

1 **Dietary shifts may underpin the recovery of a large carnivore population**

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15 **Abstract**

16 Supporting the recovery of large carnivores is a popular yet challenging endeavour.
17 Estuarine crocodiles in Australia are a large carnivore conservation success story, with the
18 population having extensively recovered from past heavy exploitation. Here, we explored if
19 dietary changes had accompanied this large population recovery by comparing the isotopes
20 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in bones of crocodiles sampled 40 to 55 years ago (small population) with bones
21 from contemporary individuals (large population). We found that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were
22 significantly lower in contemporary crocodiles than in the historical cohort, inferring a shift in
23 prey preference away from marine and terrestrial food webs. We propose that an increase in
24 intraspecific competition within the recovering crocodile population, alongside an increased
25 abundance of feral ungulates occupying the floodplains, may have resulted in the crocodile
26 population shifting to feed predominantly upon terrestrial food sources. The number of feral
27 pigs consumed to sustain and grow crocodile biomass may help suppress pig population growth
28 and increase the flow of terrestrially derived nutrients into aquatic ecosystems. The study
29 highlights the significance of prey availability in contributing to large carnivore population
30 recovery.

31

32 **Keywords:** Stable Isotope Analysis, *Crocodylus porosus*, estuarine crocodile, dietary niche,
33 trophic cascade, apex predator.

34 **Introduction**

35 Across the globe, large-bodied carnivores have been extirpated from much of their
36 original ranges [1]. Most have experienced substantial population declines and range
37 contractions due to conflict with humans, loss of habitat, and reduction in prey availability [2-
38 5]. Large carnivore loss is an issue because there is a growing body of evidence that healthy
39 populations of these animals are necessary to maintain biodiversity and ecosystem function
40 through “top-down” forces, such as predation, intraspecific competition, and trophic cascades;
41 as well as “bottom-up” forces, such as primary production, nutrient dynamics, and energy
42 cycles [1, 6-9]. Moreover, large carnivores tend to be iconic species, and as such, there is a
43 considerable global effort to conserve and recover their populations [10].

44 Nevertheless, numerous cases remain where large carnivores have not recovered
45 despite concerted efforts. In some cases, the reasons for this are precipitated by humans, whilst
46 for others, recovery has been unsuccessful due to increased competition for limited resources
47 as the population grows [11].

48 The estuarine crocodile (*Crocodylus porosus*) is Australia’s large-carnivore restoration
49 success story. Unregulated hunting in the mid-20th century drove the species to the brink of
50 extinction, with only a few thousand individuals existing in the wild by the 1970s [12].
51 Regulation in the global trade of crocodile skins and national protection has allowed the
52 Australian estuarine crocodile population to recover to pre-exploitation levels with little
53 additional intervention [13]. The recovery rates in crocodile populations around Australia have
54 been mixed. Some regions, like the Northern Territory (NT), have seen rapid recoveries [14],
55 whilst populations in other states like Queensland (QLD) and Western Australia (WA) have
56 shown limited recovery over the same period [15, 16]. Crocodile population abundance is
57 strongly linked with habitat type, catchment areas, and climate seasonality [17]. Nonetheless,
58 the quality and quantity of the diet presumably play a significant role in the rate of recovery.
59 Understanding the role prey has played in allowing the estuarine crocodile to achieve this
60 population growth may assist us to better understand the relationship between prey competition
61 and large carnivore population recovery.

62 Estuarine crocodiles are a large-bodied (<500 kg adult body wt.) generalist carnivore,
63 and although they lack the high metabolic requirements of endothermic carnivores, an estuarine
64 crocodile must feed upon the equivalent of ~ 4% of its body weight every week to maintain

65 body mass [18]. Therefore, a 1,000 kg crocodile would need to eat ~40 kg of protein per week
66 for maintenance, growth, and reproduction [18]. Crocodiles' biomass in the NT rivers has
67 increased over the past 50 years, from virtually zero in the early 1970s to almost 500 kg of
68 crocodile per kilometre of river in 2020 [14]. This level of prey protein needs to have been
69 acquired somewhere.

70 Here, we assessed if the dietary isotopic niche of the estuarine crocodile population has
71 changed from a time when population densities were very low (< a few thousand) to the current
72 population size (>100,000 adults) [14]. We measured the ratio of stable isotopes ($\delta^{13}\text{C}$ and
73 $\delta^{15}\text{N}$) in bone from museum specimens collected 40 to 55 years ago and compared this with
74 contemporary individuals and potential prey items. Isotopic variations in bones have been used
75 to study animal diets [19], including the balance between freshwater, marine, and terrestrial
76 food sources [20, 21]. We hypothesised that as populations grew, the increased competition
77 between crocodiles may have resulted in a greater reliance upon readily available alternative
78 prey sources.

79

80 **Methods**

81 *Sample collection*

82 Estuarine crocodile bone samples were collected from north-western areas of the
83 Northern Territory (Australia) between Darwin Harbour and the East Alligator River (~300km
84 apart). Historical bone samples were collected from the Museum and Art Gallery of the
85 Northern Territory (MAGNT, Darwin, NT, Australia). Those bones were from crocodiles
86 caught and killed between 1968 and 1986 from various parts of the Northern Territory (n =
87 22). Some crocodiles were very large and were likely older than 50 years of age upon death
88 [22]. Sampled crocodile bones were from animals with total body lengths ranging between 120
89 and 513 cm. The sampled bones were not treated or preserved and cleaned by macerating the
90 bones in water. Samples were taken from the left front leg humerus. First, an ~ 0.2 mm outer
91 bone layer was scraped away using a scalpel, and then ~ 0.2 g of bone was scraped out and
92 placed in a sample vial for the analysis.

93 The contemporary cohort of bone samples was collected from crocodiles trapped and
94 removed from around Darwin as part of the crocodile management program in 2016 [23]. These

95 crocodiles ranged from 115 to 330 cm total body length (n = 24). A small section from the left
96 humerus bone was removed and prepared as the museum specimens. The bones were not
97 acidified before analysis as carbonate removal has been shown to have minimal effect upon
98 reptile bone $\delta^{13}\text{C}$, but instead, a correction factor was used ($\times 1.2 + 2.1$; [24]). The mean C/N
99 ratios for the bone samples was 3.4 ± 0.2 S.D. (range = 3.1 to 4.3), and only 3 out of 48 samples
100 had C/N ratios above a proposed threshold of 3.6 for intact collagen [25].

101 Bone samples from both cohorts were freeze-dried at -40°C (Dynamac Freeze Dryer)
102 for 48h and then homogenised to a fine powder using an electric ball-mill grinder (RETSCH
103 Mixer Mill MM400). A small sub-sample from each was weighed (0.8 – 1.0 mg) into tin
104 capsules and analysed for stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) at the Stable Isotope Core Laboratory
105 at Washington State University (WA, USA). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from those bone
106 samples were corrected using diet-tissue discrimination factors (1.4 ‰ and 3.0 ‰, respectively)
107 [24, 26, 27]. The carbon isotope values were adjusted for the Suess effect by applying a
108 correction factor to the “historic” cohort based on the atmospheric CO_2 [28] for each
109 crocodile’s year of death.

110 Body size is known to influence $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in crocodylians. Our
111 “contemporary” cohort had a reduced range of body sizes compared to the “historic” cohort.
112 To provide a more comparative range of body sizes, we sourced additional stable isotope values
113 from Adame et al. [26]. These estuarine crocodiles (n = 41, size range = 83.5 to 420 cm, details
114 in Table S1 of Supplementary Materials) were captured from the same region between 2012
115 and 2014 (details on scute tissue isotopic discrimination in [26]). We first tested if it was a
116 valid assumption to combine these two groups of crocodiles, whose collagen had been sampled
117 from different tissues (bone and scute). The first method performed was an analysis of
118 covariance (ANCOVA) in R [29]. The models assessed the dependent variables $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
119 against the interaction between the factors “group” and “total body length” (covariate). The
120 second method was a Bayesian framework to assess the stable isotope niche region and
121 pairwise niche overlap. Isotopic values were plotted using the package nicheROVER [30] in R
122 (Supplementary Materials, Fig. S1). Given the lack of statistical difference (Supplementary
123 Materials, Table S2; Fig. S1), the stable isotope data from all contemporary crocodiles were
124 combined (n = 65) for comparison with the “historic” cohort.

125 ANCOVA was used to test for differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between the historic and
126 contemporary samples across the size ranges, with the stable isotope values as dependent

127 variables and the interaction between cohort and total body length (covariate) as factors (Table
128 1). Isotopic niche widths and overlap were calculated using the package SIBER (Stable Isotope
129 Bayesian Ellipses in R) [31] in R (Fig. S2, Supplementary Materials).

130

131 **Results and Discussion**

132 Our key finding was that contemporary estuarine crocodiles have significantly lower
133 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than those sampled 40 to 55 years ago (Fig. 1). Collagen has a slow tissue
134 turnover rate, and these isotopic values reflect the individual's broad diet over several years
135 [24, 32]. Estuarine crocodiles are highly mobile individuals and regularly travel over 400 km
136 in a few weeks, connecting populations across broad regions [33, 34]. In the study region,
137 individuals regularly travel between coastal, estuarine, and freshwater environments [35].
138 Therefore, our SIA results present a broad picture of the isotopic landscape of the estuarine
139 crocodile population over two different periods. We found that individuals became more ^{13}C -
140 and ^{15}N -enriched as they grew, and this relationship was similar between "historical" and
141 "contemporary" cohorts (Fig. 1, Table 1). Ontogenetic changes in isotopic values are common
142 in crocodylians [27, 36-39] and other large carnivores that show indeterminate growth [40] and
143 are suggested to reflect changes in diet and metabolism as the animal grows [38, 41]. This
144 evidence suggests that the $\delta^{15}\text{N}$ of estuarine crocodiles scaled similarly with body size class
145 under high (contemporary) and low (historic) population densities. However, the baseline shifts
146 in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ suggest that the contemporary diets of crocodiles include a significantly
147 greater contribution from terrestrial sources (Fig. 2).

148 The observed decreases in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of contemporary crocodiles (Fig.
149 1) are inconsistent with potential isotopic shifts in terrestrial and floodplain basal resources
150 (e.g., invasion of C4 grasses [42]). Similarly, they are inconsistent with a shift in $\delta^{15}\text{N}$ baseline
151 values from rising point-source nitrogen loads in the region [43, 44]. We argue that the
152 observed differences in isotope values between historical and contemporary crocodiles reflect
153 a major shift in prey from predominantly riverine-marine to predominantly terrestrial food
154 resources (Fig. 2). As a population, historic crocodiles appear to have fed upon a broader
155 diversity of prey than contemporary individuals. Crocodiles are generalised feeders and eat
156 whatever prey is available to them. Whilst this may reflect a decreased availability of marine-

157 based and other prey, we propose that the dietary shift was more likely due to increased
158 availability of a single terrestrial prey (e.g., feral pigs, *Sus scrofa*) [18, 26, 45].

159 Over the last 50 years, management interventions have significantly reduced the local
160 population of Asian water buffalo (*Bubalus bubalis*; 5.6 to < 0.1 buffalo per km²) [46]. There
161 is sparse survey data for feral pig abundance over a comparative period. Still, anecdotal
162 observations suggest feral pigs are at far higher densities than before buffalo eradication [47],
163 and empirical studies demonstrate that buffalo removal results in growth in local feral pig
164 abundance [48]. Buffalo are large herding animals and could be eaten only by very large
165 crocodiles, whereas the reduced size of feral pigs and their wallowing behaviour near
166 waterbodies make them ideal prey across a broader size of estuarine crocodiles. Pigs are prolific
167 breeders compared to buffalo and native animals like kangaroos, providing a quickly
168 replenished and highly nutritious food supply for crocodiles.

169 If feral pigs are indeed the primary prey source maintaining and growing this large
170 estuarine crocodile population, then the increase in crocodile biomass would be accompanied
171 by terrestrial sourced nutrients being deposited into the freshwater environment. This is
172 supported by stable isotope values reflecting high terrestrial inputs (specifically feral pigs),
173 even for crocodiles with a body size too small to capture live pigs [26]. Our results reveal that
174 this terrestrial contribution was not as high 40 to 50 years ago, supporting the hypothesis that
175 crocodylians create habitat linkages and nutrient fluxes between terrestrial and aquatic food
176 chains [49-51]. Although feral pig abundance has been reported to have increased in the past
177 40 years [47], it is plausible that crocodiles may suppress feral pig populations. Therefore,
178 estuarine crocodiles may mitigate feral pigs' adverse ecological and agricultural impacts [52].

179 In conclusion, the extensive recovery of the estuarine crocodile population in the study
180 area appears to have been supported by access to an increased abundance of invasive terrestrial
181 prey (i.e., feral pigs). The differences in population recovery rates in other parts of Australia
182 [15, 16] may be attributed to lower terrestrial prey densities, where riparian habitats do not
183 support such large feral pig populations. As the crocodile population grew, inter-specific
184 competition caused individuals to move back into inland floodplains, where they were
185 previously extirpated by hunters [12, 14, 53]. Before the arrival of large exotic terrestrial
186 vertebrates, crocodiles likely fed upon native terrestrial herbivores. However, these do not
187 occur in similar densities within floodplains nor inhabit swamp areas that would make them as
188 accessible to crocodiles. Further research is required to better understand the link between prey

189 availability, bioenergetics, and crocodile population growth. Crocodile prey choice may be
190 enriching oligotrophic freshwater systems with terrestrially derived nutrients, like what has
191 been observed for large vertebrates in other ecosystems [54]. What this means for the ecology
192 of Australian river-floodplain ecosystems remains unknown and requires future investigation.

193

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203

204 **Ethical Statement**

205 The historical cohort of estuarine crocodile bones used in the present study was sampled
206 from museum specimens (Museum and Art Gallery of the Northern Territory; MAGNT). The
207 contemporary cohort of bone samples was donated by the Department of Environment, Parks
208 and Water Security (DEPWS) from crocodiles removed for human safety [23, 55]. The
209 sampling of crocodile scute was conducted under the Griffith University animal's ethics
210 protocol approved by the Animal Ethics Committee (ENV/08/11/AEC) and Kakadu National
211 Park permit guidelines (RK 786) [25].

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374 **Tables:**

375 **Table 1:** Summary of performed statistics tests (ANCOVA).

Model coefficients	<i>Dependent variable: $\delta^{15}N$</i>				<i>Dependent variable: $\delta^{13}C$</i>			
	Sum Sq	Df ^d	F-value	<i>P</i> ^e	Sum Sq	Df ^d	F-value	<i>P</i> ^e
<i>Intercept</i> ^a	81.1	1	85.496	< 0.001	4728.2	1	1044.606	< 0.001
<i>Cohort</i> ^b	5.6	1	5.937	0.017	77.2	1	17.054	< 0.001
<i>Body length</i> ^c	16.7	1	17.567	< 0.001	105.7	1	23.355	< 0.001
<i>Cohort:body length</i>	0.7	1	0.782	0.379	5.3	1	0.167	0.283

^a *Intercept* represents a crocodile from the “contemporary” cohort of total body length = 83.5cm.
^b *Cohorts* = “Historic” and “Contemporary”. ^d *Df* = degrees of freedom.
^c *Body length* = Total body length (cm). ^e *P* = significant at <0.05.

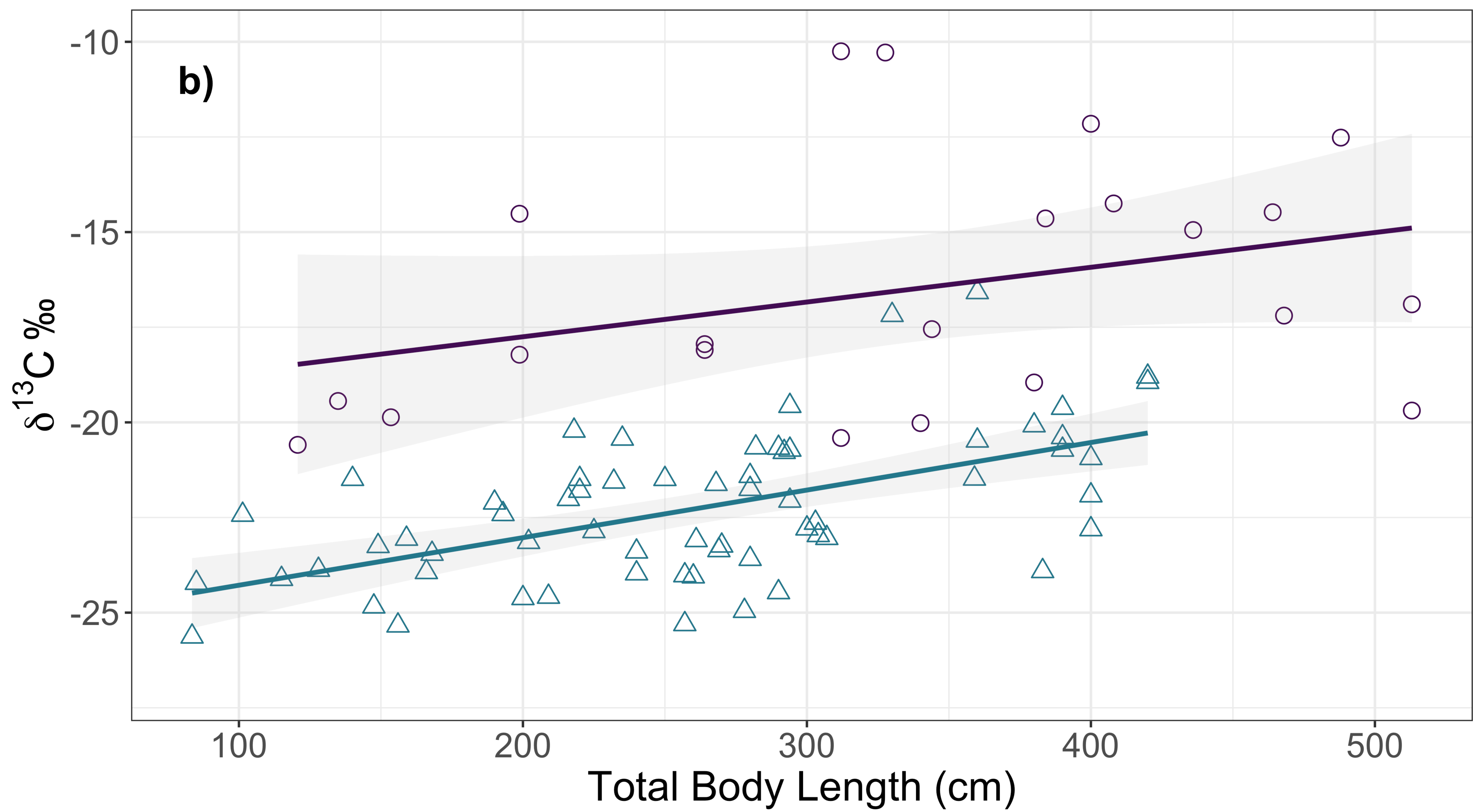
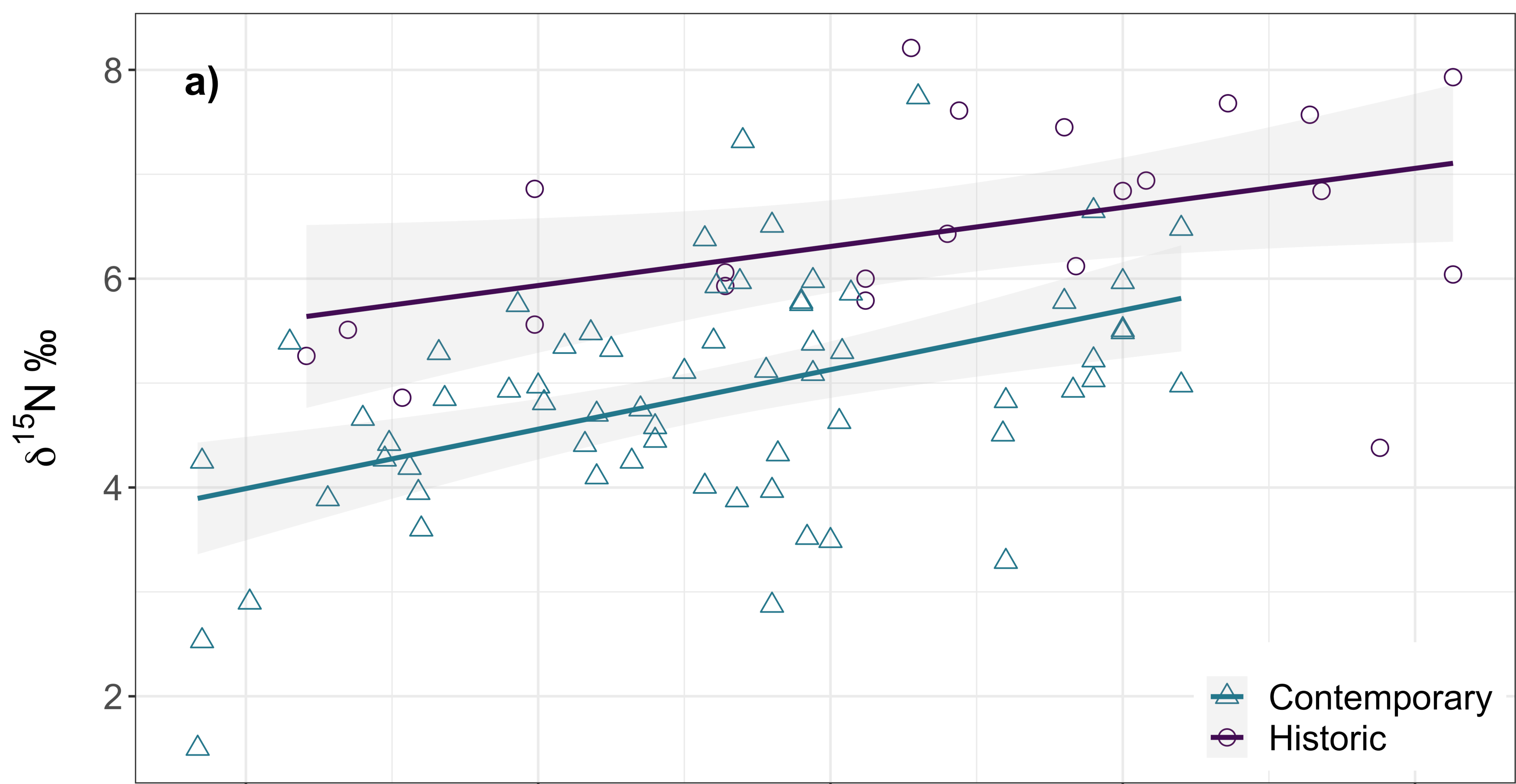
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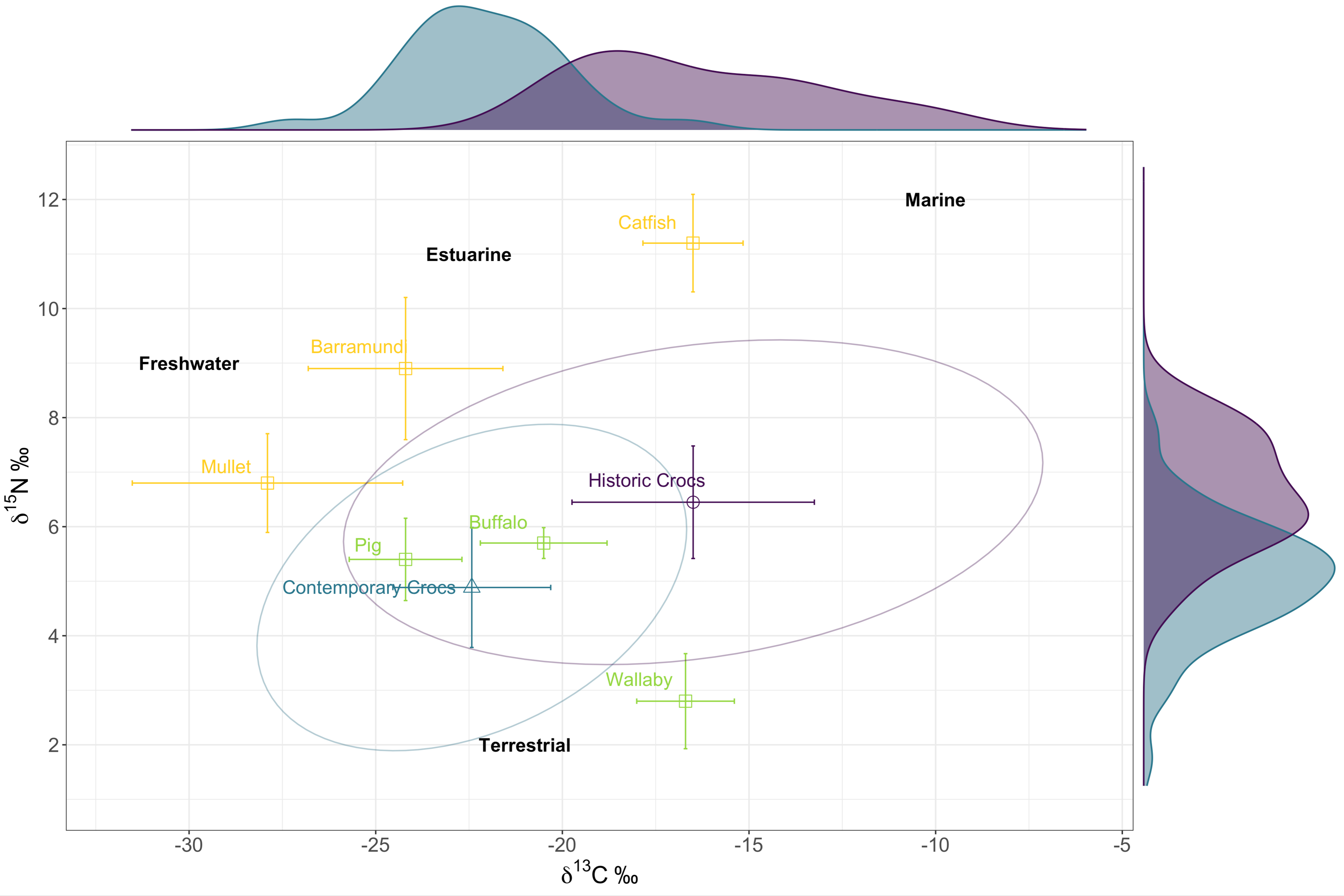
377 **Figure legends**

378 **Figure 1:** Relationship between (a) $\delta^{15}\text{N}$ (‰) and (b) $\delta^{13}\text{C}$ (‰) and total body length of
379 estuarine crocodiles (*Crocodylus porosus*). Purple circles denote specimens collected between
380 1968 and 1986 (n = 22), and blue triangles represent samples collected between 2012 and 2016
381 (n = 65). Shaded area denotes 95% confidence intervals.

382

383 **Figure 2:** Stable isotope biplot of estuarine crocodiles (*Crocodylus porosus*). Purple denotes
384 samples collected between 1968 and 1986 (n = 22) and blue represents samples collected
385 between 2012 and 2016 (n = 65). Marginal density plots represent the data distribution and
386 ellipses show the standard Bayesian areas corrected for the sample size for each cohort.
387 Standardised ellipses area and Layman metrics are shown in Fig. S2 (Supplementary
388 Materials). The isotopic ratios for wallaby *Macropus agilis* (n = 19), buffalo *B. bunalis* (n = 8),
389 pig *S. scrofa* (n = 57), mullet *Liza ordensis* (n = 82), barramundi *Lates calcarifer* (n = 170),
390 and catfish *Netuma thalassina* (n = 5) were sourced from [25]. Data points are mean \pm s.d.





SUPPLEMENTARY MATERIALS

Dietary shifts may underpin the recovery of a large carnivore population

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Stuart E. Bunn, Hamish A. Campbell

Methodology

Stable Isotope Analysis

The crocodile bone samples used in the present study were analysed for stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) at the Stable Isotope Core Laboratory at Washington State University (WA, USA). The samples for carbon and nitrogen isotopic analysis were converted to N_2 and CO_2 with an elemental analyser (ECS 4010, Costech Analytical, Valencia, CA); these two gases were separated with a 3m GC column and analysed with a continuous flow isotope ratio mass spectrometer (Delta PlusKP, Thermofmnigan, Bremen) [1, 2]. Isotopic reference materials were interspersed with samples for calibration. Contribution of ^{17}O was corrected by the IPMS software using the Santrock correction [3].

Carbon isotopic results were reported in ‰ relative to VPDB (Vienna Peedee belemnite) by assigning a value of +1.95‰ to NBS 19 CaCO_3 [4]. Current NIST calibration of VPDB uses BS 19 and L-SVEC as anchor points. The carbon isotopic compositions of internationally distributed isotopic reference materials, had they been analysed at the Stable Isotope Core Laboratory with the study's samples, were [5]:

NBS 19	CaCO_3	+1.95 (exactly)
NBS 18	CaCO_3	-5.01
IAEA-CO-1	CaCO_3	+2.49
L-SVEC	Li_2CO_3	-46.6
RM 8542	Sucrose	-10.45
USGS24	Graphite	-16.05
NBS 22	oil	-30.03
USGS40	glutamic acid	-26.39
USGS41	glutamic acid	+37.63
IAEA-CO-9	BaCO_3	-47.32 (exactly)

The 2-sigma uncertainty of carbon isotopic results is 0.5‰, unless otherwise indicated. The study bone samples were normalized using two of internal standards. These standards were previously calibrated to NBS 19, RM 8542, and IAEA-CO-9, as defined above.

24 **Tables:**

25 **Table S1:** Summary of crocodile data used in the present study.

<i>Cohort/Group</i>	<i>n</i>	<i>Mean total body length (cm)</i>	<i>Minimum total body length (cm)</i>	<i>Maximum total body length (cm)</i>	<i>Tissue sampled</i>
Historic	22	337.5	120.7	513.0	Bone
Contemporary 1	24	212.1	115.0	330.0	Bone
Contemporary 2 *	41	283.8	83.5	420.0	Scute

* Sourced from Adame et al., 2018 [6].

26

27 **Table S2:** Summary statistics of ANCOVA performed in R to test for differences in isotopic
 28 ratios between 2 groups of modern crocodiles: “Contemporary 1” (Darwin Harbour crocodiles) and
 29 “Contemporary 2” (sourced from Adame et al. [6]). Body length = crocodile total body length (cm).

Model coefficients	<i>Dependent variable: $\delta^{15}N$</i>				<i>Dependent variable: $\delta^{13}C$</i>			
	Sum Sq	Df^b	F	P^c	Sum Sq	Df^b	F	P^c
<i>Intercept^a</i>	12.5	1	17.202	< 0.001	1164.46	1	403.483	< 0.001
<i>Group</i>	0.003	1	0.004	0.952	0.00	1	0.001	0.975
<i>Body length</i>	12.1	1	16.761	< 0.001	10.97	1	3.799	0.056
<i>Group:body length</i>	1.7	1	2.320	0.133	0.35	1	0.120	0.730

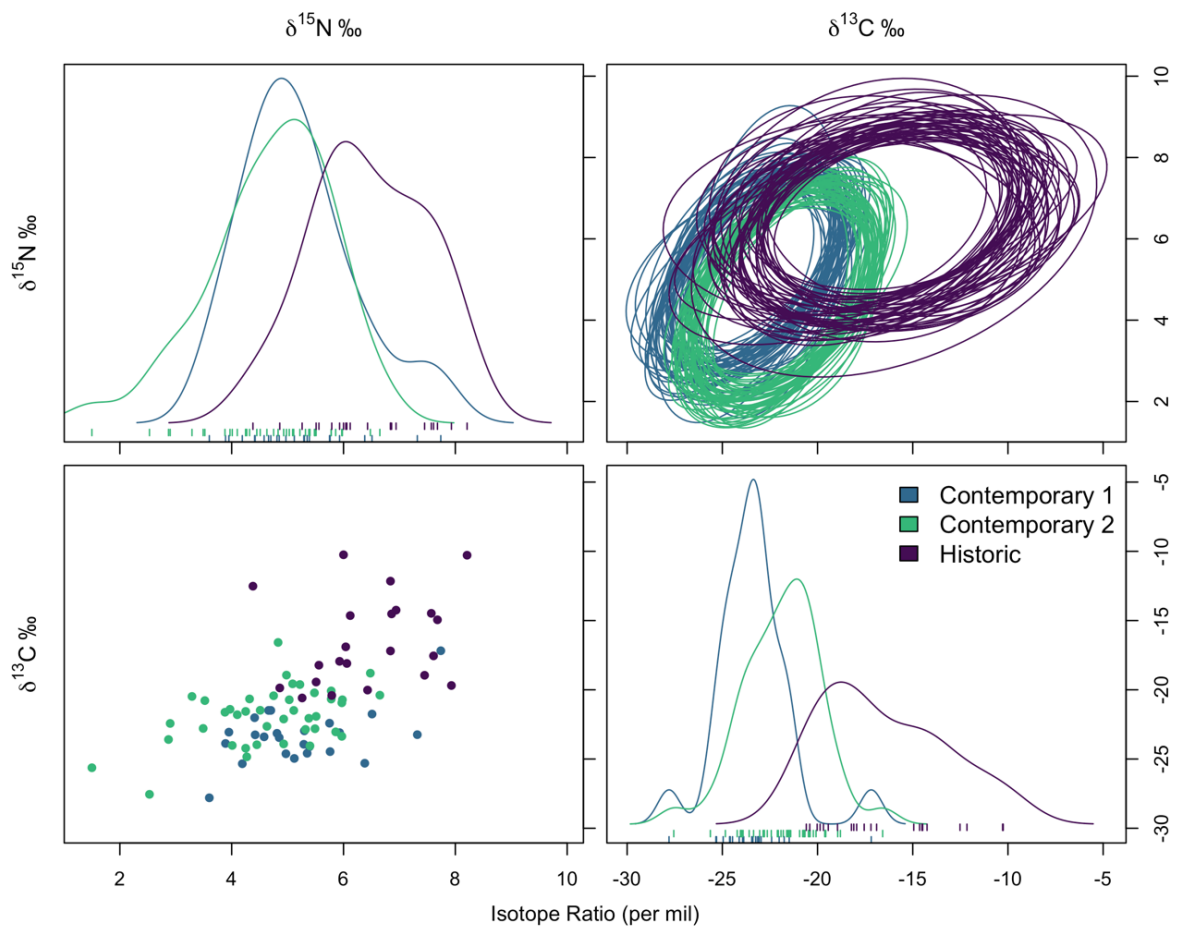
^a Intercept represents a crocodile from the “contemporary1” group of total body length = 115.0 cm.

^b Df = degrees of freedom.

^c P = significant at <0.05.

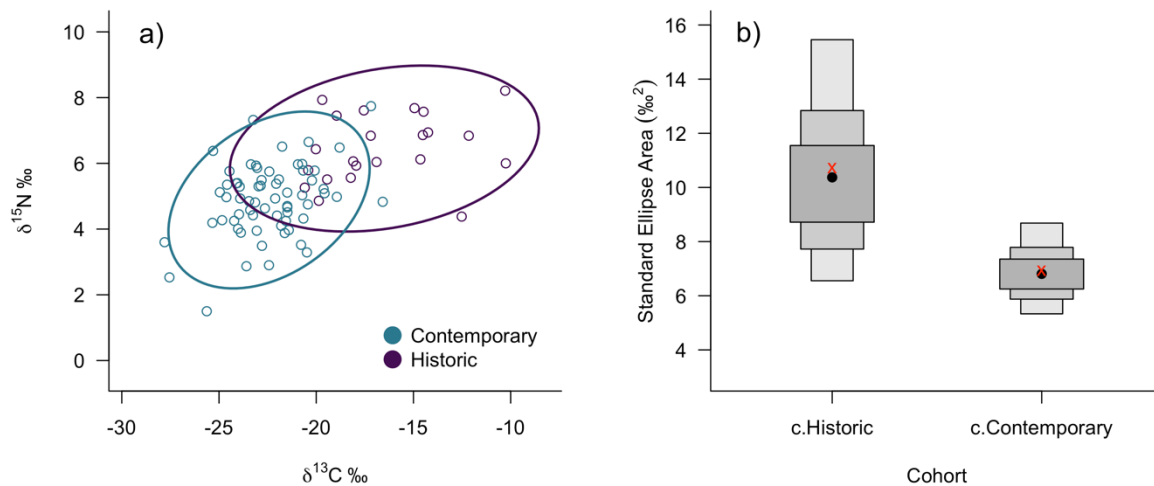
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31 **Figures:**



32

33 **Figure S1:** Stable isotope biplots of estuarine crocodiles (*Crocodylus porosus*) from “historic”
34 cohort (purple, n = 22) and each of the two groups (“contemporary 1” in blue, n = 24; and
35 “contemporary 2” sourced from Adame et al. [6] in green, n = 41) that later composed the
36 “contemporary”. Panels a) and d) show the density distributions, b) represents the niche plot,
37 and c) shows the raw data for each pairwise combination of isotope data generated by the
38 Bayesian analysis using the package nicheROVER [7] in R [8].



39

40 **Figure S2:** a) Isotope biplot for “historic” and “contemporary” experimental cohorts of
 41 estuarine crocodiles (*Crocodylus porosus*), with standard ellipse areas corrected for sample
 42 size (SEAc). The overlap of the maximum likelihood of the fitted standard ellipses is 53.5%.
 43 b) Standardised ellipses area for each experimental cohort. Red crosses indicate the point
 44 estimates of the Layman metrics for each cohort. Plots were generated using the package
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46

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