

1 **A Comparative Analysis of Marine Mammal Tracheas**

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3 ^{1*}Colby Moore, ¹Michael Moore, ²Stephen Trumble, ³Misty Niemeyer, ¹Betty Lentell,
4 ⁴William McLellan, ⁴Alexander Costidis, ^{1,5**} Andreas Fahlman

5
6 ¹ *Woods Hole Oceanographic Institution, 266 Woods Hole Road, MS50, Woods Hole,*
7 *MA, 02543, USA.*

8 ² *Baylor University, Department of Biology, One Bear Place #97388, Waco, TX, 76706,*
9 *USA.*

10 ³ *International Fund for Animal Welfare, 290 Summer Street, Yarmouth Port, MA, 02675,*
11 *USA.*

12 ⁴ *University of North Carolina Wilmington, Department of Biology and Marine Biology,*
13 *601 S. College Road, Wilmington, NC, 28403, USA.*

14 ⁵ *Texas A&M University-Corpus Christi, 6300 Ocean Drive, Corpus Christi, TX, 78412,*
15 *USA.*

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17
18
19
20 **Current address: Baylor University, Department of Biology, One Bear Place #97388,*
21 *Waco, TX, 76706, USA.*

22 ***Corresponding author email: andreas.fahlman@tamucc.edu*

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32 **Summary**

33 In 1940, Scholander suggested that stiffened upper airways remained open and
34 received air from highly compressible alveoli during marine mammal diving. There are
35 little data available on the structural and functional adaptations of the marine mammal
36 respiratory system. The aim of this research was to investigate the anatomical (gross) and
37 structural (compliance) characteristics of excised marine mammal tracheas. Here we
38 defined different types of tracheal structures, categorizing pinniped tracheas by varying
39 degrees of continuity of cartilage (categories 1-4) and cetacean tracheas by varying
40 compliance values (categories 5A and 5B). Some tracheas fell into more than one
41 category, along their length, for example, the harbor seal (*Phoca vitulina*) demonstrated
42 complete rings cranially, and as the trachea progressed caudally tracheal rings changed
43 morphology. Dolphins and porpoises had less stiff, more compliant spiraling rings while
44 beaked whales had very stiff, less compliant spiraling rings. The pressure-volume (P-V)
45 relationships of isolated tracheas from different species were measured to assess
46 structural differences between species. These findings lend evidence for pressure-induced
47 collapse and re-inflation of lungs, perhaps influencing variability in dive depth or
48 ventilation rates of the species investigated.

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60 **Keywords**

61 Diving, Lung collapse, Pressure-volume, Compliance, Diving physiology, Alveolar
62 compression

63 **1. Introduction**

64 The unique distribution of cartilage in the trachea and bronchi of marine
65 mammals was noted by Scholander (1940), detailing that the extent and length of
66 cartilage varied between species. Scholander noted that dolphins, porpoises, and sea lions
67 (*Zalophus californianus*) all have cartilage extending far to the alveolar sac, thus
68 supporting the hypothesis that the cartilaginous trachea may play a role in alveolar
69 compression and collapse (Kooyman and Sinnett, 1979), acting as either rigid reinforced
70 space for respiratory air or compliant collapsible tissue during compression. Scholander
71 (1940) suggested that the anatomy of the respiratory system of marine mammals would
72 allow alveolar collapse and cessation of gas exchange upon deep dives. The model
73 proposed by Scholander suggested compression of the respiratory system would force air
74 from the alveoli and alveolar ducts into the rigid, cartilaginous bronchi and trachea.
75 Depending on the mechanism of alveolar compression, this may reduce the ventilated
76 alveolar surface area and increase the thickness of the alveolar membrane, thereby
77 reducing the gas diffusion rate (Bostrom et al., 2008). This would eventually result in
78 atelectasis, or alveolar collapse (commonly referred to as lung collapse), as all alveolar
79 air is pushed into the upper airways (bronchi and trachea), thus terminating gas exchange.
80 Upon ascent, the alveoli are reinflated, an apparently effortless action for marine
81 mammals, yet problematic in humans (Fahlman, 2008).

82 Tracheal rigidity facilitates the rapid and more complete emptying of the lungs as
83 compared with terrestrial mammals (Denison et al., 1971; Bostrom et al., 2008). The
84 modified trachea also allows for high ventilation rates during the surface interval, and
85 tidal volumes that are close to the vital capacity, resulting in efficient gas exchange and
86 faster replenishment of O₂ stores and removal of CO₂ at the surface (Scholander, 1940;
87 Olsen et al., 1969; Kooyman and Sinnett, 1979; Kooyman and Cornell, 1981).

88 Recent theoretical models suggest that, in addition to the relative volume between
89 the upper and lower respiratory system, the compliance of the trachea is important in
90 determining the lung collapse depth (Bostrom et al., 2008) and subsequent levels of gas
91 exchange at pressure (Fahlman et al., 2009). Lung diffusion measurements in harbor seals
92 and California sea lions concurred that the diffusion rate is directly related to the diving
93 lung volume and the ambient pressure (Kooyman and Sinnett, 1982). Given the predicted

94 lung volumes and diffusion rates, theoretical models (Bostrom et al., 2008) allow
95 predictions as to how pressure affects the volume in the various compartments of the
96 respiratory system and theoretically how pulmonary shunts might develop. However,
97 these models are influenced by compliance estimates for the various portions of the
98 respiratory system and little mechanical information currently exists for the upper
99 airways of marine mammals (Sokolov et al., 1968; Cozzi et al., 2005; Bagnoli et al.,
100 2011), making predictions for respiratory changes uncertain.

101 The relationship between pressure and volume gives an estimate of the
102 compliance of the respiratory tract and has been successfully performed on excised lungs
103 from terrestrial mammals (Bachofen et al., 1970), with few data documenting compliance
104 of marine mammal lungs (Denison et al., 1971; Piscitelli et al., 2010; Fahlman et al.,
105 2011). The compliance of the trachea has been suggested to affect the amount of air
106 displaced from the lungs (Bostrom et al., 2008), and thereby the depth where the alveoli
107 collapse and gas exchange ceases. The alveolar collapse depth and cessation of gas
108 exchange should occur at a shallower depth for a mammal with a more rigid trachea
109 (Bostrom et al., 2008). Our aim was to assess the airway compliance of several species of
110 marine mammals as compared to gross morphological observations, encompassing both
111 shallow and deep diving cetaceans and pinnipeds, in an attempt to link form and function
112 and provide more detail on the role of the trachea during diving.

113

114 **2. Material and methods**

115 *2.1 Animals*

116 By-caught marine mammals were recovered in association with fishing gear from
117 the Northeast Fisheries Observer Program (NEFOP). Animals were kept on ice when
118 available and transported to the Woods Hole Oceanographic Institution (WHOI), Marine
119 Research Facility, within 24 hours of landing. Animals were stored in a 4°C chiller prior
120 to necropsy. Stranded animals were collected by the International Fund for Animal
121 Welfare (IFAW) staff and volunteers and transported to WHOI as soon as possible, and
122 also stored at 4°C prior to necropsy. The California sea lions in this study were collected
123 by the Marine Mammal Center (Sausalito, CA) and the excised tracheas were shipped
124 chilled to WHOI for compliance studies. Both the True's beaked whale (*Mesoplodon*

125 *mirus*) and Gervais' beaked whale (*Mesoplodon europaeus*) were stranded in northern
126 North Carolina, and transported to the North Carolina State Veterinary School in Raleigh.
127 By-caught and stranded marine mammals used for compliance measurements were
128 recovered at a decomposition code of 2. In addition, three species of terrestrial mammals
129 were utilized in compliance and morphology data collection (dog, cattle and pig).

130

131 *2.2 Morphometrics*

132 Upon arrival, the sex was determined and each animal was weighed (± 0.2 kg)
133 using a Tri-coastal, class III scale (model # LPC-4) (Table 1). Routine morphometric
134 measurements were also completed. These included standard length, girth, flipper length,
135 dorsal fin (dolphin and porpoise) or hind flipper length (seals). During the necropsy, the
136 trachea was removed as detailed below.

137

138 *2.3 Protocol*

139 The entire respiratory system was excised (lungs, bronchi and trachea) from each
140 carcass. The structural properties of the lungs were measured and have been discussed in
141 a separate publication (Fahlman et al., 2011). The trachea was separated from the lungs
142 and excess associated tissue was removed. Removal of associated tissue could affect
143 compliance data, and that was not accounted for in this study. The trachea included all
144 tissue caudal to the larynx and just cranial to the first bronchial bifurcation. Because the
145 relative length of the odontocete trachea is much shorter than that of the pinniped and
146 was found in association with an accessory bronchus, the conducting airway was excised
147 closer to the secondary bronchial bifurcation (Fig. 1).

148 Tracheal gross morphology was determined by examination of the entire length of
149 each trachea as well as cross sectional views at cranial, mid and caudal areas. Five
150 tracheal categories were assigned to describe major differences in gross morphology
151 (Figs 2-5; Table 2). Tracheal categories were determined by continuity of cartilage for
152 pinnipeds and compliance values for cetaceans.

153 For pinnipeds specifically, if complete rings were present, a portion of the trachea
154 was designated as category 1 (Fig. 2). If an overlay of cartilage, or "slip", was present in
155 cross sections, where during compression one side of cartilage can easily slide over the

156 other, reducing internal volume, the trachea was categorized as 2 (Fig. 3). When a “slip”
157 was accompanied by a large gap in cartilage, it was defined as category as 3 (Fig. 4). The
158 4th tracheal category (Fig. 5) consisted of a “horseshoe” shaped tracheal ring: incomplete
159 cartilage rings separated by one “gap”. Tracheal category 5, consisted solely of cetacean
160 tracheas, comprised of spiraling rings divided into two subcategories, *A* and *B* (Fig. 6;
161 Table 2), because of similar gross morphology with only compliance differences (*A* was
162 less stiff/complaint, and *B* was rigid).

163 To estimate compliance, excised tracheas were placed on a tray and intubated
164 using a human (Hudson RCI, Teleflex Medical Inc.) or veterinary (Equine Nasotracheal
165 Tube, Jorgensen Laboratories) endotracheal tube of suitable size. The endotracheal tube
166 was attached to a system of 3-way valves with a volumetric syringe of suitable size (3-60
167 ml). Ends of the trachea were sealed shut with a Twixit clip (Linden Sweden, Inc.). The
168 compression at the end of the trachea by the Twixit clip, as well as the addition of the
169 endotracheal tube, affected the volume measurement slightly. This was accounted for
170 during measurements of the floodable volume by assuming that both instruments are not
171 collapsible, measuring additional volume and subtracting that volume from the end
172 measurements.

173 The compliance of the trachea, or in some cases sections of the trachea, was
174 estimated by determining the P-V relationship. The trans-luminal pressure difference (ΔP
175 = $P_{\text{trach}} - P_{\text{amb}}$, cmH₂O), the differential pressure between the inside of the trachea (P_{trach})
176 and the ambient pressure (P_{amb}), was measured using a differential pressure transducer
177 (MPX type 339/2, Harvard Apparatus) connected to an amplifier (Tam-A, Harvard
178 Apparatus) and the data collected on a laptop using an A/D card (USB 1208LS,
179 Measurement Computing) sampling at 2 Hz. All compliance measurements were
180 performed laying the trachea horizontally on a tray in air. The total volume injected or
181 removed from the trachea to reach a ΔP of 50 cmH₂O or -50 cmH₂O, respectively, was
182 divided in 4-5 equal increments. The trachea was then inflated or deflated with these
183 volumes using a volumetric syringe. A minimum of three leak-free inflation/deflation
184 curves was recorded for each sample. After an injection or removal of a bolus of air, the
185 volume was held constant until the pressure stabilized before the next step-wise change in
186 tracheal volume. The tracheal compliance appeared to change along the length of the

187 trachea in some species, e.g. harbor seal. In those species, the tracheal compliance was
188 separately measured at the upper and lower section of the trachea (Table 3).

189 The volume change (ΔV) was expressed as a fractional change from the internal
190 (floodable) volume of the relaxed trachea (i.e. $\Delta P=0$). For inflation and deflation
191 experiments, ΔP was expressed as the pressure change between P_{amb} and P_{trach} . As a
192 convention, ΔP and the volume used for inflation (ΔV) was expressed as a positive value
193 while both ΔP and ΔV were negative during deflation trials. As the animal dives two
194 forces will balance P_{amb} ; 1) the pressure in the airways (e.g., P_{trach}) and 2) the structural
195 properties of the trachea and alveolar space (Bostrom et al., 2008). Thus, changes in the
196 internal volume of the respiratory system will depend on the balance between these
197 pressures. In the current study, we investigated the structural properties by measuring the
198 P-V relationship of the trachea. The transpulmonary pressures in the mammalian lung
199 seldom exceed 30 cmH₂O (2.93 kPa). Thus, we only exposed the tracheas to transtracheal
200 (ΔP) pressures up to about 4 kPa, as those pressures were within the physiological range,
201 and much higher differential pressures would result in trauma (Brown and Butler, 2000).

202 The volume of the relaxed trachea (floodable volume) was measured by
203 immersing the trachea horizontally in a water bath to avoid tissue expansion or change in
204 length (Table 1). One end was sealed with a twixit clip, the trachea flooded with water,
205 and the total volume of water measured. Floodable volume was measured in triplicate by
206 weighing a filled volumetric flask.

207

208 *2.4 Data processing and statistical analysis*

209 The room temperature, and the ambient pressure were used to convert all volumes
210 to standard temperature pressure dry (STPD). It was assumed that air used to inflate the
211 excised trachea was completely saturated with water vapor.

212 The relationship between pressure and volume was determined using a
213 Generalized Linear Model (GLM), with relative volume as the dependent variable, ΔP ,
214 body mass, and species as independent variables, and animal ID as a random variable
215 (Littell et al., 1998). The slope of each P-V relationship represented the elastic properties
216 of the trachea, with increasing slope representing a more compliant tissue. To compare
217 compliance among species, the 95% confidence limit (CL) was estimated for the slope.

218 The standard error of the mean (SEM) for each parameter was used to determine the 95%
219 CL and differences among species were assessed by determining which samples had
220 overlapping CL's. Parameters were fitted using R (R: A Language and Environment for
221 Statistical Computing, R Foundation for Statistical Computing, version 2.5.1, 2007).

222 In this study P-values ≤ 0.05 were considered significant. Data were presented as
223 mean values \pm standard deviation (SD) unless otherwise stated.

224

225 **3. Results**

226 *3.1 Tracheal category types*

227 We described five pinniped species, four cetacean species and three species of
228 terrestrial mammals in total during this study (n=32, Table 1). All tracheas were fresh
229 with the exception of one cetacean trachea (Gervais' beaked whale) that was fixed in
230 formalin and thus was used for morphology descriptions only (not included in Table 1 or
231 Fig. 7A,B). The fin whale (*Balaenoptera physalus*) was too large for compliance
232 measurements and was also used for morphology descriptions only (not included in Table
233 1 or Fig.7A,B). Four distinct gross tracheal structures, or categories, were observed for
234 the pinniped species and one for cetaceans (Table 2). As such, five tracheal structures
235 were designated in marine mammals (category 1 through 5; Figs 2-6; Table 2).

236

237 *3.2 Overview of P-V relationships*

238 The P-V relationship was plotted for each species and the results are detailed in
239 Fig. 7A,B. There was good reproducibility in the P-V relationship between tracheas for
240 the California sea lion (data not shown). As the size of the trachea varied with body mass,
241 the volume was expressed as a percentage of the floodable volume. In this study we
242 compared the compliance during inflation and deflation for each species (Fig. 7A,B).
243 Compliance is a numerical value for the slope, therefore the numerical value for the
244 compliance of inflation and deflation of each trachea was considered here (Fig. 7A,B).

245

246 *3.3 Form and function of pinnipeds*

247 *Harbor seal and gray seal*

248 The harbor seal and gray seal (*Halichoerus grypus*) were grouped together
249 morphologically, as they had similar external tracheal anatomy, where there appeared to
250 be two distinct areas lengthwise of rigidity which determined the areas of measured
251 compliance in this study (Fig. 8). Further histology would be needed to determine the
252 type and amount of tissue present between rings lengthwise (versus cross sectional), thus
253 potentially indicating differing flexibility between the two regions. The cranial end to the
254 middle section appeared to be a stiff, rigid area, with minimal tissue separating the
255 cartilage rings (Fig. 8). However, the middle and caudal sections were “accordion”-like
256 in structure, suggesting a lengthwise change in morphology and compliance, as the
257 trachea progressed (Fig. 8; Table 3).

258 Specific to the harbor seal, three different cross sectional tracheal categories were
259 revealed based on macroscopic differences in cartilage continuity (Figs 2-4, 8; Table 2),
260 despite the two distinct regions (lengthwise) of differing rigidity confirmed by
261 compliance measurements (Table 3). A cross section of the cranial portion revealed that
262 the cartilaginous rings of the harbor seal trachea were complete throughout their
263 circumference, thus forming a complete circle of cartilage with no connective tissue
264 bridges (Fig. 2). We categorized this cranial section of complete rings as category 1 (Fig.
265 2). Mid sections of the harbor seal trachea were incomplete, forming a gap as well as a
266 “slip” (Category 3; Fig. 4). Caudal cross-sections of the harbor seal trachea conformed to
267 category 2 morphologically (Fig. 3).

268 As previously mentioned, the gray seal trachea also displayed (external
269 lengthwise) a distinct cranial area of relative rigidity, becoming more “accordion”-like in
270 structure moving towards the lungs (Fig. 8). Cross sectional examination revealed
271 complete cartilage rings cranially with a distinct change in morphology as the trachea
272 progressed towards the lungs, specifically demonstrated by a “slip” (Fig. 2,8). Thus, the
273 gray seal began cranially as category 1 and changed morphologically to category 2 in mid
274 and caudal regions (Fig. 2,8; Table 2).

275 For both the harbor seal and gray seal, the “slip” allowed for variation in volume
276 over a ΔP in the physiological range (up to 4 kPa). At higher differential pressures, the
277 trachea stiffened, specifically when deflated (data not shown). The compliance in the
278 gray seal trachea varied with inflation and deflation and also demonstrated age related

279 changes (Fig. 7A,B). Therefore, the gray seals were divided into three age groups (Hg1,
280 Hg2 and Hg3). Interestingly, older gray seals (Hg1: Fig. 7A,B) demonstrated higher
281 compliance values on both inflation and deflation, as compared to the younger gray seals
282 (Hg2 and Hg3: Fig. 7A,B). One gray seal (DO7662) trachea was frozen and compliance
283 measurements were taken pre and post-freeze. Compliance results were identical to the
284 other gray seal in its age class (DO6322), indicating that freezing the tracheas prior to
285 measurements appeared not to affect the validity of the data, although longer freezing
286 intervals may affect the results.

287 Overall, both the harbor seal and gray seal demonstrated similar rigidity in
288 tracheal compliance (Fig. 7A,B). In addition, both species also demonstrated a change in
289 morphology as the trachea approached the lungs, both beginning cranially with a
290 complete cartilage ring and developing a break in cartilage in more caudal regions (Fig.
291 8). Further compliance measurements in all three cross sectional areas of the harbor seal,
292 as opposed to the two areas measured, may have revealed slightly different compliance
293 measurements, however this could also have proved more difficult with smaller tracheal
294 pieces to measure.

295

296 *Harp seal*

297 The cartilaginous rings of the harp seal (*Pagophilus groenlandica*) were
298 discontinuous around the circumference of the trachea along the entire length of the
299 trachea (Category 2; Table 2). During compression, cartilage sides were able to “slip”
300 over each other reducing the circumference of the airway.

301 The harp seal showed no distinct variation in tracheal compliance along its entire
302 length. The overlapping tracheal structure allowed expansion or compression as the
303 connective tissue stretched, and alternatively as one side was able to “slip” over the other.
304 During inflations, the connective tissue separating the ends of the incomplete cartilage
305 circumference stretched (Fig. 7B). In other words, the harp seal trachea was
306 comparatively very compliant when inflated. Similarly, the trachea was also very
307 compliant during deflations, but at very low volumes, or at differential pressures > 4 kPa,
308 it became rigid and non-compliant (data not shown, out of physiological range). During
309 an inflation of the harp seal trachea, ΔP initially increased sharply but then gradually

310 declined to a stable value within 10-15 sec. This was not seen in the gray seal, where the
311 ΔP stabilized immediately following inflation. This was probably due to the elastic
312 structure of the harp seal trachea that kept the discontinuous rings together. Thus, the
313 excised trachea of the harp seal acted as an elastic buffer, dampening the pressure
314 changes.

315

316 *Northern elephant seal*

317 Elephant seals (*Mirounga angustirostris*) were classified as category 4 (Table 2).
318 The trachea was “horseshoe” shaped, where cartilage rings were incomplete but not
319 overlapping. In all cases, this incomplete morphology continued the length of the trachea
320 as it progressed caudally.

321 During both inflation and deflation experiments, the elephant seal tracheal
322 compliance data values (Fig 7A,B) were between those of other pinniped species. In
323 other words, compliance values for the elephant seal were neither the highest (harp seal)
324 nor the lowest (harbor seal) among pinnipeds (Fig 7A,B).

325

326 *California sea lion*

327 California sea lions demonstrated a cylinder of overlapping discontinuous
328 cartilage rings fused by connective tissue, progressing from the larynx to the first
329 bronchial bifurcation (Fig. 9; Table 2). The sea lion tracheal morphology was classified
330 as category 2, as discontinuous cartilaginous rings formed a “slip” where sides
331 overlapped one another (Table 2).

332 During compression the lateral sides of the sea lion trachea overlapped throughout
333 the tracheal length. The sea lion trachea exhibited tissue involution cranially which
334 continued in the mid-section until the caudal regions where the trachea separated into two
335 fused primary bronchi that traveled towards the lungs (Fig. 9). This was indicative of the
336 thoracic inlet region in otariids. Both sea lion bronchi displayed overlaying cartilage, or
337 “slip” features as well, allowing both tubes to compress separately. Thus, each bronchial
338 tube was also considered to be in category 2, although not measured separately or as part
339 of the true trachea.

340 The discontinuous cartilaginous rings of the sea lion allowed for medium
341 compliance relative to other species in this study (Fig. 7A,B), perhaps due to the primary
342 bifurcation being located more cranially. In other words, either the two bronchi were
343 elongated or the true trachea was shortened affecting overall compliance (Fig. 7A,B). In
344 addition, similar compliance values were found for both the inflation and deflation
345 measurements (Fig. 7A,B).

346

347 *3.4 Form and function of odontocetes*

348 The trachea of the harbor porpoise (*Phocoena phocoena*) was conical and wide
349 (Fig. 1), similar to other odontocetes investigated in this study. An accessory bronchus
350 was observed cranial to the main tracheal bifurcation, and joined to the cranial section of
351 the right lung in all odontocete species studied (Fig. 6) as well as one baleen whale, the
352 fin whale. All cetacean species examined were classified as category 5 due to overall
353 similarity in gross structure. Compliance values revealed two subcategories for cetaceans:
354 ones with (A) compliant and (B) rigid/less compliant spiraling rings (Fig. 6; Table 2).
355 Tracheal rigidity was observed in one beaked whale species: True's beaked whale
356 (Category 5B; Table 2), where as harbor porpoise and dolphin species demonstrated
357 greater compliance, or were less rigid overall (Category 5A; Fig. 6; Table 2). The
358 Gervais' beaked whale trachea was not analyzed for compliance, just morphology, as it
359 was in a fixed state of preservation (Fig. 6). Any microscopic discontinuities in the
360 cartilage of cetacean tracheas were not distinguished here, and further histology would be
361 needed to confirm any microscopic discontinuity suggested by the observed compliance
362 differences between subcategories. This is a future aim for further study.

363 The common dolphin (*Delphinus delphis*) and white-sided dolphin
364 (*Lagenorhynchus acutus*) tracheas were more compliant during deflation (Fig. 7A) than
365 when inflated (Fig. 7B). The harbor porpoise demonstrated the opposite, being more
366 compliant on inflation than deflation (Fig. 7A,B). The True's beaked whale had lower
367 compliance values on inflation and deflation than any of the other odontocete species
368 investigated (Fig. 7A,B).

369

370 *3.5 Form and function of terrestrial animals*

371 The dog (*Canis lupis familiaris*) trachea was determined to be category 4 as
372 cartilaginous rings were incomplete and a gap was present between ends of the cartilage,
373 forming a “horseshoe” (Table 2). The gap was present through the entire length of the
374 trachea. Dogs, although not the closest land relative to pinnipeds, showed similar tracheal
375 morphology to the elephant seal (Table 2). Compliance values for both inflation and
376 deflation for the dogs did not vary much amongst themselves (Fig. 7A,B).

377 The pig (*Sus scrofa*) trachea was grouped in category 2 (Fig. 3; Table 2), as there
378 were incomplete cartilaginous rings with distinct overlapping of cartilaginous sides,
379 forming the characteristic “slip” feature seen in some pinniped species. Examined species
380 sharing this category were distal portions of the harbor seal trachea and the entire length
381 of the sea lion and harp seal tracheas. The pig trachea demonstrated the highest values of
382 compliance on inflation, although deflation data were not measured (Fig. 7A,B).

383 The cattle (*Bos primigenius*) tracheas had incomplete rings with a common dorsal
384 ridge unique to this species in this study. Thus, cattle were grouped in category 2 (Table
385 2), as it was the closest categorical group for this morphology. Compliance values for
386 cattle indicated similar compliance values on inflation and deflation (Fig. 7A,B). The
387 most compliant trachea for terrestrial mammals was the pig followed by similar
388 compliance values for the dog and cows (Fig. 7A,B).

389

390 **4. Discussion**

391 In this study, we provided detailed descriptions and categorically classified the
392 varied respiratory anatomy of some marine mammal species with different diving
393 behaviors and lineages. Specifically, we described gross and structural properties of
394 several pinniped and cetacean tracheas. Results suggested that there was great variety in
395 tracheal morphology and physical properties among pinniped species. For example,
396 tracheas were found to have complete, incomplete and/or changing cartilage rings
397 throughout the trachea. However, less variation based on compliance data and
398 morphology was demonstrated in the odontocete trachea, even given their equally diverse
399 diving behavior. For example, all cetaceans demonstrated similar spiraling cartilage rings
400 and the addition of an accessory bronchus. Although microscopic differences may exist,

401 cetacean gross morphology was similar. This finding suggested that evolutionary
402 pressures other than diving adaptation could be involved.

403 Our experimental approach had limitations, specifically regarding decomposition
404 of the collected tissues and the longitudinal change in tracheal length during the
405 compliance measurements that proved difficult to quantify. Thus, the tracheal compliance
406 in live animals may prove to be different. We suggest that future experiments attempt *in*
407 *situ* inflations and deflations in order to account for the dynamics of the thoracic wall,
408 visceral mass, etc. Although we did not find inter-specific variation in tracheal rings apart
409 from age-related changes, further histology and greater sample size may have indicated
410 otherwise. Our study was different from the approach by Cozzi et al. (2005), where strain
411 curves were used to assess the compliance of the trachea in the striped dolphin (*Stenella*
412 *coeruleoalba*). Our approach allowed a simultaneous assessment of both compression
413 and expansion of the trachea. Despite these limitations, there was good reproducibility
414 between P-V curves from different individuals within the same species and our method
415 proved to be useful for indicating functional differences in tracheal samples, especially
416 when matched to gross anatomy. In addition, we categorized tracheas based on visual
417 observation. Undiscovered microscopic differences in pinniped and cetacean tracheas
418 may be revealed with future histology, which may help to improve category descriptions.
419 Given the limited data pertaining to marine mammal respiratory behavior, this work can
420 be considered an addition to the growing field of knowledge in respiratory adaptations for
421 marine mammals.

422

423 *4.1 Tracheal form and function*

424 Previous studies have published accounts of the anatomical characteristics of the
425 trachea in marine mammals (Scholander, 1940; Slijper, 1962; Sokolov et al., 1968;
426 Kooyman and Sinnett, 1979; Bagnoli et al., 2001; Cozzi et al., 2005). In this study, we
427 separated the various structural characteristics observed into five distinct categories
428 depending on the continuity of cartilage throughout the circumference of the tracheal
429 rings and compliance values, thus correlating form and function in a more complete
430 descriptive manner. For all phocid seals in this study (harbor seal, gray seal, harp seal and

431 elephant seal), the P-V curve matched well to observable morphology, and illustrated the
432 efficiency of P-V calculations when describing morphology.

433

434 *Pinnipeds – Phocidae*

435 The harbor, gray, bearded (Sokolov et al., 1968) and ribbon (Sokolov et al., 1968)
436 seals all demonstrated changing tracheal morphology as the trachea progressed towards
437 the lungs (Table 2). However, other pinniped species (California sea lion, elephant and
438 harp seal) had a consistent morphology the entire length of their trachea. One hypothesis
439 for a changing morphology may be that “slips” present caudally result in a stiffer region
440 cranially and more compliant region caudally. The caudal incomplete rings add
441 compliance to an overall rigid trachea, thus allowing a small degree of deeper diving
442 behavior.

443 Harbor and gray seals are often sympatric in their North Atlantic range and
444 reports have indicated that average dive depths for both species are relatively shallow,
445 however they sometimes perform deep dives (mean depth: 12-40 m and 48-51 m
446 respectively) (Thompson and Fedak, 1993; Boness et al., 1994; Thompson et al., 1996;
447 Lesage et al., 1999; Gjertz et al., 2001; Vincent et al., 2002; Moore et al., 2009, Table 4).
448 If morphological traits limit physiology we would expect these species to express similar
449 tracheal anatomy and compliance. The compliance indicated a rather stiff trachea during
450 deflation (compression) in young animals of both species (Figure 7A,B). In the gray seal,
451 the compliance increased with age. The trachea of the harp seal had even greater
452 compliance as compared with the harbor and gray seal (Fig. 7A,B) and was classified in
453 category 2 (Table 2). Although harp seals tend to maintain the majority of dives in
454 shallow water, they are capable of dives to even greater depth as compared with the
455 harbor and gray seals (Lydersen and Kovacs, 1993; Folkow et al., 2004, Table 4).

456 The tracheal morphology of the Northern elephant seal was “horseshoe” shaped
457 throughout the length of the trachea (Table 2). The morphology was different as
458 compared with other pinnipeds in the current study (Table 2), but similar to that of the
459 bearded and ribbon seals (Sokolov et al., 1968), the dog and the human (Netter, 2011).
460 Despite the morphological differences, the tracheal compliance was high in this deep
461 diving species (LeBoeuf et al., 2000; Kuhn et al., 2009; Robinson et al., 2012, Table 4).

462 The relative lack of cartilage throughout the circumference of the trachea may have aided
463 in the higher compliance, as compared with the harbor and gray seals. In addition, the
464 greater thickness and prominence of connective tissue in the gap between cartilaginous
465 rings as compared to the harp seal's thin connective tissue bridge, may explain its lower
466 compliance values as compared to the harp seal (Fig. 7A,B).

467 If tracheal compliance has important consequences for alveolar collapse (Bostrom
468 et al., 2008), the results in the phocids suggest that the alveolar collapse depth should
469 occur at higher pressure in deep diving species. There are several possible explanations
470 for this unexpected result. Theoretical models suggest access to the lung helps to lower
471 the end-dive N₂ levels during deep dives, and thereby reduce the risk of bubbles to form
472 (Fahlman et al., 2009; Hooker et al., 2010). Additionally, the compliance of the excised
473 trachea may not accurately describe the compliance *in situ* in a live animal.

474

475 *Pinnipeds- Otariidae*

476 The sea lion trachea has been previously described by Sokolov et al. (1968) and
477 was shown to remain consistent in length regardless of neck length, whereas the longer
478 neck length was correlated with longer bronchial tubes (Sokolov et al., 1968). The sea
479 lion trachea was equally compliant during inflation and deflation, and had properties that
480 fell in between those of the phocids (Fig. 7A,B). The dive depth of the California sea lion
481 is deeper than the harbor and gray seals but shallower than the elephant seal (Feldkamp et
482 al., 1989; Weise et al., 2006, Table 4).

483

484 *Odontocete tracheal categories*

485 Gross anatomical features of all the cetacean tracheas were very similar. Tracheas
486 maintained the same general shape and all cetacean tracheas also displayed an accessory
487 bronchus leading to the upper right lobe of the lung (Fig. 6). The tracheas of all cetaceans
488 sampled appeared to be composed of a relatively short, single cylinder of tightly
489 compact, spiraling, irregularly shaped tracheal rings, which fuse with adjacent cranial and
490 caudal rings at one or more locations. Although the two ends of each ring may not meet
491 to form a complete ring, the firm fusion to adjacent rings forms a seemingly more robust
492 structure than the generic gap or "slip" type of discontinuous tracheal rings seen in other

493 mammals. Since all cetacean tracheas examined showed this type of morphology, a single
494 category was used for cetaceans in this paper (Fig. 6; Table 2).

495 The P-V relationships were very different for the white-sided dolphin as
496 compared with the other odontocetes, especially the True's beaked whale (Fig. 7A,B).
497 Therefore, we divided the cetacean tracheas into two subcategories based on the
498 compliance (*A* and *B*). Variation in compliance may be due to microscopic differences in
499 tracheal morphology, specifically continuity, thickness and longitudinal area of cartilage.
500 Any discontinuities in cartilage rings likely aided in ease of compression under pressure
501 and may explain the differences in compliance values. However, histology is required to
502 support that claim.

503 Beaked whales are known deep divers (Hooker and Baird, 1999; Baird et al.,
504 2006; Tyack et al., 2006), yet have relatively rigid tracheas. The stiffer trachea would
505 cause the alveoli to collapse at a shallower depth and reduce gas exchange during deeper
506 dives (Bostrom et al., 2008). This was the opposite of what was seen in the pinniped
507 tracheas, where deeper diving animals had a more compliant upper airway and shallow
508 diving animals tended to have more rigid tracheas. However, marine mammals may also
509 alter alveolar collapse by altering the respiratory volume during diving and dive with
510 either a partially or completely filled lung (Scholander 1940; Kooyman et al., 1970;
511 Hooker et al., 2005) or exhale during ascent (Hooker et al., 2009).

512

513 *The role of an accessory bronchus in diving*

514 All cetaceans examined in this study shared a common characteristic: an
515 accessory bronchus. This anatomical feature is not found in the pinniped, but has been
516 described in Artiodactyl species (Nickel et al., 1979). Pigs, although not the closest
517 terrestrial relative, are related to cetaceans and similarly demonstrated an accessory
518 bronchus. Its presence could be significant to lung function, as an additional bronchus
519 may allow for altered ventilatory flow rates, and/or alterations in internal tracheal and
520 lung pressures during inhalation and exhalation.

521 In humans, the presence of this additional bronchus is an anomaly and associated
522 with respiratory disease (Manjunatha and Gupta, 2010). There is an increased prevalence
523 of disease when the tracheal bronchus is associated with the right lobe (Barat and

524 Konrad, 1987). However, the accessory bronchus invests a distinct lung lobe in humans
525 and pigs, yet cetaceans lack lung lobes (Berta et al., 2006), perhaps limiting the
526 detrimental effects associated with the human clinical condition. Given the universal
527 prevalence of the accessory bronchus in cetacean species, this anatomical feature may
528 have an important function. For example, it has been shown that cetaceans have strong
529 musculature, among them the scalenus, intercostals and rectus abdominus to power
530 respiratory flow rates up to 160 l sec^{-1} (Kooyman and Cornell, 1981; Cotton et al., 2008).
531 The accessory bronchus may aid the generation of these flow rates. In addition, cetaceans
532 have cartilage extending down to the alveolar sac (Kooyman and Sinnett, 1979) as well
533 as additional elastic fibers and musculature in the conducting airways (Denison et al.,
534 1971; Kooyman and Sinnett, 1982) that allow extreme respiratory flow rates (Kooyman
535 et al., 1975) while at the surface. Future imaging work to look at the airway dynamics
536 may help elucidate if the accessory bronchus has an important function and if it acts to
537 further enhance the respiratory adaptations of cetaceans. Finally, it is also possible that
538 although this anatomical feature coincidentally aids in compression and recruitment of
539 the alveoli, its presence arises as a conserved evolutionary trait.

540 In an evolutionary context, the smaller odontocete species (harbor porpoise,
541 white-sided dolphin, and common dolphin) in this study shared the same basic gross
542 tracheal structure as the fin whale, a baleen whale and of the mysticete lineage of
543 cetacean species. Thus, the accessory bronchus likely was conserved in cetacean
544 evolutionary history, and was maintained on both sides of the odontocete-mysticete
545 evolutionary tree.

546

547 *4.2 The trachea's role in lung function and as a diving adaptation*

548 While Scholander (1940) suggested that the trachea of marine mammals was a
549 rigid structure, his observations were mainly based on the gray seal and bottlenose whale
550 (*Hyperoodon ampullatus*). While this appears to be valid for the gray seal and possibly
551 the bottlenose whale (which is in the beaked whale family) we suggest that not all
552 tracheas of marine mammals can be considered incompressible. That the trachea is in fact
553 compressed during diving has been shown in live submerged Weddell (*Leptonychotes*
554 *weddelli*) and elephant seals (Kooyman et al., 1970). We further propose that there may

555 be great variation between species and that this variation may affect compression of the
556 respiratory system, the depth of alveolar collapse, and gas dynamics during diving
557 (Bostrom et al., 2008; Fahlman et al., 2009).

558 There are inconsistent data with regards to hypothetical modeled depth of alveolar
559 collapse for marine mammals. Ridgway and Howard (1979) estimated collapse and
560 termination of gas exchange at 70 m, whereas a more recent calculation, which accounted
561 for the dive response, suggested that the alveolar collapse depth might occur at depths
562 greater than 70 m (Fahlman et al., 2006). Pulmonary shunt measurements in the harbor
563 seal and California sea lion infer that complete alveolar collapse may not occur until
564 depths greater than 150 m even at diving lung volumes as low as ~20% of the total lung
565 capacity (Kooyman and Sinnett, 1982). More recent studies, measuring changes in
566 arterial partial pressure of O₂ during a dive, have suggested alveolar collapse for sea lions
567 to be approximately 225 m (McDonald and Ponganis, 2012). It should be noted that the
568 degree of pulmonary filling at the onset of a dive might vary considerably between
569 pinnipeds and cetaceans; consequently, the depth of alveolar collapse may similarly vary
570 considerably within and between species.

571 Recent studies suggest that the structural characteristics of the respiratory tract are
572 important in determining the alveolar collapse depth in marine mammals (Bostrom et al.,
573 2008). With all other variables equal, a more compliant trachea pushes the collapse depth
574 deeper. The reason is that the upper respiratory system compresses concurrently with the
575 lower sections of the respiratory system, reducing the available volume of alveolar air
576 circulation (Bostrom et al., 2008). This may seem counterintuitive as an adaptive
577 advantage, as a faster alveolar collapse could limit N₂ absorption, however there may also
578 be occasions where gas exchange at a deeper depth could actually be beneficial. One such
579 example being that a deeper depth of collapse has been modeled to reflect lower end dive
580 N₂ levels (Fahlman et al. 2009). Regardless, the diving lung volume and the relative size
581 of the conducting airways (trachea and bronchi) and alveolar space are important in
582 determining the collapse depth, as well as behavioral adjustments while the animal is
583 diving. Thus, anatomical differences in tracheal structure may correlate with life history
584 and diving ability.

585 There were important differences in tracheal compliance between shallow diving
586 species of pinniped (harbor and gray seal) versus deeper diving species (elephant seal),
587 lending more evidence to the notion that although diving ability is not entirely dictated by
588 tracheal morphology, anatomy may play an important role in lung function during a dive.
589 As an animal dives, air from collapsing alveoli is pushed up into the trachea, but if the
590 trachea is also compliant, like the alveoli, and they both compress under pressure, this
591 will alter the alveolar collapse depth (Bostrom et al., 2008). For example, the tracheal
592 compliance for compression was significantly higher in the deeper diving elephant seal as
593 compared with the comparatively shallow diving gray and harbor seals. This suggests
594 that the alveolar collapse depth may be deeper in the elephant seal. It is possible that this
595 unexpected result represents tracheal compliance of juvenile animals, or that the *in vivo*
596 compliance, when the trachea is surrounded by tissue, is different. However, theoretical
597 results suggest that a deeper alveolar collapse depth during deep dives reduces end-dive
598 venous N₂ tension (P_{N₂}) and thereby the risk for bubbles to form during ascent (Fahlman
599 et al., 2009).

600 Caution should be taken when correlating dive ability to tracheal morphology, as
601 dive data may show great variability among individuals and may be dependent on
602 particular range, animal behavior, size and age. Simple morphological comparisons
603 between shallow diving species of cetacean and deep diving cetaceans (beaked whales)
604 may not be appropriate, as they have demonstrated vastly different dive behavior. In
605 addition, other anatomical features of the trachea may also affect compliance. For
606 example, connective tissue or the endotracheal venous plexus, which may fill and
607 displace the intra-tracheal air volume (Cozzi et al., 2005; Costidis and Rommel, 2012). It
608 would make sense that associated tissues as well as engorgement would affect
609 compliance in measurements taken *in situ* rather than in excised tracheas. Furthermore,
610 different tissue types may affect compliance values differently. This is a limitation of this
611 study that could be further addressed in future work by repeating measurements *in situ*
612 and carefully examining the associated tissues.

613 Our data suggested that in the pinniped model, deeper divers had a more
614 compliant trachea, while the opposite was true for the cetacean. As far as gas exchange is
615 concerned, this suggests different strategies for pinnipeds as compared with cetaceans.

616 Differences could also be linked to diverging behaviors such as exhalation versus
617 inhalation before a dive (Snyder, 1983). It is possible that this reflects the need to limit N₂
618 absorption in deep-diving cetaceans diving on inspiration as compared with deep-diving
619 pinnipeds that commonly exhale before diving (Snyder, 1983).

620 Unlike most terrestrial species, the marine mammal respiratory system and
621 specifically its behavior under pressure, remains obscured from general knowledge
622 (Bagnoli et al., 2011). Future work is aimed at expanding these data to encompass many
623 more species of marine mammals, with specific concentration on histology. Results may
624 lend more insight into lung mechanics during deep dives. In addition, a wider range of
625 ages will likely reveal more detail pertaining to the degree of tracheal compliance as
626 related to ontogeny of diving in these species. It is hopeful that further investigation into
627 the compliance and microscopic anatomical characteristics of the respiratory tract of
628 these divers will lead to a more comprehensive knowledge of their respiratory system.

629

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641

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778

779 **Figure Legends.**

780

781 **Fig. 1.** Excised harbor porpoise (DO8803) trachea, cut cranially at larynx and caudally
782 after first bifurcation.

783

784 **Fig. 2.** Category 1 tracheal model- complete cartilage rings at the cranial cut (arrow
785 indicates cross sectional area taken) found in both gray and harbor seals.

786

787 **Fig. 3.** Category 2 tracheal model- slip feature present in cartilage ring at the caudal cut
788 (arrow indicates cross sectional area taken).

789

790 **Fig. 4.** Category 3 tracheal model- slip feature and gap present in cartilage rings at the
791 mid cut (arrow indicates cross sectional area taken).

792

793 **Fig. 5.** Category 4 tracheal model- a gap of connective tissue/musculature, i.e.
794 “horseshoe” shaped cartilage rings.

795

796 **Fig. 6.** Category 5 cetacean tracheal model- blue arrow indicates location of the
797 accessory bronchus found in all cetacean species examined (Odontocete and Mysticete).

798 Sub category *A* refers to the compliant/less rigid spiraling cartilage rings found in dolphin
799 species and the harbor porpoise. Sub category *B* refers to the less compliant/rigid
800 spiraling cartilage rings found in beaked whale species.

801

802 **Fig. 7. A** (Deflation) and **B** (Inflation): Elastic properties (compliance) of marine
803 mammal tracheas. The compliance was determined as the slope of the relationship
804 between pressure and volume by fitting the data with a GLM using individual animal as a
805 random factor. Pressure was expressed as the absolute trans-mural pressure and volume
806 as a percent change from the internal volume when the trans-mural pressure was 0 kPa.
807 Numbers above the error bars indicate tracheal category as defined by Table 2. Species
808 *Pv* = *Phoca vitulina*, *Hg*= *Halichoerus grypus*, *Pg* = *Pagophilus groenlandicus*, *Ma*=
809 *Mirounga angustirostris*, *Zc*=*Zalophus californianus*, *Dd* = *Delphinus delphis*, *La* =
810 *Lagenorhynchus acutus*, *Pp* = *Phocoena phocoena*, *Mm*= *Mesoplodon mirus* *Clf*= *Canis*
811 *lupis familiaris*, *Ss*=*Sus scrofa*, *Bp*=*Bos primigenius*. *Hg1* (adult) includes DO7662Hg
812 and DO6322Hg, *Hg2* (juvenile) is DO5257Hg and *Hg3* (YOY) is H-0066Hg (Table 1).
813 *Hg* represents an average of all gray seals analyzed. *Bp1* is an adult cow, *Bp2* a calf and
814 *Bp* the average value.

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816 **Fig. 8.** Lengthwise change in morphology of harbor seal and gray seal tracheas.
817 Horizontal arrows indicate 3 different cross sectional morphologies (cranial, mid and
818 caudal) in the harbor seal trachea and 2 in the gray seal trachea. Vertical arrows indicate
819 two regions of differing rigidity lengthwise for both harbor and gray seals.

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821 **Fig. 9.** California sea lion trachea and representative graphic. Arrows indicate location of
822 trachea and elongated fused bronchial tubes.

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