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# **[MUSCLE PHYSIOLOGY AND AEROBIC DIVE LIMIT IN EMPEROR PENGUINS]**

*Muscle physiology during diving, what determines the end of a dive for the penguin, Spheniscidae?*

## **Abstract**

The Emperor penguin is capable of diving to extreme extent. It can dive up to 27.6 minutes and dive to approximately 550 meters in depth. It is the most accomplished diver of all the penguin species.

Studies on the dive limit of the Emperor penguin discovered a crossover from aerobic metabolism to anaerobic metabolism. And depending on whether the Emperor was measured in man-made diving holes or in free-diving state, dive duration lasts on aerobic metabolism for 5.6 and 8 minutes respectively. After this amount of time, the Emperor relies on anaerobic metabolism. Anaerobic metabolism, however, is less efficient in creating energy (releases only 1/18<sup>th</sup> of energy otherwise provided by aerobic metabolism) and creates the product lactic acid. At a certain point, this build up of lactic acid must be washed out, thus limiting diving duration.

Anaerobic metabolism has been proven to be less efficient, even so, approximately 35-55% observed dives of the Emperor are reported to use it as a main energy source. The increasing amount of lactic acid should trigger the penguin to terminate the dive, however, there is no net increase in blood lactate concentrations during the dive. The product, lactic acid is created, however the muscles are cut off from blood circulation during a dive, preventing the lactic acid from entering the rest of the body.

Once the Emperor, in a man-made diving hole, passes 7 minutes of diving, a lactic acid concentration of 5 mmol liter<sup>-1</sup> is measured. In order to washout these amounts back to resting lactic acid concentrations (1.2-2.7 mmol liter<sup>-1</sup>) a post-dive interval of approximately 40 minutes is needed. The Emperors, however, rarely exceed a PDI of 5 minutes.

The goal of this thesis was to determine what would terminate a dive. In order to answer this, more study has to be done on the negative build up of lactic acid in the muscles and its effects on diving abilities.

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## Introduction

There is great interest in the diving abilities and restraints of diving sea-birds, with a particular interest in the Emperor penguin (*Aptenodyptes forsteri*). Their dives often last the longest and reach to deepest depths compared to the rest of the penguin species. Current record sets the Emperor penguin to a diving duration of 27.6 minutes (Sato et al. 2011) and another record dive reached approximately 550 meters in depth (Wienecke et al. 2007).

However, their oxygen stores have shown that a record dive like that, could not be performed on just aerobic metabolism (Butler 2004, Butler 2006). The Emperor penguin would have to rely on anaerobic metabolism as well (Carbone and Houston 1996).

Anaerobic metabolism results in an increase in blood lactate concentrations (Ponganis et al. 2010) which results in longer post-dive resting periods, in order to washout the lactate build-up in its system (Meir et al. 2008, Ponganis et al. 2011). The increase in blood lactate would then prove to be a trigger for the Emperor penguin to return to the surface (Ponganis et al. 2011). As an increase in blood lactate concentrations urges the animal to refresh its oxygen stores (Ponganis et al. 2010). However, Emperor penguins that rely on anaerobic metabolism during a dive have not shown an increase in blood lactate concentrations (Ponganis et al. 2010).

Aerobic dive limit has been introduced to determine the metabolism of the penguin during a dive, whether it was based on aerobic or anaerobic processes (Butler 2004, 2006).

A dive is considered to cross the aerobic dive limit duration once the post-dive lactate concentrations are higher than that of the pre-dive/resting blood lactate concentrations (Butler 2006, Meir et al. 2008, Ponganis et al. 2010). Aerobic dive limit (ADL) therefore depends on the amount of oxygen stores and oxygen consumption rate during the dive (Butler 2006).

Oxygen depletion in the pectoral muscles is a reason for switching to anaerobic metabolism (Carbone and Houston 1996, Butler et al. 2006). The muscles are the largest oxygen consumers during a dive and therefore most likely the first to be depleted (Carbone and Houston 1996).

Aerobic dive limit differs between the penguin species. The Emperor penguin has an aerobic dive limit of 5.6 - 8 minutes (Ponganis et al. 2010, Sato et al. 2011) while, for instance, the Little penguin (*Eudyptula minor*) has an ADL of 44 seconds (Ropert-Coudert et al. 2006).

The Emperor penguin has maximized its dive up to 27.6 minutes, even though it has an ADL of 5.6-8 minutes, the last 20 minutes of that dive would have to rely on anaerobic metabolism, even though the use of this type of energy can't be maintained indefinitely (Butler 2004, Butler 2006, Ponganis et al. 2010). And anaerobic metabolism is far less efficient in yielding energy than aerobic metabolism. Only 1/18<sup>th</sup> of the total amount of ATP is derived from anaerobic metabolism (Boyd 1997). It would therefore seem unlikely that the diver would rely on anaerobic metabolism for such a long period of time, except if the reward, for example; being able to reach prey rich densities, is great enough.

How are they capable of maximizing their dives to such an extent? What have they thus far achieved to expand their diving duration? Are they relying on anaerobic metabolism for such a long period of time?

**Main question:** Muscle physiology during diving, what determines the end of a dive for the penguin, *Spheniscidae*?

### Sub-questions:

1. Are observed dive durations exceeding calculated and measured aerobic dive limit?
2. Are the penguins relying on anaerobic metabolism, is there an increase in lactate concentrations, once they exceed DLT/cADL?
3. How are muscle myoglobin concentrations reflected in aerobic dive limit?
4. Are observed and measured DLT/cADL maximized by the penguins? Or are they capable of expanding even further?
5. What determines the end of a dive?

## ***Aerobic dive limit***

The term, aerobic dive limit (ADL), is one that is misunderstood very often. It implies a dive that is only limited by oxygen, and this is not entirely true (*Butler 2006*). Aerobic dive limit is, in fact, affected more by depletion in glucose stores than depletion of actual oxygen stores (*Kooyman 1989*).

ADL also implies that a dive can't be continued after that given amount of time, unless there is an immediate increase in anaerobic metabolism and its' product, namely lactate (*Carbone and Houston 1996, Ponganis 2010*). For the Emperor penguins however, neither of the above are true. So what defines the aerobic dive limit for the Emperor penguin?

Throughout the studies there are two types of aerobic dive limit. Because ADL has only been measured in the Emperor penguin, other penguin species have a calculated ADL (cADL). And cADL is defined by the (expected) amount of oxygen stores available in the penguin divided by the (expected) rate of oxygen consumption during diving (*Butler 2004*).

Oxygen consumption rate is based on heart rate, which is linked to metabolic rate, and the activity of locomotory/pectoral muscles during the dive (*Boyd 1997*).

Exceeding cADL defines a complete oxygen depleted state. However, an overall depletion of oxygen stores would lead to certain death, cADL is therefore more a guide line to expected diving durations rather than actual limits (*Butler 2006*).

However the measured ADL in Emperor penguins was exceeded as well, approximately 35-55% of observed dives during a study. Complete exhaustion of all available oxygen stores could not be the case in true ADL, something which the term 'ADL' does imply. Therefore it was chosen to be renamed (*Butler 2004*), to avoid further confusion, instead the term Diving Lactate Threshold (DLT) was introduced.

When the penguin exceeds DLT, the influx of blood lactate is higher than the efflux, causing an increase of blood lactate concentrations (compared to resting concentrations) (*Butler 2004*). Therefore exceeding this line means only an increase in blood lactate concentrations, and their increasing need for anaerobic metabolism.

Dive duration is determined by the available amount of oxygen stores and the rate they are consumed during a dive. Available oxygen stores, such as pre-dive air sac volume, are also determined by the amount of myoglobin and hemoglobin concentrations (*Kooyman and Ponganis 1998*).

## ***Penguins and aerobic dive limit***

The Emperor penguin is the only species of penguin in which diving lactate threshold was determined. Its DLT is approximately 5.6 – 8 minutes (*Ponganis et al. 2010*). For the other penguin species, cADL was calculated, with the use of their expected air-sac volume and consumption rate (*Butler 2004*).

However, there is much uncertainty on their actual oxygen consumption rate during the dive (*Nagy et al. 1984, Kooyman and Ponganis 1998*). For instance, an overestimation of the oxygen consumption of the Jackass penguin (*Spheniscus demersus*), lead to believe that the aerobic dive limit of a similar sized penguin species, the Humboldt penguin (*Spheniscus humboldti*), was approximately 0.4 minutes. If true, the Humboldt penguin would be incapable of reaching required diving depth and have insufficient time to search for prey (*Kooyman 1989*). A closer look at the cADL of the Humboldt penguin concluded that it was, in fact, approximately 2.3-2.5 minutes (*Butler et al. 1984, Kooyman 1987*).

That particular study assumed that the Jackass penguin, as well as the similar sized Humboldt penguin, could reach up to ten times their metabolic resting rate during a dive (*Nagy et al. 1984*). For comparison, a 'Tour de France' cyclist would use a ten-fold resting metabolic rate to perform (*Kooyman and Ponganis 1998*).

The Chinstrap penguin (*Pygoscelid antarcticus*) has a diving metabolic rate approximately 4.68-5.55 times the resting metabolic rate (Moreno et al. 1996), similar to the Adélie penguin (5.4 times the resting metabolic rate).

Penguin species	% oxygen in Lungs	% oxygen in Blood	% oxygen in Muscles	DLT/cADL (minutes)	Maximum VO <sub>2</sub> (ml O <sub>2</sub> kg <sup>-1</sup> min <sup>-1</sup> )	Maximum observed dive depth (meters)	% dives >DLT (cADL)
<b>Emperor</b> <i>Aptenodytes forsteri</i>	19 <sup>6</sup>	34 <sup>6</sup>	47 <sup>6</sup>	5.6 - 8 max: 27.6 <sup>3</sup>	52-53 <sup>12</sup>	550 <sup>8</sup>	35-55 <sup>20</sup>
<b>King</b> <i>Aptenodytes patagonicus</i>	17-23 <sup>6,14</sup>	29-37 <sup>6,14</sup>	40 - 54 <sup>6,14</sup>	2-3 max: 7.5 <sup>11</sup>	<69 <sup>6</sup> 45-58 <sup>7</sup>	318.4 <sup>11</sup>	20-40 <sup>11</sup>
<b>Adélie</b> <i>Pygoscelid adeliae</i>	32-45 <sup>6,9</sup>	29-38 <sup>6,9</sup>	26-29.5 <sup>6,9</sup>	1.1 - 1.9 <sup>1</sup> max: 5	55-63 <sup>9</sup>	145.9 <sup>11</sup>	14-54 <sup>1,9</sup>
<b>Gentoo</b> <i>Pygoscelid papua</i>	34 <sup>14</sup>	26 <sup>14</sup>	40 <sup>14</sup>	1.1-2.5 <sup>1,9,10</sup> max: 6	63 <sup>9</sup>	135 <sup>19</sup>	35-62 <sup>9,13</sup>
<b>Chinstrap</b> <i>Pygoscelid antarcticus</i>	-	-	-	1.1 - 2.1 <sup>1,9</sup> max: 3	57 <sup>9</sup>	70 <sup>19</sup>	4-37 <sup>9</sup>
<b>Humboldt</b> <i>Spheniscus humboldti</i>	-	-	-	1,5 - 2.5 <sup>9,21</sup>	-	53 <sup>9</sup>	-
<b>Macaroni</b> <i>Eudyptes chrysolophus</i>	-	-	-	1.5 <sup>10,15</sup> max: 6	27 <sup>15</sup>	-	7 <sup>15</sup>
<b>Rockhopper</b> <i>Eudyptes chrysocome</i>	-	-	-	1.2 max: 3 <sup>16,17</sup>	-	109 <sup>18</sup>	-
<b>Little (Blue)</b> <i>Eudyptula minor</i>	-	-	-	0.3 <sup>5</sup> max: 1.5 <sup>2</sup>	45 <sup>2</sup>	66.7 <sup>2</sup>	2 <sup>2</sup>

**Table 1.** Percentage of total oxygen stores over the lungs, blood and muscles. DLT=Diving Lactate threshold, either measured or 'expected' values.  $\text{ml O}_2\text{kg}^{-1}\text{min}^{-1}$  indicator of metabolic rate, oxygen consumption during a dive. Either calculated or expected. Maximum depth observed during a few studies. Percentage of dives that exceed diving lactate threshold durations. There are still many blanks present, and not all penguin species have been measured.

Information provided by: Chappell et al. 1993<sup>1</sup>, Ropert-Coudert et al. 2006<sup>2</sup>, Sato et al. 2011<sup>3</sup>, Kooyman & Kooyman 1995<sup>4</sup>, Betghe et al. 1997<sup>5</sup>, Kooyman & Ponganis 1998<sup>6</sup>, Fahlman et al. 2007<sup>7</sup>, Wienecke et al. 2007<sup>8</sup>, Culik et al. 1994<sup>9</sup>, Croxall et al. 1988<sup>10</sup>, Sato et al. 2002<sup>11</sup>, Kooyman & Ponganis 1994<sup>12</sup>, Boyd 1997<sup>13</sup>, Butler 2001<sup>14</sup>, Green et al. 2003<sup>15</sup>, Wilson et al. 1997<sup>16</sup>, Tremblay et al. 2003<sup>17</sup>, Chérel et al. 1999<sup>18</sup>, Trivelpiece et al. 1986<sup>19</sup>, Ponganis et al. 2010<sup>20</sup>, Kooyman 1989<sup>21</sup>, Butler 1982, Kooyman 1987, Ponganis and Kooyman 2000, Ponganis et al. 1997, Ponganis et al. 1999.

Distribution of oxygen in the three main oxygen stores; lungs, blood and muscles, differs amongst the penguin species (table 1). The lungs of the Emperor penguin store the least amount of oxygen of all the other penguin species that have been measured (Kooyman and Ponganis 1998).

The smaller penguin species, Gentoo and Adélie, distribute their oxygen more evenly over the three main oxygen stores (Kooyman and Ponganis 1998, Culik et al. 1994, Butler 2001).

Emperor and King penguins (*Aptenodytes patagonicus*) are deep diving penguin species, and store the highest percentage of their oxygen in the muscles. Emperor and King penguins have chosen for large oxygen stores in the muscle to maintain rapid swimming as they push themselves to the limits in order to reach depths they could otherwise not exploit. They frequently rely on anaerobic metabolism due to their mass-related high metabolic rate (Kooyman and Ponganis 1998).

The Adélie and Gentoo penguin can be classified as a middle group regarding diving depth, their maximum diving depth ranges from 135-146 meters (Sato et al. 2002, Trivelpiece et al. 1986). They have a higher percentage of their oxygen stored in the lungs (Kooyman and Ponganis 1998, Culik et al. 1994, Butler 2001). The Chinstrap, Humboldt, Macaroni, Rockhopper and Little

penguins are shallow diving species and rarely reach or exceed 100 meters in diving depth (Ropert-Coudert et al. 2002, Culik et al. 1994, Cherel et al. 1999, Trivelpiece et al. 1986). Their oxygen distribution has not been measured as of yet. However, they are shallow divers and it can be argued that they are therefore less sensitive to positive buoyancy (Carbone and Houston 1996, Sato et al. 2002) and would be more likely to have larger amount of oxygen stored in the lungs. Yet their cADL's are relatively low, this could imply that they are either less capable of storing high amounts of oxygen stores (compared with deep-diving penguin species), or perhaps that their oxygen consumption rate is relatively high (Kooyman and Ponganis 1998, Butler 2006).

## **Muscle physiology**

The locomotory muscles are the largest oxygen consumers during a dive and they are the first of the oxygen stores to be depleted (Carbone and Houston 1996).

Once the muscle oxygen store is depleted, the penguin changes to mostly anaerobic metabolism for energy (Williams et al. 2012). The energy required to proceed diving (ATP) can be maintained through phosphocreatine (PCr) hydrolysis and glycolysis (Gly). The elongation of an anaerobic dive depends on PCr and Gly stores as well as its ability to store oxygen in the muscles, for which myoglobin is needed (Williams et al. 2012).

The Emperor penguin has the highest amount of myoglobin, of all measured animals (Ponganis et al. 1997). The Emperor has a myoglobin concentration of 6.4g per 100g muscle tissue (Ponganis et al. 1997). This is twice as large as the Adélie penguin, which has a myoglobin content of 3.0g per 100g muscle tissue (Weber et al. 1974).

Perhaps the Adélie does not require the excessive use of its muscles as the Emperor does, or it might be that the Adélie penguin has a higher amount of phosphocreatine and glycogen stores to compensate (Ponganis et al. 1997). The Adélie stores the highest amount of oxygen in its lungs, as shown in table 1 and the Emperor relies more on its muscles for oxygen storage (Kooyman and Ponganis 1998, Butler 2001).

Once the muscle oxygen stores of the Emperor penguins are depleted, blood lactate concentrations do not increase above resting levels (Ponganis et al. 2003, Ponganis et al. 2010, Sato et al. 2011). Thus the products of anaerobic metabolism must be contained within the muscles, preventing it from entering the blood flow. This can only be accomplished if the muscles are cut off from blood circulation during the dive (Ponganis et al. 2003).

This assumption was supported by studying the pectoral muscle temperature in the Emperor penguin. During a dive the average temperature of the locomotory muscle would increase, and during the post-dive surface interval, the average temperatures would decrease again. This pattern is consistent with what would be predicted if there were no to little muscle blood flow during dives (Ponganis et al. 2007, Meir et al. 2008, Williams et al. 2011).

### **Post-dive lactate concentrations**

Lactate is always present in the blood. During resting the influx of blood lactate is equal to the efflux of blood lactate, there is no net increase in concentration (Butler 2004, Butler 2006).

Aerobic dive limit is described as the point in dive duration after which lactate concentrations are higher than resting lactate concentrations, 1.2-2.7 mmol liter<sup>-1</sup> (Ponganis et al. 1997, Williams et al. 2012). If anaerobic metabolism was needed, an increase in lactate would be measured. And active but non-perfused muscle is most likely the source of lactate concentration accumulation when surpassing the DLT. This increase in blood lactate does not occur during the dive, but rather during the post-dive resurface, when tachycardia (180-220 bpm) takes place, causing a wash out of lactate product in the muscles (Ponganis et al. 2003, Meir et al. 2008, Ponganis et al. 2009).

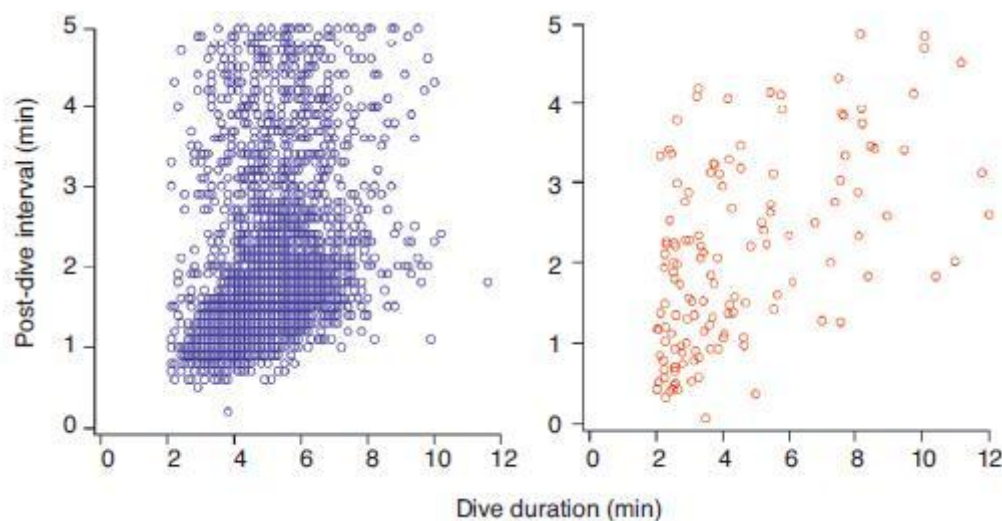
Blood samples were taken every two hours to determine blood lactate concentrations (*Ponganis and Kooyman 1997*). Post-dive blood samples were taken approximately five minutes after the bird resurfaced, in most cases only one post-dive blood sample was taken. Just a few cases had multiple blood samples taken over the course of an hour (*Ponganis and Kooyman 1997*).

The myoglobin oxygenation of the Emperor does not suffer the negative effects of lactate concentration increase. Therefore metabolic acidosis does not impair myoglobin function under conditions of prolonged physical effort, such as diving (*Tamburrini et al. 1999*). The muscles are capable of maintaining high levels of lactate without causing disturbance.

The highest concentration of blood lactate in the Emperor penguin measured was 7-9.5 mmol liter<sup>-1</sup> plasma lactate (*Kooyman and Ponganis 1994*). This was measured in an Emperor penguin using its maximum metabolic rate while swimming in a channel. This most certainly does not depict real life situations. This level of blood lactate is considered to portray the maximum blood lactate increase possible for the Emperor. An increase of 6 mmol liter<sup>-1</sup> blood plasma lactate would require at least 40 minutes of resurface time (post-dive interval) to washout (*Kooyman and Ponganis 1995*). All dives longer than 7 minutes had lactate concentrations greater than 5 mmol liter<sup>-1</sup> (*Ponganis et al. 1997*). But as graph 1 shows, post-dive interval does not come near 40 minutes of recovery time, suggested by *Kooyman and Ponganis (1998)*.

Post-dive interval, also known as PDI, is the resting time needed to wash out all anaerobic products after a dive and replenish the penguin to pre-dive state (*Kooyman and Kooyman 1995*). PDI is assumed to increase once the Emperor penguin exceeds DLT. However, increase in dive duration does not seem to correlate with an increase in PDI (*Sato et al. 2011*).

Emperor penguins show no increase in blood lactate during a dive because the muscles are cut off from blood circulation. Muscle myoglobin is not affected by the increase in lactate and post-dive intervals do not increase once the Emperor exceeds DLT.



**Graph 1.** Post-dive interval duration set to dive duration. Left graph depicts the free swimming Emperor penguin, the right graph shows the Emperor penguin in a man-made diving hole. There is no correlation between increasing dive duration and post-dive interval (*Sato et al. 2011*).

## ***Emperor penguin exceeds expected diving duration***

The Emperor penguin exceeds, 35-55% of dives made, the diving lactate threshold (*Ponganis et al. 2010*). Lactate concentrations have proven not to increase during a dive (*Ponganis et al. 2010, Sato et al. 2011*), ruling them out as a possible trigger to resurface. Perhaps the penguins are



taking more measurements in order to minimize their oxygen consumption rate, such as accomplish even lower diving metabolic rates than currently expected of them (*Kooyman and Ponganis 1994*). Or perhaps drop heart rate frequencies even further than taken into account (*Green et al. 2003, Meir et al. 2008*).

### ***Diving metabolic rate***

Diving metabolic rate is reflected as the rate of oxygen consumption, noted as  $VO_2$ . An overestimation of the diving metabolic rate of the Humboldt penguin lead to believe it had a cADL of 0.4 minutes. (*Nagy et al. 1984*).

The diving metabolic rate of a free-diving Emperor penguin was calculated to be  $52\text{ml O}_2\text{kg}^{-1}\text{min}^{-1}$  (*Kooyman and Ponganis 1994*). A near ten-fold of the resting metabolic rate, hence perhaps this is an overestimation of the actual diving metabolic rate. In which case it would explain why the Emperors often exceed the DLT.

Emperor penguins, in man-made diving holes, have a similar metabolic rate of a resting and hand-fed Emperor penguin (*Ponganis et al. 2010*). This translates to a rate of  $5.5\text{-}6.2\text{ ml O}_2\text{ kg}^{-1}\text{min}^{-1}$  (*Kooyman and Ponganis 1994, Ponganis et al. 2010*).

Perhaps diving metabolic rate of the Emperor penguin is overestimated, as it was in the Humboldt penguin. This would explain the high percentage of dives that exceed DLT of 5.6 minutes without visible consequence (*Ponganis et al. 2010*). And supports the idea that the dives are mostly aerobic, because anaerobic metabolism is less efficient in creating energy (*Boyd 1997*).

### ***Diving heart rate***

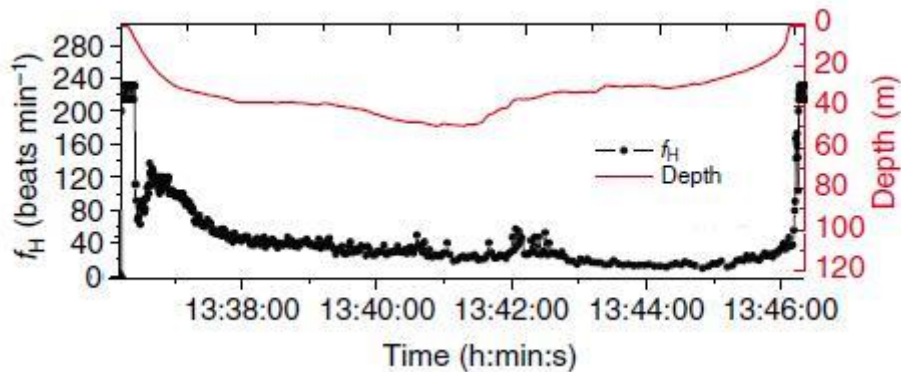
During a dive, heart rate declines rapidly and, at certain points in the diving activity, reach extreme low frequencies. A decrease in heart rate translates to a decrease in cardiac output and blood tissue perfusion (*Meir et al. 2008*). The oxygen consumption rate decreases along with the heart rate (*Green et al. 2005*). Bradycardia may decrease metabolic rate during diving, but can't account for large changes in metabolic rate (*Kooyman and Ponganis 1998*). Heart rate and stroke frequency are not correlated (*Meir et al. 2008*).

When planning to dive, the Emperor penguin increases its heart rate, a pre-dive tachycardia of 180-220 beats per minute, and declines once the penguin submerges (*Kooyman et al. 1992b, Meir et al. 2008*). This pre-dive tachycardia increases the oxygen intake (*Kooyman et al. 1992b*).

The first interval at two-three minutes into the dive, average heart rate lies between 60-100 bpm. The average heart rate decreases even further as the penguin reaches the DLT barrier. The heart rate decreases even further after the dive exceeds the DLT barrier, sometimes reaching mere 6 bpm, true bradycardia (*Green et al. 2003, Froget et al. 2004, Ponganis et al. 2008, Meir et al. 2008*).

Once the penguin heads back to the surface, tachycardia kicks in (180-220 bpm) and lasts until the first phases of reaching the surface (*Kooyman et al. 1992b, Meir et al. 2008*). This pattern can be seen in graph 2.





**Graph 2.** Heart rate and depth set to time of the Emperor penguin (Meir et al. 2008). This is an example of a dive exceeding DLT.

Overall, the average heart rate of the Emperor penguin is much lower in dives that exceed DLT (average heart rate approx.  $41 \pm 1$  bpm) than dives that remain within the DLT duration (average heart rate approx.  $85 \pm 3$  bpm) (Meir et al. 2008). This is relatively low compared to an average heart rate of the Adélie penguin (*Pygoscelis adeliae*), which is approximately 107 bpm (Culik 1992).

The readjustment in heart rate, seen in the first few minutes of the dive, could be a sign of blood being pumped back to the muscle after the initial descent, a period of time in which stroke frequency needs to be high to overcome positive buoyancy. This surge of blood to the muscles might also explain the delayed increase of muscle temperature (Froget et al. 2004, Meir et al. 2008).

This certain heart rate pattern, however, is only found in deep dives. It is most likely present to provide a last surge of blood to the already tired muscles, after fighting against positive buoyancy, which was created by the increase in lung oxygen volume (Sato et al. 2002, Froget et al. 2004). But this short surge of blood to the muscles contradicts the statement that the muscles are cut off from the blood circulation during the dive (Ponganis et al. 2010).

### ***Hypothermia in penguins***

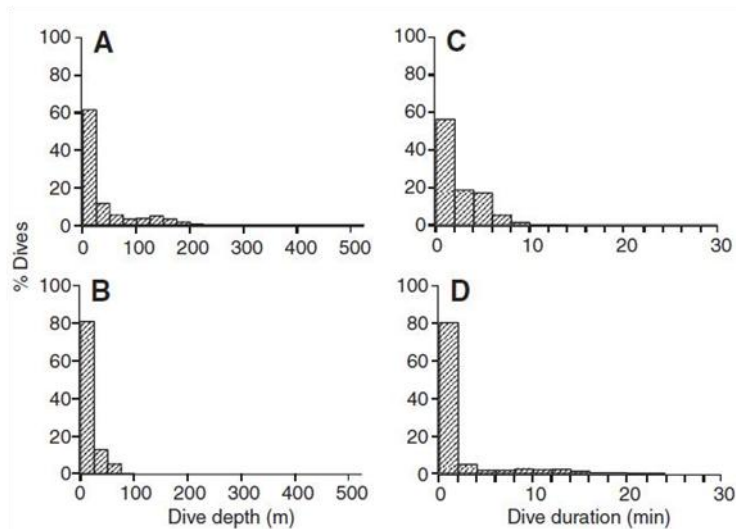
Average body temperatures of the Emperor penguin decrease as the dive continues, during the post-dive interval the penguin generates heat again, driving body temperatures back to normal (Butler 2004).

Body temperature may also have an effect on oxygen store utilization (Kooyman and Ponganis 1998). A reduction in metabolic rate may either be a result or a cause of changes in body temperature. Therefore, body temperatures of multiple Emperors were measured, and results revealed that body core temperatures remained approximately the same as that of resting penguins (Ponganis et al. 2001, Ponganis et al. 2003). The extremities of the penguin did decline during the dive, with an average decrease of approximately 10-20 °C (Handrich et al. 1997). The Emperor does conserve energy by minimizing heat loss through its extremities, but does not seem to lower its metabolic rate, hence the steady body core temperatures. Even during a dive of approximately 23.1 minutes, core temperatures remained within the 36.3-36.7 °C range (Ponganis et al. 2010), this is equal to a penguin in rest.

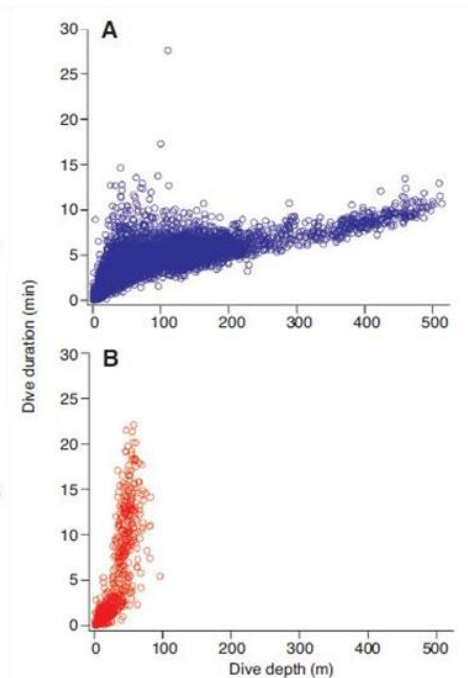
Body temperatures are not just affected by metabolic rate, they are also driven by; ingestion of cold prey, high convective losses during swimming, insulation. And this needs to be thoroughly investigated before the reasons of such temperature changes are understood (Kooyman and Ponganis 1998).

## Diving behavior

Studies on the diving abilities of Emperor penguins have focused on using man-made diving holes for achieving measurements. The man-made diving hole makes it easier to control when to start measuring the dive and when to end it (Sato *et al.* 2011). However, there seems to be a difference in diving abilities when measured in man-made diving holes or when measured with free-diving Emperor penguins (Sato *et al.* 2011).



**Graph 3.** Percentage of observed dives of emperor penguins versus dive depth (A,B) and dive duration (C,D) at sea (A,C) and in a diving hole (B,D) (Sato *et al.* 2011).



**Graph 4.** Dive duration against dive depth of emperor penguins at sea (A) and diving holes (B) (Sato *et al.* 2011).

Free-diving Emperor penguins have longer diving durations and go to further depths than Emperor penguins diving in diving holes, see graph 3 (Sato *et al.* 2011). Graph 4 sets diving duration against dive depth. In the case of the free-diving Emperor penguins, there is a slow increase in diving duration once reaching to greater depths. However the Emperor penguins in man-made diving holes show that dive duration does not increase the diving depth (Sato *et al.* 2011).

Emperor penguins in diving holes were measured to have longer dives than free-diving penguins. Because these penguins do not dive to greater depth, it is suggested that they rely less on their pectoral muscles, their oxygen stores do not deplete as soon as in free-diving Emperor penguins (Carbone and Houston 1996, Sato *et al.* 2011) This assumption is counterfeited for DLT of free-diving penguins was higher than DLT of penguins in diving holes. Free diving penguins maintain a DLT of approximately 8 minutes (Sato *et al.* 2011). The Emperors in diving holes however did show a decrease in stroke frequency as opposed to free-diving Emperor penguins. But then post-dive resting periods were not significantly different from each other (Sato *et al.* 2011). Thus free-diving Emperor penguins dive to greater depth, with a higher stroke frequency, have a higher DLT, have shorter diving durations, but have similar post-dive resting periods as diving hole Emperors. This most certainly shows that the two studying methods show two very different ways of diving in Emperor penguins.

### ***'The Emperor penguin plans dive'***

The Emperor penguin plans its dive (Sato et al. 2011). Mean maximal air volumes for deep dives were approximately 83% greater than those during shallow dives (Sato et al. 2011). They calculated the remaining amount of air in the respiratory system after the dive. They knew the swim speed, depth and body angle of the penguin while passively gliding to the surface at the end of the dive. With the help of buoyancy they calculated the remaining amount of air in the respiratory system. This is based on the assumption that the penguins do not exhale while submerged. The Emperor penguins that dived up to 300 meters were found to inhale more air before diving. The penguins that dived to 400-500 meters depth had a lower air sac volume, this corresponds to them exhaling when submerged (Sato et al. 2011).

The King penguin has also been observed to change its amount of oxygen according to its dive. Average air-volumes in the lungs ranged from 70 ml for shallow dives (<50 meters) to 125 ml for deep dives (>100 meters) (Wilson 2003, Ponganis et al. 2010).

This assumption was also confirmed by a study on Little penguins (Ropert-Coudert et al. 2006). The Little penguin chose to pursue a prey towards greater depth than usual, and showed an increase in effort to reach the surface. The early part of the ascent to the surface after the dive needed to be strong in flipper frequency to overcome the negative buoyancy. The Little penguin had too little positive buoyancy to ascend passively, as shallow-diving sea-birds usually do (Sato et al. 2002). It was concluded that the Little penguin did not expect to dive as far and long as it did (Ropert-Coudert et al. 2006).

### ***The unexpected dive***

One study recorded an extraordinary dive of the Little penguin (*Eudyptula minor*). It reached a diving depth of 66.7 meters and a diving duration of 90 seconds (Ropert-Coudert et al. 2006). This exceeded, by far, the calculated ADL of 44 seconds (Bethge et al. 1997).

There was a sudden change in descending rate measured, with average descend rate of 1.11 ms<sup>-1</sup> until the 50.7 meter limit, followed by a sudden increase to 2.53 ms<sup>-1</sup> (Ropert-Coudert et al. 2006). This was measured along with an increase in flipper beating activity, this pattern reflects a possible scenario of the Little penguin spotting a prey and pursuing it to greater depth. The penguin decided to dive longer because of a last minute prey sighting.

This Little penguin, in particular, crossed a thermocline, which would not happen during a normal dive. Average sea temperature dropped from 15.7°C to 14.4°C (Ropert-Coudert et al. 2006). A few other dives were observed to cross this thermocline, however the time spent at that depth was relatively short, perhaps they are avoiding colder waters to reduce thermoregulatory costs (Ropert-Coudert et al. 2006).

## ***Optimal diving models***

Optimal diving models mainly focus on exchange of respiratory gasses during a dive. The importance of buoyancy, prey density and predatory threat are left out of the equation (Halsey and Butler 2006). Optimal diving models state that the dive is only maximized if all the oxygen stores are nearly fully depleted as the diver resurfaces. The diver thereby maximized its foraging time underwater. And according to optimal diving models, decreasing oxygen concentrations is the main reason for a dive to end. However according to Houston (2011) that is not the case. He states that other reasons, for example, finding acceptable prey could trigger a 'premature' dive termination. This would not render the dive less successful than one which fully depletes its oxygen stores (Houston 2011).

Dives that end before reaching cADL/DLT are considered to be suboptimal according to the present diving model. However, the increasing, uncomfortable urge to breathe is not taken into

account, along with predation risk, ingestion of food, prey density and giving up times (*Culik and Wilson 1994, Shiomi et al. 2012*) as reasons to terminate a dive before reaching cADL. The ability to shut certain regions of the body from blood circulation during a dive is also not taken into account in optimal diving models (*Butler 2004*). And it is certain that the Emperor penguin shuts its muscles off from blood circulation (*Ponganis et al. 2010*).

### ***Two diving models***

There are two types of diving, when exceeding DLT/cADL. The switch model and the mixed metabolism model (*Carbone and Houston 1996*). The switch model is based on aerobic metabolism, until the stores are depleted (exceeding DLT/ADL) and then fully rely on anaerobic metabolism. The mixed metabolism model relies on both aerobic and anaerobic metabolism even before lactate concentrations increase.

The switch model seems less likely to occur in nature (*Carbone and Houston 1996*). The muscles account for the highest percentage of oxygen storage and consumption during a dive. The muscles are most likely to be depleted first, while lung and blood oxygen storage remain. Therefore there could not be a total switch from aerobic metabolism to anaerobic metabolism. The trigger needed for the switch model does not occur while diving, for the increase in lactate concentrations only takes place during the post-dive washout (*Kooyman and Ponganis 1998*).

Current optimal diving models lack certain important factors, such as prey density, predation risk, ingestion of food and giving up times (*Culik and Wilson 1994*). In order to state whether a dive is optimal or not, these factors have to be taken into account.

## ***Discussion***

### ***Are observed dive durations exceeding calculated and measured aerobic dive limit?***

The Emperor penguin exceeds DLT in numerous occasions, approximately 35-55% of the observed dives (*Ponganis et al. 2010*). The Little penguin, however, does not exceed calculated ADL often, only 2% of the dives have been observed to exceed cADL (*Ropert-Coudert et al. 2006*). This seems to be different within the penguin species. However it is evident that deep-diving penguin species do exceed their DLT/cADL more often than shallow diving penguins (*Chappell et al. 1993, Culik et al. 1994, Boyd 1997, Sato et al. 2002, Green et al. 2003, Ropert-Coudert et al. 2006, Ponganis et al. 2010*). This observation is strange as *Boyd (1997)* suggested that anaerobic metabolism provides the diver with just 1/18<sup>th</sup> of the available ATP, and is therefore an inefficient way of gaining energy.

### ***Are the penguins relying on anaerobic metabolism, is there an increase in lactate concentrations, once they exceed DLT/cADL?***

Increase in blood lactate concentrations does occur once the penguin exceeds DLT/cADL (*Ponganis et al. 1997, Kooyman and Ponganis 1998, Ponganis et al. 2010*). However, this only occurs during the post-dive interval (*Sato et al. 2011*). The muscles are the largest oxygen consumers (*Carbone and Houston 1996*) and they are cut off from blood circulation during the dive, this causes lactic acid increase within the muscles which is released during the post-dive interval (*Ponganis et al. 2010*).

Average blood lactate concentrations, in Emperor penguins, increase to 5 mmol liter<sup>-1</sup> after diving for 7 minutes. A post-dive interval of approximately 40 minutes is needed to washout a blood lactate concentration of 6 mmol liter<sup>-1</sup>. However their post-dive intervals are nowhere near this duration. As to what the exact reason is for this, has yet to be studied.

### ***How are muscle myoglobin concentrations reflected in aerobic dive limit?***

The highest amount of myoglobin concentration is found in the Emperor penguin, with a myoglobin concentration of 6.4g per 100g muscle tissue (*Ponganis et al. 1997*). Adélie penguins

are, compared to Emperors, medium-divers. Their myoglobin concentrations are indeed much lower, concentrations are approximately 3.0g per 100g muscle tissue (Weber *et al.* 1974). Myoglobin concentrations have not been measured in the other penguin species, therefore can't be compared to full extent. However, the shallow diving penguin species have a different oxygen distribution amongst the three oxygen stores: lungs, blood and muscles. The medium-diving penguin stores a higher amount of oxygen in its lungs, while the deep diving penguin stores the highest amount of oxygen in the muscles (Culik *et al.* 1994, Kooyman & Ponganis 1998, Butler 2001). This is facilitated by their higher myoglobin concentrations.

### ***Are observed and measured DLT's and ADL's maximized by the penguins? Or are they capable of expanding even further?***

The Emperor penguin has minimized its average diving heart rate, in order to maximize its diving duration. Once a dive reaches DLT, average heart rate can drop as low as 6 bpm (Meir *et al.* 2008). Average

Body core temperatures are kept at steady level. The penguin does shut off its extremities from the rest, and their average temperatures decrease substantially.

It is hard to tell whether they have fully maximized their dive. Their post-dive intervals remain very short, compared to their diving duration, this suggests that the penguins are capable of prolonging their dives even further. However, measured lactic acid concentrations indicate that they are relying on anaerobic metabolism, and create concentrations that require longer periods of rest (>6 mmol liter<sup>-1</sup> requires at least 40 minutes of PDI). Perhaps studies have focused too much on post-dive intervals, and not on the behavior of the penguin when it was not diving for prolonged periods of time. How is the penguin making up for its negative build up of lactate? Muscle oxygenation is unaffected by increased lactic acid concentrations, but does that mean that the penguin can perform dives and increase lactic acid concentrations above the leftover concentrations from the previous dive?

### ***What determines the end of a dive?***

It is not easy to determine what in particular ends a dive, that certainly depends on the given circumstances. The Emperor penguin has shown different diving behavior in man-made diving holes than dive behavior of diving in man-made diving holes free-diving Emperors (Sato *et al.* 2011). Free-diving Emperors are not saving energy by lowering their stroke frequency, and still show a higher DLT than Emperors in diving-holes (8 to 5.6 minutes respectively) (Sato *et al.* 2011).

Optimal diving models consider depleting oxygen stores as the most important reason for terminating a dive. And divers who terminated their dive with available oxygen stores are considered to dive suboptimal. These models have not taken: prey density, giving up times and increasing predatory threat, into account. As well as the increasing, uncomfortable urge to breathe which comes along with decreasing oxygen stores. And of course, the probable negative build up of lactic acid in the muscles due to past dives.

In order to truly answer if we have observed maximum diving ability of the penguin species, more studies have to focus on free-diving behavior. This is most like natural circumstances and shows a more honest diving behavior than when measured in a man-made diving hole. The diving hole, however, is very successful in order to 'strain' the animal to fullest abilities. That will, however, not determine what their reason is for terminating a dive.

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