by wax esters instead of triacylglycerols (Koopman, 2007;
305 Litchfield, Greenberg, & Mead, 1976; Singleton et al., 2017), and this different composition of the
306 tissue probably affects the patterns of lipid deposition and mobilisation. While the blubber of
307 ziphiids has been shown to display stratification of wax esters, the overall lipid content appears to
308 be uniform (Koopman, 2007; Litchfield et al., 1976; Singleton et al., 2017). So, had shallow biopsy
309 samples been taken from these animals, lipid content estimates would be representative of the total
310 lipid across all layers. However, as ziphiid blubber does not show the same layered lipid deposition
311 and mobilization characteristics seen in other cetacean species, it suggests that the tissue is perhaps
312 not being used in the same way.

313 **Lipid content variation as an indicator of body condition: Is it a useful marker?**

314 The wide variation in lipid content both within and between the balaenopterid samples is likely a
315 result of the life-history strategies of these species. Balaenopterids cycle their energy stores during
316 seasonal migrations that link temporally and spatially separated breeding and feeding seasons
317 (Koopman, 2007). As they are adapted to cycle their fat stores, the range in blubber lipid content
318 is likely to vary much more than for species that do not undertake prolonged fasting periods, and
319 thus live within much narrower limits of stored energy reserves. This was highlighted in the larger
320 dataset of morphometric measures from the SMASS with the 10-fold difference between the
321 girth/length index of individuals in the thinnest and fattest condition. Cause of death was retained
322 following model selection, showing that individuals that died as a result of acute trauma had a
323 higher lipid content across the full blubber depth compared to chronically debilitated animals. This
324 is to be expected as chronically debilitated animals have depleted their fat reserves following a
325 more gradual decline in health and perhaps reduced foraging opportunities compared to individuals
326 that died as a result of an acute traumatic event.

327 Neither sex nor age class were retained as important explanatory variables which was surprising
328 given previous evidence of variation in fat stores across sex and reproductive classes in
329 balaenopterids (Aguilar & Borrell, 1990; Lockyer et al., 1985). However, these data were
330 dominated by juvenile minke whales, and for this reason, little can be inferred about the effect of
331 age class on blubber lipid content. Similarly, as the animals had probably not reached sexual
332 maturity, the lack of differences between the sexes could indicate that sex differences only appear
333 once animals start to deposit and mobilize fat stores associated with the costs of reproduction.
334 Finally, while the full blubber depth samples could be used to differentiate between gross
335 differences as a result of different cause of death categories, only the lipid content of the middle

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336 layer was significantly correlated with morphometric body condition. This is consistent with
337 previous reports that the middle layer of fin and sei whale blubber is made of loose, fatty tissue,
338 and was the most variable in thickness across individuals compared to the other two layers
339 (Lockyer et al., 1985). The lack of correlation between the outer layer lipid content and
340 morphometric condition further highlights that total lipid content in shallow depth biopsy samples
341 does not provide information on individual body condition in these species.

342 Model selection for the ziphiid data retained no covariates as important explanatory variables.
343 There were also no significant correlations between the lipid content in any blubber layer and
344 morphometric condition. Total lipid content from biopsy samples in these species therefore
345 provides no information on individual body condition. One potential reason for the lack of
346 variation seen here could be a consequence of the dorsal site of the blubber sample. Dorsal blubber
347 samples were taken immediately caudal to the dorsal fin in order to investigate the potential use of
348 biopsy samples which target this area of free-ranging animals. Whaling data and strandings data
349 have provided valuable physiological information about topographical variation in blubber lipid
350 stores in both the large mysticetes and small odonotocetes. To date, however, there have been no
351 such studies to investigate the variation in ziphiids, a much less well-understood family of
352 cetaceans. The posterior dorsal area of mysticetes is a major site for lipid storage in both the
353 blubber and the muscle (Lockyer et al., 1985), but this may not be the case for beaked whales,
354 particularly if they are more similar to small odontocetes. In small odontocetes, the blubber in the
355 thoracic-abdominal region is hypothesized to play an important role in insulation and energy
356 storage, whereas the region posterior to the dorsal fin is thought to primarily act to maintain
357 hydrodynamic and locomotory functions and is metabolically inert (Gómez-Campos, Borrell,
358 Correas, & Aguilar, 2015; Koopman, 1998; Koopman et al., 2002; Tornero, Borrell, Forcada,
359 Pubill, & Aguilar, 2004).

360 Some areas of the body are therefore more important for energy storage than others, and this
361 variation is not necessarily captured in the dorsal sampling site of biopsy samples for some species.
362 An important next step is to analyse the blubber lipid content across multiple sample sites in
363 ziphiids to assess if their patterns of lipid stores are more similar to those observed in large
364 mysticetes or small odontocetes. This would help to determine if the lack of relationship between
365 lipid content and metrics of body condition and other covariates of interest used here are a result
366 of the dorsal sampling site. Or, perhaps, ziphiid blubber does not act as an energy storage organ
367 with clear patterns of lipid deposition and mobilization in the same way as other cetacean species.

368 The apparent absence of variation in total lipid content both within and between the ziphiids could
369 also be a result of the high wax ester content of the blubber (Hooker, Iverson, Ostrom, & Smith,
370 2001; Koopman, 2007; Litchfield et al., 1976; Singleton et al., 2017). It is possible that ziphiid
371 blubber lipid storage properties differ from other species because wax esters have different
372 chemical properties to the triacylglycerols. For example, wax esters are less rapidly metabolized
373 and less efficiently hydrolysed than triacylglycerols (Place, 1992; Pond, 1998) which makes them
374 poor energy reserves. Wax esters also have lower densities (specific gravities) than triacylglycerols

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375 (Sargent, 1978), and are less sensitive to phase change with changing temperature (Nevenzel,
376 1970), both of which affect overall tissue buoyancy. Wax esters in the blubber have also been
377 suggested to play a potentially important role in thermoregulation as they provide better insulation
378 than triacylglycerols (Bagge, Koopman, Rommel, McLellan, & Pabst, 2012; Singleton et al.,
379 2017). So, stores of wax esters may provide various elements of physiological or mechanical
380 advantage in terms of buoyancy and insulation which contribute to their extreme deep-diving
381 abilities. Currently, there is no evidence to suggest that any of the beaked whale species experience
382 substantial or predictable fasting cycles, or periods of food shortage when lipid reserves might be
383 drawn upon (MacLeod, 2018). These species may therefore prioritize maintaining consistent
384 blubber hydrodynamic, thermal and buoyancy parameters which comes at the expense of having
385 variable fat deposits for energy storage. As such, small changes in the blubber lipid content may
386 considerably affect their overall buoyancy and diving capabilities, so they exist within a small
387 range (Miller et al., 2016). The very narrow range in the girth/length condition estimates using the
388 larger morphometric dataset from the SMASS further supports the possibility that these species
389 survive within very narrow body fat limits.

390 **Cetacean blubber: Much more than just an energy store**

391 There was no relationship between blubber thickness and blubber lipid content in either species
392 group. Results of this study therefore support the idea that blubber thickness alone is an inadequate
393 index of fat stores as the lipid content of the tissue varies independently of its thickness (Aguilar,
394 Borrell, & Gomez-Campos, 2007; Dunkin, McLellan, Blum, & Pabst, 2005; Evans et al., 2003).
395 There are two possible reasons for this lack of a relationship: either the small sample size of this
396 dataset prevented the detection of any correlations between lipid content and blubber thickness,
397 or, the other functional roles of the blubber not involved in energy storage also affect blubber
398 thickness. This is a small sample size of 17 individuals, and it is possible that the lack of correlation
399 between lipid content and blubber thickness could be because there was not a great enough range
400 in blubber thicknesses or lipid content within each species group. However, as the ziphiids sampled
401 covered the range of potential morphometric body condition estimates from the larger SMASS
402 dataset, and the balaenopterid samples showed a huge range in lipid content from ~2% to ~76%,
403 this is probably not the case.

404 Various other studies have also shown that blubber thickness was a poor index of nutritive
405 condition as it was not correlated with changes in overall body fat reserves (Aguilar et al., 2007;
406 Caon, Fialho, & Danilewicz, 2007; Evans et al., 2003; Gómez-Campos et al., 2015; Koopman,
407 2007; Read, 1990; Ruchonnet et al., 2006). It is well recognized across all species that in addition
408 to its role as a deposit of energy, blubber also serves important functions as an insulator, it is
409 involved in active thermoregulation, it streamlines the body, facilitates hydrodynamic locomotion,
410 contributes to water balance, and provides buoyancy (Iverson, 2009). As such, the extent to which
411 blubber lipids can be mobilized is limited by these other functional considerations. For this reason,
412 attempts should be made to move away from using blubber thickness, and total blubber lipid
413 content as an indicator of condition, and alternative methods should be investigated across all

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species. There are likely very complex relationships between body size, thermal habitat, lipid stratification, blubber thickness, and metabolism that warrant further investigation.

Conclusions and future directions

Total lipid content measured in shallow depth blubber biopsy samples from the dorsal area in balaenopterids and ziphiids is not a useful tool to estimate overall body condition. In balaenopterids, it provides no information on the lipid energy reserves in the middle and inner layers that are involved in lipid deposition and mobilization. In ziphiids, it shows no variation between individuals to distinguish between animals in either good or poor health. Varying habitats and life history strategies have shaped blubber composition characteristics which likely result in the species-specific differences in lipid content seen here. The relative importance of different blubber functions across different species also means that if the ecology and hence physiology of a species requires stability to be maintained in one blubber function, it will influence the variability possible in others. As a result, quantifying total lipid alone from biopsy samples obtained from live animals provides little information on an individual's overall health or energy reserves. In addition, a number of studies have now shown that the lipid content measured in a biopsy sample is not reflective of that of the tissue when sampled at necropsy (Krahn et al., 2004; McKinney et al., 2014; Ryan, McHugh, O'Connor, & Berrow, 2012). The discrepancies are thought to be a result of lipid loss during dart retrieval as the adipocytes are disrupted upon dart impact.

Further work should prioritize measuring other components of blubber biopsies as potential markers of condition. For example, blubber cellularity, specifically adipocyte size and number shows some potential as a biomarker of adiposity (Castrillon, Huston, & Bengtson Nash, 2017). Using the proportions of different saturated / unsaturated fatty acids or the proportion of triaclyglycerols to wax esters, for example, could also be investigated as a more reliable marker. Proteomic and transcriptomic approaches to examine changes in proteins of interest, and gene expression profiles in blubber tissue are also being investigated to identify important markers and regulators of lipid metabolism and energy homeostasis (Kershaw, Botting, Brownlow, & Hall, 2018; Khudyakov, Champagne, Meneghetti, & Crocker, 2017; Martinez et al., 2018).

Finally, as well as using biomarkers to estimate the energy reserves of individuals through biopsy sampling, morphometric measurements using photogrammetry are also a potentially valuable tool for body condition estimates in some species. The use of unmanned aerial vehicles (UAVs) to obtain full body aerial images of individuals has recently made aerial photogrammetry techniques much more accessible (Durban, Fearnbach, Barrett-Lennard, Perryman, & Leroi, 2015; Durban et al., 2016). A small number of studies have used high-resolution images collected from UAVs to investigate population-level changes in body condition (Christiansen, Dujon, Sprogis, Arnould, & Bejder, 2016; Christiansen et al., 2018; Fearnbach, Durban, Ellifrit, & Balcomnb, 2018). By combining new methods in blubber biomarker research and aerial photogrammetry, better measures of the health of individuals will be developed as tools for integration into long-term monitoring protocols for populations. Tracking the health and overall body condition of free-

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452 ranging cetaceans in this way is important for assessing the impacts of anthropogenic disturbance
453 on these species. This will help identify populations that are particularly at risk, which is crucial
454 for driving population conservation efforts and priorities.

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465 **REFERENCES**

466 Ackman, R. G., Eaton, C. A., & Jangaard, P. M. (1965). Lipids of the fin whale (*Balaenoptera*
467 *physalus*) from North Atlantic waters: Fatty acid composition of whole blubber and blubber
468 sections. *Canadian Journal of Biochemistry, 43*(9), 1513-1520. doi: 10.1139/o65-169
469 Ackman, R. G., Hingley, J. H., Eaton, C. A., Logan, V. H., & Odense, P. H. (1975). Layering and
470 tissue composition in the blubber of the northwest Atlantic sei whale (*Balaenoptera*
471 *borealis*). *Canadian Journal of Zoology, 53*(9), 1340-1344. doi: 10.1139/z75-159
472 Aguilar, A., & Borrell, A. (1990). Patterns of Lipid Content and Stratification in the Blubber of
473 Fin Whales (*Balaenoptera physalus*). *Journal of Mammalogy, 71*(4), 544-554. doi:
474 10.2307/1381793
475 Aguilar, A., Borrell, A., & Gomez-Campos, E. (2007). The reliability of blubber thickness as a
476 measure of body condition in large whales. . *59th Annual Meeting of the Scientific*
477 *Committee of the Interactional Whaling Commission (SC/59/017)*
478 Bagge, L. E., Koopman, H. N., Rommel, S. A., McLellan, W. A., & Pabst, D. A. (2012). Lipid
479 class and depth-specific thermal properties in the blubber of the short-finned pilot whale
480 and the pygmy sperm whale. *The Journal of Experimental Biology, 215*(24), 4330-4339.
481 doi: 10.1242/jeb.071530
482 Baird, R. W., Webster, D. L., McSweeney, D. J., Ligon, A. D., Schorr, G. S., & Barlow, J. (2006).
483 Diving behaviour of Cuvier's (*Ziphius cavirostris*) and Blainville's (*Mesoplodon*
484 *densirostris*) beaked whales in Hawai'i. *Canadian Journal of Zoology, 84*(8), 1120-1128.
485 doi: 10.1139/z06-095
486 Balcomb, K. C., & Claridge, D. (2001). A mass stranding of cetaceans caused by naval sonar in
487 the Bahamas. *Journal of Science, 5*, 2 - 12.
488 Beck, G. G., Smith, T. G., & Hammill, M. O. (1993). Evaluation of Body Condition in the
489 Northwest Atlantic Harp Seal (*Phoca groenlandica*). *Canadian Journal of Fisheries and*
490 *Aquatic Sciences, 50*(7), 1372-1381. doi: 10.1139/f93-157

14

491 Bêty, J., Gauthier, G., & Giroux, J. (2003). Body condition, migration, and timing of reproduction
492   in snow geese: a test of the condition-dependent model of optimal clutch size. *American*
493   *Naturalist. 162*, 110-121.
494 Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., &
495   White, J.-S. S. (2008). Generalized linear mixed models: a practical guide for ecology and
496   evolution. *Trends in Ecology & Evolution, 24*(3), 127-135. doi: 10.1016/j.tree.2008.10.008
497 Caon, G., Fialho, C. B., & Danilewicz, D. (2007). Body Fat Condition in Franciscanas (*Pontoporia*
498   *blainvillei*) in Rio Grande do Sul, Southern Brazil. *Journal of Mammalogy, 88*(5), 1335-
499   1341. doi: 10.1644/06-mamm-a-364r.1
500 Castrillon, J., Huston, W., & Bengtson Nash, S. (2017). The blubber adipocyte index: A
501   nondestructive biomarker of adiposity in humpback whales (*Megaptera novaeangliae*).
502   *Ecology and Evolution*, n/a-n/a. doi: 10.1002/ece3.2913
503 Christiansen, F., Dujon, A. M., Sprogis, K. R., Arnould, J. P. Y., & Bejder, L. (2016). Noninvasive
504   unmanned aerial vehicle provides estimates of the energetic cost of reproduction in
505   humpback whales. *Ecosphere, 7*(10), e01468. doi: doi:10.1002/ecs2.1468
506 Christiansen, F., Vivier, F., Charlton, C., Ward, R., Amerson, A., Burnell, S., & Bejder, L. (2018).
507   Maternal body size and condition determine calf growth rates in southern right whales.
508   *Marine Ecology Progress Series, 592*, 267-281.
509 Coleman, R. A., & Lee, D. P. (2004). Enzymes of triacylglycerol synthesis and their regulation.
510   *Progress in Lipid Research, 43*(2), 134-176. doi: https://doi.org/10.1016/S0163-
511   7827(03)00051-1
512 Dunkin, R. C., McLellan, W. A., Blum, J. E., & Pabst, D. A. (2005). The ontogenetic changes in
513   the thermal properties of blubber from Atlantic bottlenose dolphin *Tursiops truncatus.*
514   *Journal of Experimental Biology, 208*(8), 1469-1480. doi: 10.1242/jeb.01559
515 Durban, J. W., Fearnbach, H., Barrett-Lennard, L. G., Perryman, W. L., & Leroi, D. J. (2015).
516   Photogrammetry of killer whales using a small hexacopter launched at sea. *Journal of*
517   *Unmanned Vehicle Systems, 3*, 131-135.
518 Durban, J. W., Moore, M. J., Chiang, G., Hickmott, L. S., Bocconcelli, A., Howes, G., . . . LeRoi,
519   D. J. (2016). Photogrammetry of blue whales with an unmanned hexacopter. *Marine*
520   *Mammal Science, 32*(4), 1510-1515. doi: doi:10.1111/mms.12328
521 Evans, K., Hindell, M. A., & Thiele, D. (2003). Body fat and condition in sperm whales, *Physeter*
522   *macrocephalus*, from southern Australian waters. *Comparative Biochemistry and*
523   *Physiology Part A: Molecular & Integrative Physiology, 134*(4), 847-862. doi:
524   http://dx.doi.org/10.1016/S1095-6433(03)00045-X
525 Fearnbach, H., Durban, J. W., Ellifrit, D. K., & Balcomnb, K. C. (2018). Using aerial
526   photogrammetry to detect changes in body condition of endangered southern resident killer
527   whales. *Endangered Species Research, 35*, 175-180.
528 Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification
529   of total lipides from animal tissues. *Journal of Biological Chemistry, 226*, 497-509.
530 Frantzis, A. (1998). Does acoustic testing strand whales? *Nature, 392*, 29. doi: 10.1038/32068
531 Friedlaender, A. S., Johnston, D. W., Tyson, R. B., Kaltenberg, A., Goldbogen, J. A., Stimpert, A.
532   K., . . . Nowacek, D. P. (2016). Multiple-stage decisions in a marine central-place forager.
533   *Royal Society Open Science, 3*(5). doi: 10.1098/rsos.160043
534 Galgani, J., & Ravussin, E. (2008). Energy metabolism, fuel selection and body weight regulation.
535   *International journal of obesity (2005), 32 Suppl 7*(Suppl 7), S109-S119. doi:
536   10.1038/ijo.2008.246

15

537   Gómez-Campos, E., Borrell, A., & Aguilar, A. (2011). Assessment of nutritional condition indices
538   across reproductive states in the striped dolphin (*Stenella coeruleoalba*). *Journal of*
539   *Experimental   Marine   Biology   and   Ecology,   405*(1–2),   18-24.   doi:
540   http://dx.doi.org/10.1016/j.jembe.2011.05.013
541   Gómez-Campos, E., Borrell, A., Correas, J., & Aguilar, A. (2015). Topographical variation in lipid
542   content and morphological structure of the blubber in the striped dolphin. *Scientia Marina,*
543   *79*(2), 189 - 197.
544   Hall, A. J., McConnell, B. J., & Barker, R. J. (2001). Factors affecting first-year survival in grey
545   seals and their implications for life history strategy. *Journal of Animal Ecology, 70*(1), 138-
546   149. doi: 10.1111/j.1365-2656.2001.00468.x
547   Hauksson, E., Víkingsson, G. A., Halldórsson, S. D., Ólafsdóttir, D., & Sigurjónsson, J. (2011).
548   Preliminary report on biological parameters for NA minke whales in Icelandic waters.
549   *International Whaling Commission Reports, SC/63/O15*.
550   Heithaus, M. R., Frid, A., Wirsing, A. J., Dill, L. M., Fourqurean, J. W., Burkholder, D., . . . Bejder,
551   L. (2007). State-dependent risk-taking by green sea turtles mediates top-down effects of
552   tiger shark intimidation in a marine ecosystem. *Journal of Animal Ecology, 76*, 837–844.
553   Heyning, J. E. (2002). Cuvier's Beaked Whale. In T. A. Jefferson (Ed.), *Encyclopedia of Marine*
554   *Mammals* (pp. 305-207): Academic Press.
555   Hooker, S. K., & Baird, R. W. (1999). Observations of Sowerby's Beaked Whales, *Mesoplodon*
556   *bidens*, in the Gully, Nova Scotia. *Canadian Field Naturalist, 113*(2), 273-277.
557   Hooker, S. K., Iverson, S. J., Ostrom, P., & Smith, S. C. (2001). Diet of northern bottlenose whales
558   inferred from fatty-acid and stable-isotope analyses of biopsy samples. *Canadian Journal*
559   *of Zoology, 79*(8), 1442-1454. doi: 10.1139/z01-096
560   Hunt, K. E., Moore, M. J., Rolland, R. M., Kellar, N. M., Hall, A. J., Kershaw, J. L., . . . Kraus, S.
561   D. (2013). Overcoming the challenges of studying conservation physiology in large
562   whales: a review of available methods. *Conservation Physiology, 1*(1). doi:
563   10.1093/conphys/cot006
564   Iverson, S. J. (2009). Blubber. In W. F. Perrin, B. Würsig & J. G. M. Thewissen (Eds.),
565   *Encyclopedia of Marine Mammal (Second Edition)*: Academic Press.
566   Jepson, P. D., Arbelo, M., Deaville, R., Patterson, I. A. P., Castro, P., Baker, J. R., . . . Fernández,
567   A. (2003). Gas-bubble lesions in stranded cetaceans. *Nature, 425*, 575. doi:
568   10.1038/425575a
569   Kershaw, J. L., Botting, C. H., Brownlow, A., & Hall, A. J. (2018). Not just fat: investigating the
570   proteome of cetacean blubber tissue. *Conservation Physiology, 6*(1), coy003-coy003. doi:
571   10.1093/conphys/coy003
572   Khudyakov, J. I., Champagne, C. D., Meneghetti, L. M., & Crocker, D. E. (2017). Blubber
573   transcriptome response to acute stress axis activation involves transient changes in
574   adipogenesis and lipolysis in a fasting-adapted marine mammal. *Scientific Reports, 7*,
575   42110. doi: 10.1038/srep42110
576   Konishi, K. (2006). Characteristics of blubber distribution and body condition indicators for
577   Antarctic minke whales (*Balaenoptera bonaerensis*). *Mammal Study, 31*, 15-22.
578   Koopman, H. N. (1998). Topographical distribution of the blubber of harbour porpoises. *Journal*
579   *of Mammalogy, 79*, 260-270.
580   Koopman, H. N. (2007). Phylogenetic, ecological, and ontogenetic factors influencing the
581   biochemical structure of the blubber of odontocetes. *Marine Biology, 151*(1), 277-291. doi:
582   10.1007/s00227-006-0489-8

16

583 Koopman, H. N., Iverson, S. J., & Gaskin, D. E. (1996). Stratification and age-related differences
584   in blubber fatty acids of the male harbour porpoise (*Phocoena phocoena*). *Journal of*
585   *Comparative Physiology B, 165*(8), 628-639. doi: 10.1007/bf00301131
586 Koopman, H. N., Pabst, D. A., McLellan, W. A., Dillaman, R. M., & Read, A. J. (2002). Changes
587   in blubber distribution and morphology associated with starvation in the harbour porpoise
588   (*Phocoena phocoena*): Evidence for regional differences in blubber structure and function.
589   . *Physiological and Biochemical Zoology, 75*, 498-512.
590 Krahn, M. M., Herman, D. P., Ylitalo, G. M., Sloan, C. A., Burrows, D. G., Hobbs, R. C., . . .
591   Moore, C. J. (2004). Stratification of lipids, fatty acids and organochlorine contaminants
592   in blubber of white whales and killer whales. *Journal of Cetacean Research Management,*
593   *6*(2), 175-189.
594 Kuiken, T., & Hartmann, M. G. (1991). Dissection techniques and tissue sampling. *Proceedings*
595   *of the first European Cetacean Society workshop on cetacean pathology. Leiden, The*
596   *Netherlands., European Cetacean Society Newsletter No. 17 - Special issue.*, 39.
597 Litchfield, C., Greenberg, A. J., Caldwell, D. K., Caldwell, M. C., Sipos, J. C., & Ackman, R. G.
598   (1975). Comparative lipid patterns in acoustical and nonacoustical fatty tissues of dolphins,
599   porpoises and toothed whales. *Comparative Biochemistry and Physiology Part B:*
600   *Comparative Biochemistry, 50*(4), 591-597. doi: https://doi.org/10.1016/0305-
601   0491(75)90095-4
602 Litchfield, C., Greenberg, A. J., & Mead, J. G. (1976). The distinctive character of Ziphiidae head
603   and blubber fats. *Cetology, 23*, 1-10.
604 Lockyer, C. H. (1986). Body Fat Condition in Northeast Atlantic Fin Whales, *Balaenoptera*
605   *physalus,* and Its Relationship with Reproduction and Food Resource. *Canadian Journal*
606   *of Fisheries and Aquatic Sciences, 43*(1), 142-147. doi: 10.1139/f86-015
607 Lockyer, C. H. (2007). All creatures great and smaller: a study in cetacean life history energetics.
608   *Journal of the Marine Biological Association of the United Kingdom, 87*(4), 1035-1045.
609   doi: 10.1017/S0025315407054720
610 Lockyer, C. H., McConnell, L. C., & Waters, T. D. (1984). The biochemical composition of fin
611   whale blubber. *Canadian Journal of Zoology, 62*(12), 2553-2562. doi: 10.1139/z84-373
612 Lockyer, C. H., McConnell, L. C., & Waters, T. D. (1985). Body condition in terms of anatomical
613   and biochemical assessment of body fat in North Atlantic fin and sei whales. *Canadian*
614   *Journal of Zoology, 63*(10), 2328-2338. doi: 10.1139/z85-345
615 MacLeod, C. D. (2018). Beaked Whales, Overview. In B. Wursig, J. G. M. Thewissen & K.
616   Kovacs (Eds.), *Encyclopedia of Marine Mammals, 3rd Edition*: Academic Press.
617 Martinez, B., Khudyakov, J. I., Rutherford, K., Crocker, D. E., Gemmell, N., & Ortiz, R. M.
618   (2018). Adipose transcriptome analysis provides novel insights into molecular regulation
619   of prolonged fasting in northern elephant seal pups. *Physiological Genomics, 50*(7), 495-
620   503. doi: 10.1152/physiolgenomics.00002.2018
621 McKinney, M. A., Atwood, T., Dietz, R., Sonne, C., Iverson, S. J., & Peacock, E. (2014).
622   Validation of adipose lipid content as a body condition index for polar bears. *Ecology and*
623   *Evolution, 4*(4), 516-527. doi: doi:10.1002/ece3.956
624 Mead, J. G. (1989). Beaked whales of the genus Mesoplodon. In S. H. Ridgway & R. Harrison
625   (Eds.), *Handbook of Marine Mammals: River Dolphins and Toothes Whales* (Vol. 4, pp.
626   349-430). San Diego: Academic Press.

17

627   Miller, P. J. O., Narazaki, T., Isojunno, S., Aoki, K., Smout, S. C., & Sato, K. (2016). Body density
628   and diving gas volume of the northern bottlenose whale (*Hyperoodon ampullatus*). *The*
629   *Journal of Experimental Biology, 219*(16), 2458-2468. doi: 10.1242/jeb.137349
630   Møller, A. P., Christe, P., Erritzøe, J., & Mavarez, J. (1998). Condition, Disease and Immune
631   Defence. *Oikos, 83*(2), 301-306. doi: 10.2307/3546841
632   Montie, E. W., Garvin, S. R., Fair, P. A., Bossart, G. D., Mitchum, G. B., McFee, W. E., . . . Hahn,
633   M. E. (2008). Blubber morphology in wild bottlenose dolphins (*Tursiops truncatus*) from
634   the Southeastern United States: Influence of geographic location, age class, and
635   reproductive state. *Journal of Morphology, 269*(4), 496-511. doi: doi:10.1002/jmor.10602
636   Neilson, J. L., Straley, J. M., Gabriele, C. M., & Hills, S. (2009). Non-lethal entanglement of
637   humpback whales (*Megaptera novaeangliae*) in fishing gear in northern Southeast Alaska.
638   *Journal of Biogeography, 36*(3), 452-464. doi: doi:10.1111/j.1365-2699.2007.01820.x
639   Nevenzel, J. C. (1970). Occurrence, function and biosynthesis of wax esters in marine organisms.
640   *Lipids, 5*(3), 308-319. doi: 10.1007/bf02531462
641   Olsen, E., & Grahl-Nielsen, O. (2003). Blubber fatty acids of minke whales: stratification,
642   population identification and relation to diet. *Marine Biology, 142*(1), 13-24. doi:
643   10.1007/s00227-002-0934-2
644   Pitcher, K. W., Calkins, D. G., & Pendleton, G. W. (1998). Reproductive performance of female
645   Steller sea lions: an energetics-based reproductive strategy? *Canadian Journal of Zoology,*
646   *76*(11), 2075-2083. doi: 10.1139/z98-149
647   Pitt, J. A., Larivière, S., & Messier, F. (2006). Condition Indices and Bioelectrical Impedance
648   Analysis to Predict Body Condition of Small Carnivores. *Journal of Mammalogy, 87*(4),
649   717-722. doi: 10.2307/4094598
650   Place, A. R. (1992). Comparative aspects of lipid digestion and absorption: physiological
651   correlates of wax ester digestion. *American Journal of Physiology, 263*(464-471).
652   Pomeroy, P. P., Fedak, M. A., Rothery, P., & Anderson, S. (1999). Consequences of maternal size
653   for reproductive expenditure and pupping success of grey seals at North Rona, Scotland.
654   *Journal of Animal Ecology, 68*(2), 235-253. doi: 10.1046/j.1365-2656.1999.00281.x
655   Pond, C. M. (1998). *The fats of life.* Cambridge: Cambridge University Press.
656   R Core Team (2014). R: A language and environment for statistical computing. Vienna, Austria:
657   R Foundation for Statistical Computing; URL: http://www.R-project.org/.
658   Read, A. J. (1990). Estimation of body condition in harbour porpoises, *Phocoena phocoena*.
659   *Canadian Journal of Zoology, 68*(9), 1962-1966. doi: 10.1139/z90-276
660   Robbins, J., Knowlton, A. R., & Landry, S. (2015). Apparent survival of North Atlantic right
661   whales after entanglement in fishing gear. *Biological Conservation, 191*, 421-427. doi:
662   https://doi.org/10.1016/j.biocon.2015.07.023
663   Ruchonnet, D., Boutoute, M., Guinet, C., & Mayzaud, P. (2006). Fatty acid composition of
664   Mediterranean fin whale B*alaenoptera physalus* blubber with respect to body
665   heterogeneity and trophic interaction. *Marine Ecology Progress Series, 311*, 165-174.
666   Ryan, C., McHugh, B., O'Connor, I., & Berrow, S. (2012). Lipid content of blubber biopsies is not
667   representative of blubber in situ for fin whales (*Balaenoptera physalus*). *Marine Mammal*
668   *Science, 29*, 542-547.
669   Samuel, A. M., & Worthy, G. A. J. (2004). Variability in fatty acid composition of bottlenose
670   dolphin (*Tursiops truncatus*) blubber as a function of body site, season, and reproductive
671   state. *Canadian Journal of Zoology, 82*(12), 1933-1942. doi: 10.1139/z05-001
672   Sargent, J. R. (1978). Marine wax esters. *Science Progress (1933-), 65*(260), 437-458.

18

673 Shier, P. D., & Schemmel, R. (1975). Effects of Diet, Age, Strain and Anatomical Site on Fat
674   Depot Triglyceride and Fatty Acid Content in Rats. *Proceedings of the Society for*
675   *Experimental Biology and Medicine, 149*(4), 864-870. doi: 10.3181/00379727-149-38915
676 Simmonds, M. P., & Lopez-Jurado, L. F. (1991). Whales and the military. *Nature, 351*, 448. doi:
677   10.1038/351448a0
678 Singleton, E. M., McLellan, W. A., Koopman, H. N., Pokorny, A., Scharf, F. S., & Pabst, A. D.
679   (2017). Lipid composition and thermal properties of the blubber of Gervais' beaked whale
680   (*Mesoplodon europaeus*) across ontogeny. *Marine Mammal Science, 33*(2), 695-705. doi:
681   doi:10.1111/mms.12389
682 Smith, H. R., & Worthy, G. A. J. (2006). Stratification and intra- and inter-specific differences in
683   fatty acid composition of common dolphin (*Delphinus* sp.) blubber: Implications for
684   dietary analysis. *Comparative Biochemistry and Physiology Part B: Biochemistry and*
685   *Molecular Biology, 143*(4), 486-499. doi: http://dx.doi.org/10.1016/j.cbpb.2005.12.025
686 Stirling, I., Thiemann, G. W., & Richardson, E. (2008). Quantitative Support for a Subjective
687   Fatness Index for Immobilized Polar Bears. *The Journal of Wildlife Management, 72*(2),
688   568-574. doi: doi:10.2193/2007-123
689 Thomas, P. O., Reeves, R. R., & Brownell, R. L. (2016). Status of the world's baleen whales.
690   *Marine Mammal Science, 32*(2), 682-734. doi: doi:10.1111/mms.12281
691 Tornero, V., Borrell, A., Forcada, J., Pubill, E., & Aguilar, A. (2004). Retinoid and lipid patterns
692   in the blubber of common dolphins (*Delphinus delphis*): implications for monitoring
693   vitamin A status. *Comparative Biochemistry and Physiology Part B: Biochemistry and*
694   *Molecular Biology, 137*(3), 391-400. doi: http://dx.doi.org/10.1016/j.cbpc.2004.01.001
695 Tulloch, V. J. D., Plagányi, É. E., Matear, R., Brown, C. J., & Richardson, A. J. (2018). Ecosystem
696   modelling to quantify the impact of historical whaling on Southern Hemisphere baleen
697   whales. *Fish and Fisheries, 19*(1), 117-137. doi: doi:10.1111/faf.12241
698 Tyack, P. L., Johnson, M., Aguilar de Soto, N., Sturlese, A., & Madsen, P. T. (2006). Extreme
699   diving of beaked whales. *Journal of Experimental Biology, 209*(21), 4238-4253. doi:
700   10.1242/jeb.02505
701 van der Hoop, J., Corkeron, P., & Moore, M. (2016). Entanglement is a costly life-history stage in
702   large whales. *Ecology and Evolution, 7*(1), 92-106. doi: 10.1002/ece3.2615
703 Varanasi, U., & Malins, D. C. (1971). Unique lipids of the porpoise (*Tursiops gilli*): Differences
704   in triacyl glycerols and wax esters of acoustic (mandibular canal and melon) and blubber
705   tissues. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism, 231*(2), 415-
706   418. doi: https://doi.org/10.1016/0005-2760(71)90158-5
707 Verrier, D., Groscolas, R., Guinet, C., & Arnould, J. P. Y. (2011). Development of fasting abilities
708   in subantarctic fur seal pups: balancing the demands of growth under extreme nutritional
709   restrictions. *Functional Ecology, 25*(3), 704-717. doi: doi:10.1111/j.1365-
710   2435.2010.01823.x
711 Whitehead, H., & Hooker, S. K. (2012). Uncertain status of the northern bottlenose whale
712   Hyperoodon ampullatus: population fragmentation, legacy of whaling and current threats.
713   *Endangered Species Research, 19*(1), 47-61. doi: 10.3354/esr00458
714 Whitehead, H., Gowans, S., Faucher, A., & McCarrey, S. W. (1997). Population analysis of
715   Northern Bottlenose whales in the Gully, Nova Scotia. *Marine Mammal Science, 13*(2),
716   173-185. doi: 10.1111/j.1748-7692.1997.tb00625.x
717 Williams, R., Vikingsson, G. A., Gislason, A., Lockyer, C., New, L., Thomas, L., & Hammond,
718   P. S. (2013). Evidence for density-dependent changes in body condition and pregnancy

19

719   rate of North Atlantic fin whales over four decades of varying environmental conditions.
720   *ICES Journal of Marine Science: Journal du Conseil, 70*(6), 1273-1280. doi:
721   10.1093/icesjms/fst059
722 Young, R. A. (1976). Fat, Energy and Mammalian Survival. *American Zoologist, 16*, 699-710.
723 Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common
724   statistical problems. *Methods in Ecology and Evolution, 1*(1), 3-14. doi:
725   doi:10.1111/j.2041-210X.2009.00001.x

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727 **TABLES**

728 **Table 1** – Summary of the balaenopterid and ziphiid individuals used for full depth blubber sample
729 analysis. * SMASS (Scottish Marine Animal Strandings Scheme). * MICS (Mingan Island
730 Cetacean Study).

Family	Species	Number	Source
Balaenopteridae	Minke whale (*Balenoptera acutorostrata*)	6	SMASS* MICS*
	Humpback whale (*Megaptera novaeangliae*)	3	SMASS MICS
Ziphiidae	Sowerby's beaked whale (*Mesoplodon bidens*)	4	SMASS
	Cuvier's beaked whale (*Ziphius cavirostris*)	2	SMASS
	Northern bottlenose whale (*Hyperodon ampullatus*)	2	SMASS

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740 **Table 2** – Summary table of the generalised linear mixed effect model results (GLMM), and the
741 generalised linear model results (GLM) investigating the effects of various covariates on blubber
742 lipid content for the two species groups. [1]VIFs: variance inflation factors measure collinearity
743 between predictor variables. These predictor variables with high VIFs were excluded from the
744 analysis. * Indicates that the variable was retained after model selection and was statistically
745 significant at the 5% level.

		Ziphiids n = 8	Balaenopterids n = 7
GLMM	**Covariates retained following model selection**	none	blubber layer*
GLM	**Covariates discarded based on VIFs[1]**	sex, age class	girth / length
	Covariates retained following model selection	none	COD*

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747 **FIGURE LEGENDS**

748 **Fig. 1. Subsampling of the full depth blubber samples > 30mm for lipid extraction**. The left
749 panel indicates how the full depth samples were divided. The vertical dashed line shows where the
750 blubber was cut in half to generate duplicate subsamples. The horizontal dashed lines show where
751 the blubber was cut such that the outer, middle and inner layers were retained for analysis while
752 the two transitional layers were discarded. The right panel shows a frozen full depth blubber
753 sample from a juvenile humpback whale before any subsampling took place.

754 **Fig. 2. Final GLMM parameter estimates with associated error for total lipid content**
755 **through the blubber depth.** Note estimates are on the log scale as the GLMM uses a log link
756 function, and both plots are on the same scale for comparison between families. a) Ziphiids: Model
757 selection did not retain layer as an important explanatory variable as there were no significant
758 differences in lipid content between layers. b) Balaenopterids: There were significant differences
759 (indicated by the *) between the outer layer and all other layers.

760 **Fig. 3. Boxplots of lipid content across blubber layers by COD category with histograms of**
761 **the body condition estimates of the sampled animals compared to the full morphometric**
762 **dataset collected by the SMASS**. a) and b) There was no difference in total lipid content between
763 layers in the acute or chronic cases for the ziphiids. c) and d) Lipid content was significantly lower
764 across all blubber layers in the chronic compared to the acute cases (ANOVA; F = 44.71, df = 1,
765 p < 0.0001) in the balaenopterids. e) to h) Girth/length histograms for the for ziphiid (n = 42) and

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766   the balaenopterid (n = 51) data collected by the SMASS. The shaded bars indicate the range in
767   condition indices of the individuals sampled for blubber lipid extraction.

768   **Fig. 4. Lipid content in the middle blubber layer plotted against the morphometric body**
769   **condition estimates for each species group.** a) Ziphiids: There was no correlation between the
770   lipid content in the middle layer and girth/length. b) Balaenopterids: There was a significant
771   positive correlation between the lipid content in the middle layer and girth/length (p = 0.035,
772   Adjusted R^2 = 0.4).

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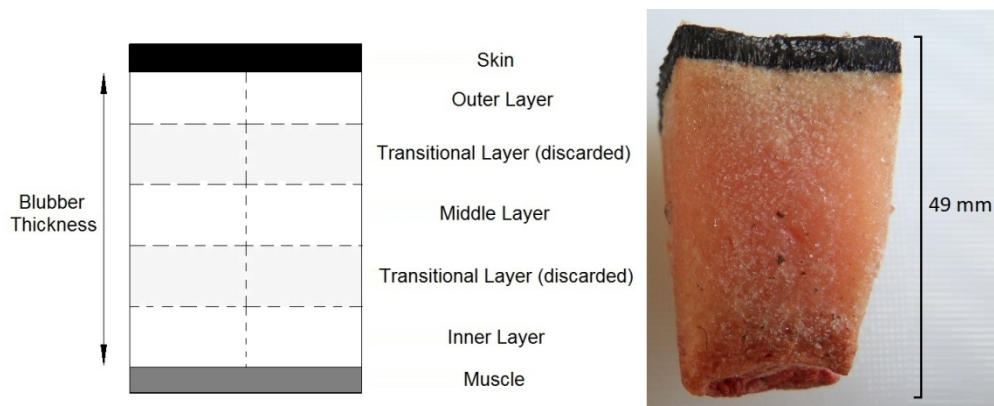9
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Fig. 1. Subsampling of the full depth blubber samples > 30mm for lipid extraction. The left panel indicates how the full depth samples were divided. The vertical dashed line shows where the blubber was cut in half to generate duplicate subsamples. The horizontal dashed lines show where the blubber was cut such that the outer, middle and inner layers were retained for analysis while the two transitional layers were discarded. The right panel shows a frozen full depth blubber sample from a juvenile humpback whale before any subsampling took place.

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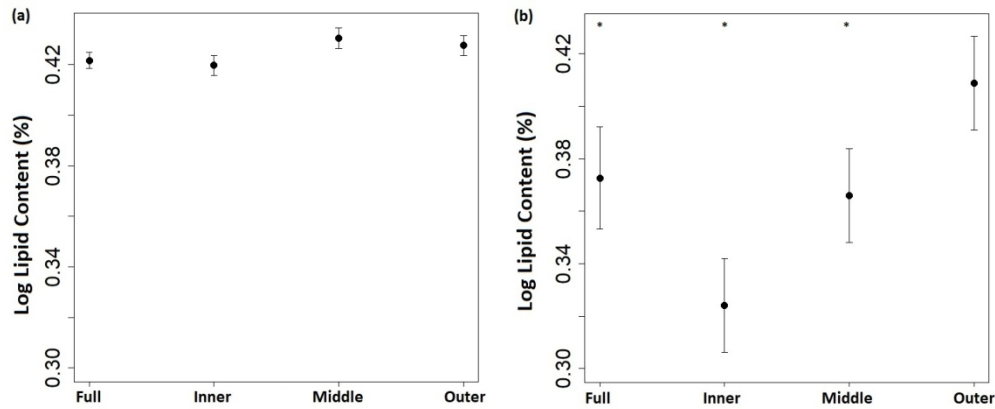47
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Fig. 2. Final GLMM parameter estimates with associated error for total lipid content through the blubber depth. Note estimates are on the log scale as the GLMM uses a log link function, and both plots are on the same scale for comparison between families. a) Ziphiids: Model selection did not retain layer as an important explanatory variable as there were no significant differences in lipid content between layers. b) Balaenopterids: There were significant differences (indicated by the *) between the outer layer and all other layers.

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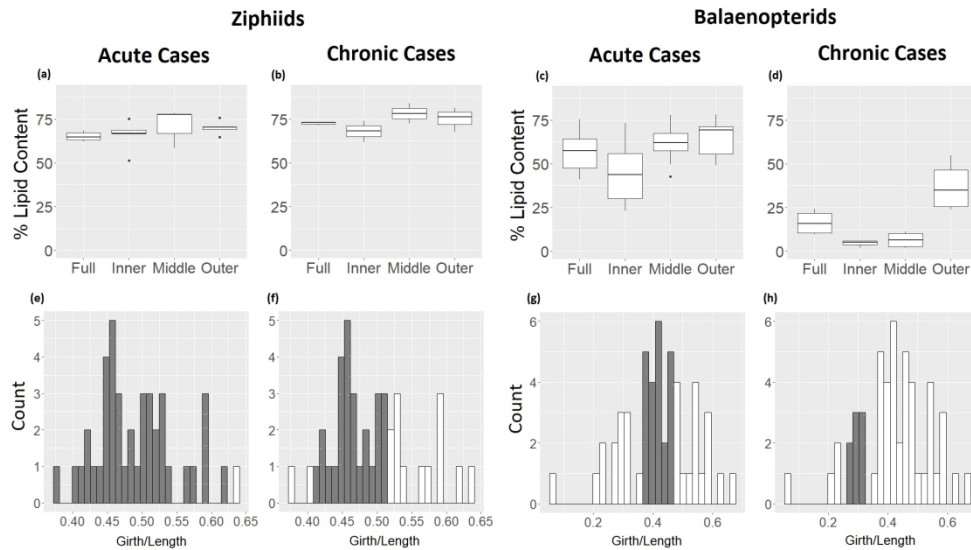43
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Fig. 3. Boxplots of lipid content across blubber layers by COD category with histograms of the body condition estimates of the sampled animals compared to the full morphometric dataset collected by the SMASS. a) and b) There was no difference in total lipid content between layers in the acute or chronic cases for the ziphiids. c) and d) Lipid content was significantly lower across all blubber layers in the chronic compared to the acute cases (ANOVA; F = 44.71, df = 1, p < 0.0001) in the balaenopterids. e) to h) Girth/length histograms for the for ziphiid (n = 42) and the balaenopterid (n = 51) data collected by the SMASS. The shaded bars indicate the range in condition indices of the individuals sampled for blubber lipid extraction.
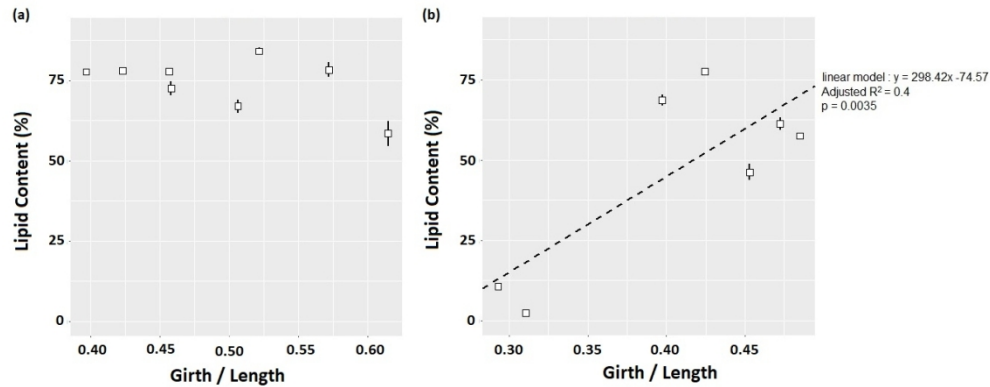
563x315mm (96 x 96 DPI)

Fig. 4. Lipid content in the middle blubber layer plotted against the morphometric body condition estimates for each species group. a) Ziphiids: There was no correlation between the lipid content in the middle layer and girth/length. b) Balaenopterids: There was a significant positive correlation between the lipid content in the middle layer and girth/length (p = 0.035, Adjusted R2 = 0.4).

359x140mm (96 x 96 DPI)