Dear Editor,

First of all, we would like to thank the reviewers for the time they spent reviewing our paper and for their constructive comments and suggestions. Comments that will lead to a substantial change in the manuscript are discussed below and all changes to the original text (including minor corrections). Please find below the response to the anonymous referee #1. Most of the corrections requested by the reviewer have taken into consideration and the changes made to the manuscript follow the recommendations of the reviewer. Below you will find the authors' response to reviewer #1 questions and comments.

We hope that these corrections will meet the requirements of the reviewer and editor.

Sincerely yours,

Nicolas Séon for all the authors.

Response to Anonymous Referee #1

General comments

This paper is welcome because it addresses a technique that has been used to estimate body temperatures of extinct animals from measurements of oxygen isotope in fossil bone and teeth. It recognises that temperature is not uniform throughout the body of aquatic vertebrates (and also terrestrial ones) and shows that isotope analysis of regional bones result in temperatures that are reasonably expected to occur in them. Two groups of extant aquatic species are chosen to represent marine mammals that show regional hypothermy in the limbs and endothermic fish that show regional endothermy by adaptively warming the red muscle, eyes and visceral organs. The results support the use of the method and are strikingly illustrated. The isotope analysis is done according to methods established in the authors' world-class laboratory. The sample sizes are adequate, carefully analysed statistically and interpreted thoughtfully. The writing is generally clear, well organised and extremely well referenced with relevant citations. There are only a few unusual expressions and typographical mistakes that may be rectified by proofreading by a native English writer.

The major problems of the paper involve (1) terminology and (2) use of references. There are suggestions below about terms that may better describe regional patterns of temperature throughout the body. Unfortunately, there appear to be several references that are not used appropriately. Classical references are fine for original ground-breaking research, but more recent papers are best for citing, because presumably they contain the foundational papers as well as recent developments. Some examples are given below.

Specific comments

30-37 Some of the citations here and in the rest of the introduction are not very useful or even appropriate. For example, in referencing ectotherms, Rodbard (1953) is a short popular article on 'warm-bloodedness', nearly 70 years old and poorly referenced. The Hight and Lowe (2007) one is more recent, but as it apparently speculates on whether elevated Tb in leopard sharks aggregating in shallow embayments is behavioural thermoregulation. Crawshaw and Hammel (1971) is about Antarctic fish. Norbert Smith's (1979) review of Tb in crocodilians has been long ago superseded (by, for example, G. Grigg and D. Kirshner 2015: Biology and Evolution of Crocodylians). Sherwin (2010) is a manual for animal husbandry and Allali et al. (2013) apparently concerns circadian clocks in camels, both not the

best references for thermal physiology. The introduction would be better if no references were given for generally accepted facts than old specific references that provide little support for the points that the authors are making. Alternatively, more recent reviews on ectothermy and endothermy could be used, for example the Oxford Scholarship 'Ecological and Environmental Physiology Series'. The authors should carefully review the appropriateness and utility of all references in the paper, not only those in the introduction.

As recommended, we have removed inappropriate references or references that are too old and associated with generally accepted facts (Rodbard, 1953; Crawshaw and Hammel, 1971; Smith, 1979; Hight and Lowe, 2007; Schmidt-Nielsen et al., 1966; Bennett et al., 1993; Sherwin, 2010; Allali et al., 2013; Nicol, 2017). We now cite more recent articles in the introduction:

Line 32: Carey et al., 1990

Line 32: McMaster and Downs, 2013

60-63 (also lines 234-235) Here it is essential to provide a citation or two for actual data for regional heterothermy in extant animals.

The following citations were added to line 63: Tomilin, 1950; Carey and Lawson, 1973; Carey, 1982

For lines 234-235,

"Detailed intraskeletal $\delta^{18}O_p$ mapping allows to document regional heterothermies in marine vertebrates. Calculated $\delta^{18}O_p$ derived temperatures are consistent with temperature heterogeneities recorded by classical methods."

Was changed into

"Detailed intraskeletal $\delta^{18}O_p$ mapping allows regional heterothermies in marine vertebrates to be documented. Calculated $\delta^{18}O_p$-derived temperatures are consistent with temperature heterogeneities recorded by classical methods (Tomilin, 1950; Carey, 1982; Graham and Dickson, 2001)" at line 251.

71-74 The groups of animals in the study are classified as either 'homeothermic endotherms' or 'poikilothermic endotherms'. Many would consider the first case misleading, because marine mammals are not wholly homeothermic, but regionally hypothermic, as the authors demonstrate. Also the second case of warm organs in fish seems like a contradiction in terms. 'Poikilotherm' meaning variable temperature is rarely used lately, and for good reason. For example, normal ectothermic fish living in the deep sea cannot be called poikilotherms because their body temperatures are constant. What is important here is that the mammals studied here have cool limbs and the fish have some warm organs. Therefore I would recommend redefining the groups in your study as two types of 'regional heterotherms'. 'Regional' is important to distinguish it from 'temporal heterotherms' which are endotherms that enter hibernation or torpor. In this study, the mammals allow the appendages to cool and the fish warm certain organs. This is simply defined and does not need special titles. You might label sections 4.2.1 'Marine mammals' and 4.2.2 'Endothermic fish'. In any case, avoid the term 'poikilotherm' and its variants.

As requested by the reviewer, the two groups initially named "homeothermic endotherms" and "poikilothermic endotherms" were respectively changed into "Marine mammals" and "Endothermic fish". The term "poikilotherm" is avoided in the rest of the text.

According to the reviewer's comment, the subtitle "4.2. $\delta^{18}O_p$ variations linked to regional heterothermies in homeothermic and poikilothermic endotherms" was replaced by "4.2. $\delta^{18}O_p$ variations linked to regional heterothermies".

144-9 Please explain in greater detail the possible reasons for the differences in predicted temperatures in teeth of fishes and mammals. One would expect that tuna teeth would form under cold conditions, because tuna respire by ram-ventilation in which the seawater constantly flows in and around their teeth. In contrast, marine mammals have closed mouths. Yet the temperatures derived from isotopes are the opposite to expectations.

The teeth of fish and mammals (especially cetaceans) record different stages of the animal's life. Although tunas breathe by ram-ventilation and therefore in cold conditions, the high efficiency of the *rete mirabile* present near the gills allows a cranial body temperature to be maintained higher than that of the surrounding water (Graham and Dickson, 2001). This explains why we get high mineralization temperature estimates from isotopes. In order to be more precise, we have added the following text to lines 212-215:

"The $\delta^{18}O_p$ values of the teeth indicate that they mineralized at a significantly higher temperature than the fins and the posterior part of the axial skeleton. This is the result of the high efficiency of the *rete mirabile* present near the gills which limits the heat losses associated with ram ventilation (Graham and Dickson, 2001). "

For dolphins, the isotopic composition of the teeth records all the animal life from its development *in utero* to its death. The main part of the tooth is mineralized during the early stages of the animal's development. We have added this following text to lines 153 - 159:

"Indeed, young dolphins breast-feed during the first 12 to 18 months of their life and ingest mother milk that is ^{18}O-enriched compared to environmental water (Wright and Schwarcz, 1998). Furthermore, odontocetes possess only one generation of teeth that grow a little bit each year until they reach their adult size. It is thus expected that the oxygen isotope composition of teeth is influenced by the ^{18}O-enriched mother milk unlike bones which are continuously remodelled, thus erasing the isotopic signal of the early animal's development. Due to the small size of the available teeth, we have sampled and analysed the whole teeth; the $\delta^{18}O_p$ values integrate the early stages of the animal's development during which it was breast-feed."

Also, please give a little more detail about remodelling of fish bone. I was not aware that this occurs (=do not know the citations), so please point out how it differs from the amniote paradigm involving secondary osteons.

Bone remodelling was documented in tunas and swordfish (Meunier and Huysseune, 1992; Atkins et al., 2014). What is striking is that bone remodelling occurs in cellular (tunas) and acellular (swordfish rostrum) bones but secondary osteons are present in both. Bone remodelling in fish is still poorly known and needs to be better evaluated and compared to that of mammals (Witten and Huysseune, 2009), which is out of the scope of this paper. From an isotopic point of view, the two processes lead to the incorporation of renewed oxygen. We have therefore added the reference (Meunier and Huysseune, 1992) so the readers can refer to it, but did not add further discussion in the text.

225-231 This final section seems to undermine the whole approach by suggesting the basis for the technique is not up to date.

The first sentence of the final section is indeed misleading and we have changed it to:

"Intra-skeletal variability resulting from regional heterothermies can lead to overestimate seawater temperature or underestimate $\delta^{18}O_{sw}$ values when applying existing fractionation equations that have been established assuming an isotopic homogeneity of the skeleton, to isolated skeletal elements (Fig. 3A, B)."

Please clarify this section by evaluating how much the equations differ and what is the magnitude of the difference.

The sentences of this section (Lines 237 to 244) already document the magnitude of the difference in both marine temperature and $\delta^{18}O_{sw}$ estimates.

Would the study be compromised in its conclusion?

Our study documents and interprets $\delta^{18}O_p$ differences in terms of regional heterothermies, so that our results and their interpretation are not compromised by pre-existing fractionation equations established for cetaceans. Nevertheless, our study draws attention to previous palaeoceanographical studies which did not consider intra-skeletal $\delta^{18}O_p$ variability caused by regional heterothermies. As a result, their palaeoenvironmental reconstructions (seawater temperature and $\delta^{18}O_{sw}$ values) might be inaccurate depending on which skeletal element they analysed and interpreted.

Technical corrections

35 Delete 'and thermolysis'. This word means breaking down tissue or cells with heat. It is not appropriate.

"And thermolysis" was removed.

38 Instead of 'non-normothermic conditions is extremely…', I suggest 'at ambient temperatures below the thermal-neutral zone can be…'.

"non-normothermic conditions is extremely…" was changed by "at ambient temperatures below the thermo-neutral zone can be extremely…"

44 Instead of 'thermometer reading' use 'thermometry'.

"Thermometer reading" was changed by "thermometry"

52 I think that both food and water would be taken in by both groups.

Following the reviewer's recommendation, the sentence: "Indeed, vertebrate $\delta^{18}O_p$ values reflect both the oxygen isotope composition of their body water ($\delta^{18}O_{bw}$), stemming from ingested water in osteichthyans or from food for marine mammals (Telfer et al.,1970; Hui, 1981; Ortiz, 2001; Rosen and Worthy, 2018)…"

Was replaced by:

"Indeed, vertebrate $\delta^{18}O_p$ values reflect both the oxygen isotope composition of their body water ($\delta^{18}O_{bw}$), originating from ingested water, food and inhaled dioxygen (Telfer et al., 1970; Hui, 1981; Ortiz, 2001; Rosen and Worthy, 2018)…"

74 See notes above about the use of 'poikilothermic'.

We have replaced the term "poikilothermic" by "endothermic fish"

141 Instead of 'results' use 'result'.

The correction has been made.

154 Use 'prey', which is the pleural.

The correction has been made.

156 Can you provide some numbers for the bone variability to compare with 0.4 for water?

Inter-bone $\delta^{18}O_p$ variability for each group of studied organisms (dolphins and endothermic fish) were added to lines 162-163.

"Nevertheless, the seasonal changes in $\delta^{18}O_{sw}$ of the water masses in which the sampled organisms fed are relatively small (± 0.4 ‰; supplementary material, table S5) and cannot fully explain the inter-bone $\delta^{18}O_p$ variability reported herein in dolphins and osteichthyans."

Was changed into

"Nevertheless, the seasonal changes in $\delta^{18}O_{sw}$ of the water masses in which the sampled marine vertebrates fed are relatively small (± 0.4 ‰; supplementary material, table S5) and cannot fully explain the inter-bone $\delta^{18}O_p$ variability reported herein in dolphins and osteichthyans (respectively 1.5 ‰ and 2.5 ‰)."

155 Use 'appendicular skeleton', not 'these skeletal regions'.

As recommended by the reviewer, we have replaced the expression "these skeletal regions" by "appendicular skeleton".

171 Use 'little', not 'few'.

"Few" was changed by "little".

177 This equation refers to temperature, but the preceding sentence refers to temperature differences. It is confusing.

In order to clarify, we replaced:

"Assuming only slight seasonal changes in marine mammal $\delta^{18}O_{bw}$ values throughout the seasons and considering the $\delta^{18}O_p$ values obtained (Table 1), our new isotope data can be used to estimate the temperature differences between limb and trunk in the sampled dolphins using the phosphate-water temperature scale published by Lécuyer et al. (2013): $T°C = 117.4 – 4.5 (\delta^{18}O_p – \delta^{18}O_{bw})$ (Eq.1)

The obtained differences in mineralization temperature are of 2 ± 0.5 °C for *D. delphis delphis*, and 1 ± 0.5 °C for *C. commersonii kerguelensis*. "

By

"The temperature differences between limb and trunk in the sampled dolphins can be calculated using differences in their $\delta^{18}O_p$ values and the phosphate-water temperature scale published by Lécuyer et al. (2013):

$T°C = 117.4 – 4.5 (\delta^{18}O_p – \delta^{18}O_{bw})$ (Eq.1)

Assuming only slight seasonal changes in marine mammal $\delta^{18}O_{bw}$ we calculated differences in mineralization temperature between limbs and trunk of 2 ± 0.5 °C for *D. delphis delphis*, and 1 ± 0.5 °C for *C. commersonii kerguelensis*."

188 Carey et al. (1984) indicate that heat in tunas is also produced in the viscera.

The reference (Carey et al. 1984) was added to the text.

201 Use 'have' not 'has'.

The correction has been made.

210 and 215 Here and earlier reference is made to thermal imaging. I would remove this, because it is irrelevant in these cases.

The text part on thermal imagery was removed.

449 Note subscript and superscript errors.

The corrections have been made.

453 It is hard to see the stars. The red around the eyes is misleading, especially since the eye is warm, but apparently not measured in this study. If the ring could be white, it would not be confused with the red on the temperature scale.

We have replaced the stars by arrows. The rings were measured, that's why it appears in red.

461 Use 'equal', not 'equals'.

The correction has been made.

465 In the footnote to the table, use 'taken' not 'taking'.

The correction has been made.