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# Adjustments of gastric pH, motility and temperature during long-term preservation of stomach contents in free-ranging incubating king penguins

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## Summary

**Male king penguins are able to store undigested food in their stomach for up to 3 weeks during their incubation fast, which evidently implies some modification of their digestive process. Using small electronic recorders, we studied the change in gastric pH, motility and temperature during the first week of food storage. The pH could be maintained at values as high as 6 throughout the incubation fast, a pH that is unfavourable for avian gastric proteinase activity. Gastric motility was never completely inhibited but could be markedly reduced. Stomach temperature was maintained at around 38°C. The fact that stomach temperature of incubating birds did not show a daily rhythmic fluctuation as seen in non-breeding birds could be due to temperature constraints on embryo development. Thus the present study demonstrates substantial adjustments of pH and gastric motility in incubating king penguins, which may contribute to the inhibition of digestive gastric processes.**

Key words: gastric pH, gastric motility, gastric temperature, stomach content preservation, penguin, *Aptenodytes patagonicus*, incubation fast.

## Introduction

One characteristic of seabirds during the breeding season is their need to transport and store food from the feeding ground back to the nesting colony on shore to feed their offspring. Food is carried in the bill, the crop or the stomach, depending on the seabird species, and the storage can be prolonged for days or weeks (Croxall, 1987). It has recently been demonstrated, however, that long-term storage of food in the stomach can also take place during the incubation period (Gauthier-Clerc et al., 2000). Indeed, preservation of undigested food for up to several weeks has been found to occur in the stomach of male king penguins *Aptenodytes patagonicus* towards the end of the incubation fast (Gauthier-Clerc et al., 2000). Such an adaptation ensures hatchling survival if the female's return is delayed. In king penguins, both mates take turns at incubating their egg, alternating periods of fasting on land and refeeding sojourns at sea (Stonehouse, 1960; Weimerskirch et al., 1992). For penguins breeding on Subantarctic Crozet Islands, the distance to the foraging zone is variable (Bost et al., 1997) as is food availability in the mesopelagic zone (El-Sayed, 1988). Consequently, foraging trip duration is highly variable as well. Generally, the male assumes the last part of the incubation and the female returns around hatching time, although her return can be delayed for over 9 days (Gauthier-Clerc et al., 2000). During this last incubation shift, males are able to fast with food still stored in their stomach (Gauthier-Clerc et al., 2000).

Such a long-term storage of food in the stomach must imply substantial modifications of the usual gastric digestive processes, since the digestive function of the stomach has been switched off and replaced by a storage function. In incubating king penguins, the well-conserved stomach content is characterized by an unchanged appearance, unchanged mass and energetic values, as well as a pH close to 4 at the beginning and the end of the incubation shift (Gauthier-Clerc et al., 2002; Thouzeau et al., 2003). These observations argue both for a modification of food breakdown in the stomach and for a delayed transfer into the intestine. One way to assess these adjustments is to study physiological parameters of gastric functions such as pH, motility and temperature.

Few studies have investigated the plasticity of seabird digestive physiology using both intra- (Wilson et al., 1989; Peters, 1997a) and interspecific (Jackson, 1992) comparisons. Intraspecific studies have been made on inshore feeders that forage close to their nest and therefore modulate their digestion for a comparatively shorter period than king penguins that do so for up to 3 weeks. Wilson and colleagues were the first to show that breeding African penguins *Spheniscus demersus* exhibit a substantial delay in gastric emptying when on shore to feed the brood (Wilson et al., 1989). Furthermore, a slowing of the digestive processes by variation in gastric pH has been observed in the Magellanic penguin (*S. magellanicus*) foraging at sea (Peters, 1997a). Lastly, modification of stomach

temperature, through adjustment of gastric motility, has been proposed to reduce the rate of digestion in several foraging penguin species (Peters, 1997a, 2004). Technical difficulties in studying physiological digestive parameters in free-living seabirds certainly explain why there have been so few studies on digestive modulation in penguins. The particular situation of long-term stomach food storage of male king penguins while on shore therefore provides a unique opportunity to better assess physiological digestive plasticity in seabirds.

Studies on digestion generally require the animals to be held under controlled conditions. However, in penguins, as with many other species, stress resulting from human disturbance with or without handling has been demonstrated to influence physiological parameters such as body temperature (Regel and Pütz, 1997), heart rate (Nimon et al., 1996) and plasma metabolites and hormones (Ménard, 1998). Thus human presence has to be minimized. In the present study we used recently developed miniature gastric probes (Peters, 1997a, 2004) to record stomach temperature and gastric pH or gastric motility in free-living birds incubating in their colony.

The purpose of our work was to test whether any adjustments occur in gastric pH, motility and temperature during food conservation in the stomach of free-ranging incubating male king penguins. To this end, we continuously monitored the changes in gastric motility, pH and temperature and compared birds incubating in the colony with non-breeders held in temporary captivity.

## Materials and methods

The study was carried out at Possession Island (46°25′S, 51°45′E), Crozet Archipelago, during the incubation period of the king penguin's breeding cycle, from December 2000 through March 2001.

### *Experiment 1: Non-breeding birds as controls for digestion*

Fifteen non-breeding adult king penguins *Aptenodytes patagonicus* (J. F. Miller) were studied in 3 series of 4–6 individuals. Non-breeders are birds not yet engaged with a mate for reproduction at the time of capture. Because it is impossible to retrieve non-breeding birds in the colony on a daily basis, non-breeders were penned together under natural climatic conditions for the duration of each experimental series. Birds were fasted for 48 h, following which they were fed once daily for 3 days with a fish diet in order to acclimate them to captivity and daily manipulation. The diet consisted of two Austral Ocean species, the mackerel icefish (*Champsocephalus gunnari*) and grey rockcod (*Notothenia squamifrons*). Before each meal, the birds were weighed to check for any changes in body mass and if necessary, the fish ration was adjusted (diet mass range: 476–940 g). Twenty-four hours after the last meal, which is a long enough period to allow complete gastric evacuation in king penguins (Jackson, 1992), each bird was given a gastric pH/temperature probe (Peters, 1997a) or a gastric motility/temperature probe (Peters, 2004), but was not provided with fish. Probes were given orally followed by a massage down the oesophagus until they passed through the stomach entrance. The first 24 h of recorded data were considered to refer to the fasting state, before birds where refed with a single meal. Three days later, the birds were reweighed and the logger was removed (see below for details). The colour of droppings was noted to determine the state of digestion, knowing that in birds excreta are whitish (due to uric acid) during feeding and greenish during fasting (Handrich et al., 1993). A large proportion of the birds regurgitated the device before the end of the experiment [57% (4 out of 7) and 50% (4 out of 8) for gastric pH/temperature probes and motility/temperature probes, respectively]. Finally, for the pH/temperature and motility/temperature probes, respectively, 3 and 4 birds retained the device for at least 1 day following the single meal and thus data obtained from these birds could be used for the analyses. These birds were used as controls for gastric pH, motility and temperature evolution during digestion.

### *Experiment 2: Breeding birds*

The female penguin undertakes the first and third incubation shifts, while the male takes the second and fourth shifts (Weimerskirch et al., 1992). For our study, males were marked with a plastic flipper band at the beginning of the second incubation shift. At the beginning of the fourth incubation shift, 15 males were equipped with a stomach probe (7 and 8 males with gastric pH/temperature probes and motility/temperature probes, respectively). For this, the bird's head was covered with a hood so as to minimize handling stress. As a precaution, the egg in the broodpatch was replaced by a dummy egg and was temporarily held in an incubator. The bird was taken away from the colony, its incubating site being marked and preserved with a piece of wood. After weighing, a stomach food sample was collected (see below for the technique used) for chemical composition determination (water, lipid and nitrogen content), and the bird was equipped with a gastric pH/temperature probe or a gastric motility/temperature probe. After this procedure, the bird was brought back to the same place in the colony and its egg restored.

The probes remained in the stomach for 7–8 days, i.e. until about the middle of the incubation fast. This duration meant that we could manipulate incubating birds without compromising their incubation success. After the 7–8 days, the bird was recaught and the probe was retrieved. A stomach food sample was collected (see below) to determine the degree of conservation.

Depending on the type of stomach probe, a specific magnetic or noose-like retrieval tool was used for recovery (Wilson et al., 1998; Wilson and Kierspel, 1998). Importantly, all equipped birds continued incubating after the end of the experiments.

### *Stomach pH/temperature probe*

Stomach probes were used that allowed gastric pH and temperature to be continuously monitored in free-ranging animals. The pH-probe (earth&Ocean Technologies, Kiel,

Germany) is a self-contained pH-meter incorporating a glass microelectrode, a separate reference electrode with a pressure balancing system and a data logger (for details see Peters, 1997a,b). The pH electrode was calibrated before and after each deployment, using traceable NIST standard reference buffers (three calibration points: pH 1.68, 4.01 and 7.01, uncertainty ± 0.02 pH units) under temperature-controlled conditions. Thus any drift could be corrected for and respective measurement uncertainties could be calculated for each deployment (see Peters, 1997a) using the program pHG (Jensen Software Systems, Laboe, Germany). Before deployment, the temperature sensor was calibrated in a water bath against a reference thermometer. The temperature range was 17–42°C with a resolution of <0.1°C (see Peters, 1997a). Data were archived in the programmable data-logger with the sampling interval set to 20 s.

### *Stomach motility/temperature probe*

A device for monitoring both stomach motion and temperature in free-ranging animals was used (earth&Ocean Technologies). The motility probe contains a piezoelectric film sensor (Peters, 2004), which allows the detection of gastric motor activity due to the electric charge generated by a dynamic strain (bending) exerted on the sensor. This type of sensor is rather independent of positioning and of a certain prebending of the sensor, rendering results from different devices comparable (Peters, 2004). The temperature measurement was the same as for the gastric pH/temperature device. Data were archived in a programmable data-logger at a sampling interval of 40 s. This sampling interval was chosen because we wanted to obtain an overall description of the stomach motion rather than the detection of every single contraction wave. Thus the recorded signals summed several contraction waves (cf. Peters 2004, for details on probe performance). The motility is expressed in relative units of the full range (0–255) of the device (Peters, 2004).

### *Food sample collection and chemical composition*

Samples of stomach contents were collected using a non-invasive tube sampling method (by sucking up material with a rubber tube, carefully introduced from the bill into the stomach). In the majority of the cases, two successive samplings were made in order to obtain food samples as representative as possible of the whole stomach contents. The two samples, each about 10–30 ml, were pooled into a tube maintained on ice and homogenized. Several aliquots were taken and frozen at –20°C until laboratory analysis in France.

Before analysis of the chemical composition of the food samples, we checked for the presence of bile pigments that reflect duodenogastric refluxes, i.e. we noted whether food samples were yellow-green. Each sample was weighed, freeze-dried and reweighed. Water content was determined by the difference between fresh and dry masses. Dry samples were ground to a fine powder for lipid and total protein analyses. Lipid content was determined gravimetrically by a method adapted from Folch et al. (1957) on 0.2 g of powder. Nitrogen content was determined using the Kjeldhal method (Hiller et al., 1948) on two 100 mg aliquots of the initial dried powder and converted to protein by multiplying by 6.25.

### *Statistical analyses*

Comparisons among days in experiment 1 were made using ANOVA for repeated measures (RM-ANOVA) followed by multiple comparison (Student–Newman–Keuls test). In experiment 2, comparisons between the onset and the end of the experiment, for body mass and chemical composition of the food, were made using a paired *t*-test. Comparison among days of the experiment for gastric temperature was made using RM-ANOVA) followed by multiple comparison (Student–Newman–Keuls test). Non-linear regression analysis was performed using the software Sigmaplot (Jandel SPSS, Chicago, USA). Data are expressed as means ± S.D.

Daytime and night-time were identified using day-length data for Crozet Archipelago for the experimental period (http://www.bdl.fr/cgi-bin/levcou.cgi).

## Results

### *Experiment 1: Non-breeding birds*

Daily mean values of pH, motility and temperature of the stomach were not significantly different before and after the feeding trial ($P>0.05$, RM-ANOVA, Table 1) except during the day of feeding, when this was related to the occurrence of minima and/or maxima of these parameters at the moment of logger administration (Figs 1, 2, Table 1). The pH showed high fluctuations in the range of between pH 2.5 and 5 several times per day during the entire course of the experiment. Maximum pH values usually occurred when stomach temperature was lowest (Fig. 1). The daily changes in gastric motility paralleled those of stomach temperature, minimum values of motility

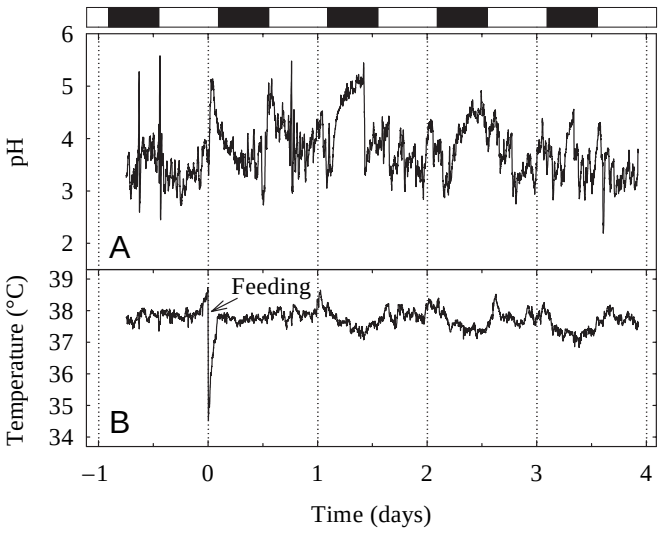


Fig. 1. Representative changes of gastric pH (A) and temperature (B) before, during and after a meal in a non-incubating king penguin (bird III). Feeding (610 g) is indicated by the arrow in B. Night-time is represented by the black parts of the upper horizontal bar.

Table 1. *Daily gastric pH, motility, and temperature, before and after a fish meal, in non-incubating king penguins*

| Parameters | Fasting day | Day after feeding 0 | 1 | 2 | RM-ANOVA |
|---|---|---|---|---|---|
| pH | | | | | |
| Daily | 2.94±0.51 (3) | 3.44±0.47 (3) | 3.18±0.89 (3) | 3.14±0.99 (2) | NS |
| Minimum | 2.29±0.65$^{a,b}$ (3) | 2.84±0.57$^{c}$ (3) | 2.40±0.84$^{b,c}$ (3) | 2.48±0.93$^{b,c}$ (2) | $P$=0.039 |
| Maximum | 3.54±0.31 (3) | 4.32±0.42 (3) | 3.92±1.08 (3) | 4.10±0.64 (2) | NS |
| Motility | | | | | |
| Daily | 26.8±6.4$^{a}$ (4) | 13.6±3.9$^{b}$ (4) | 23.3±3.9$^{a}$ (4) | 28.2±8.1$^{a}$ (3) | $P$=0.011 |
| Minimum | 6.5±2.6$^{a,b}$ (4) | 1.9±0.8$^{c}$ (4) | 3.2±1.9$^{b,c}$ (4) | 3.6±0.9$^{b,c}$ (3) | $P$=0.042 |
| Maximum | 87.5±24.9 (4) | 50.9±16.2 (4) | 81.0±41.1 (4) | 76.2±38.5 (3) | NS |
| Temperature | | | | | |
| Daily | 37.8±0.7$^{a}$ (6) | 37.5±0.9$^{b}$ (5) | 37.7±0.7$^{a}$ (6) | 38.1±0.2$^{a}$ (5) | $P$=0.007 |
| Minimum | 37.3±0.8$^{a}$ (6) | 35.5±0.8$^{b}$ (5) | 37.2±0.7$^{a}$ (6) | 37.5±0.2$^{a}$ (5) | $P$<0.001 |
| Maximum | 38.5±0.7$^{a}$ (6) | 38.1±0.7$^{b}$ (5) | 38.4±0.7$^{a}$ (6) | 38.7±0.3$^{a}$ (5) | $P$=0.005 |

Values are means ± S.D. ($N$=number of birds).

Daily pH is the daily mean of data collected every 20 s. Minimum and maximum values were obtained from mean 1 h pH, motility or temperature data.

Values in the same line followed by different letter superscripts are significantly different ($P$<0.05); NS, not significant.

For temperature, data from 6 birds instead of 7 were used because one temperature sensor was out of order from the onset of the experiment. Similarly, on day 0 after feeding we have 5 birds instead of 6 because one temperature logger was temporarily defective for an unknown reason. The number of birds decreased over days because some individuals regurgitated the device before the end of the experiment.

Motility is expressed in relative units of the full range (0–255) of the device (Peters, 2004).

occurring when the temperature was lowest (Fig. 2). The daily rhythmic fluctuation of stomach temperature was well defined, with lowest values occurring during the nocturnal period (Figs 1, 2). Except during the day of feeding (RM-ANOVA, $F_{3,21}$=32.649, $P$<0.001), the daily amplitude of temperature during the post-feeding days was not different from the previous fasting day ($P$>0.05, 1.17±0.25°C in day 1, 1.21±0.31°C in day 2, 1.16±0.30°C in day –1; Table 1).

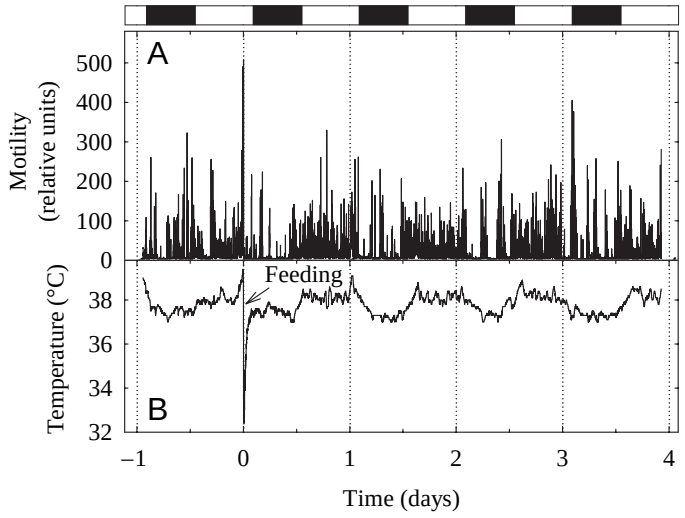


Fig. 2. Representative changes of gastric motility (A) and temperature (B) before, during and after a meal in a non-incubating king penguin (bird IV). Feeding (650 g) is indicated by the arrow in B. Night-time is represented by the black parts of the upper horizontal bar.

*Experiment 2: Breeding birds*

*Body mass changes*

The body mass of incubating birds significantly decreased over the period studied (paired $t$-test, $t$=1.471; $P$<0.001), on average by 10.4±1.4%, corresponding to a specific daily body mass loss of 15.1±2.3 g kg$^{-1}$ day$^{-1}$.

*Gastric pH evolution during the incubation fast*

Fig. 3A shows an individual example (bird 5) of pH and gastric temperature recordings during the first week of incubation. For three birds (2, 5 and, to a lesser extent, 4), pH showed unexplained fluctuations at the beginning of the recording period, so that we decided not to consider those hours concerned for further analyses (see Fig. 4).

The changes in gastric pH appeared to be highly different among incubating birds during the logger deployment (Fig. 4). For birds 1, 2, 3, 4 and 5, mean hourly pH values rarely fell below ca. pH 4 throughout the days of the experiment, i.e. values higher than the daily minimum values (range: 2.4±0.8–2.8±0.6, Table 1), and even the daily mean values (range: 3.1±1–3.4±0.5, Table 1) observed in the control group (Fig. 4). Conversely, in the two other incubating birds, the pH either decreased and was maintained at values as low as the minimum daily values observed for control birds from day 4 of the fast (bird 6) or repeatedly decreased down to these values after rather large oscillations (bird 7).

Compared to the mean value calculated for the control birds, the mean pH over the whole study period for birds 1, 2, 3, 4 and 5 was significantly higher ($P$<0.05) than

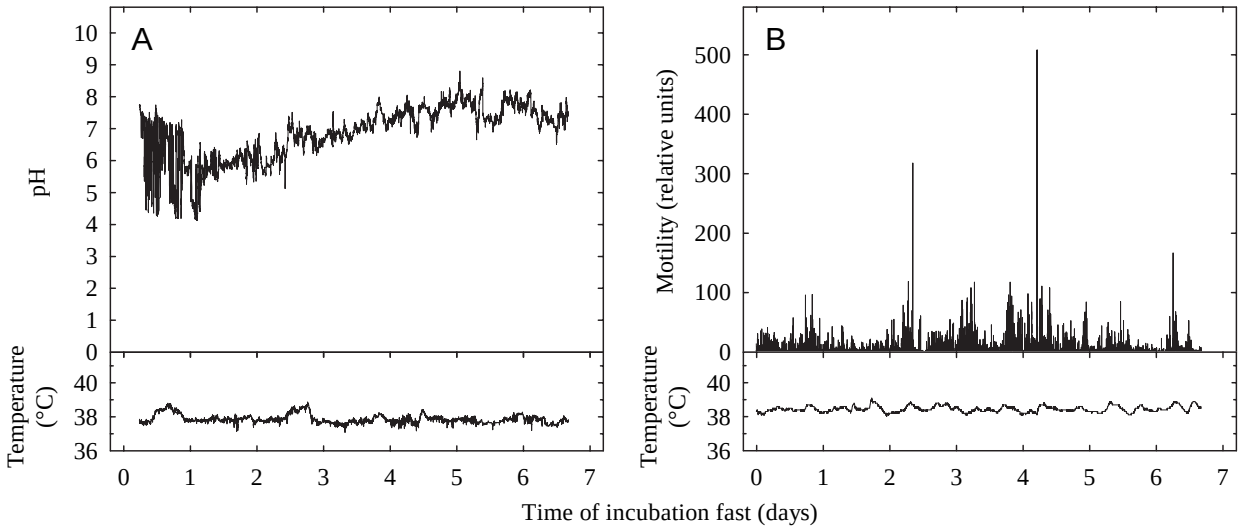


Fig. 3. Individual examples of evolution of gastric pH and temperature (A; bird 5) and of gastric motility and temperature (B; bird 11), in two incubating male king penguins.

that of control birds, with a difference of about 80%. No significant differences were found between control birds and incubating birds 6 and 7 (Fig. 5A).

*Gastric motility evolution during the incubation fast*

Fig. 3B shows an individual example of gastric motility and temperature recordings during the first week of incubation. As for pH, gastric motility evolution appeared to be very different among incubating birds (Fig. 6). Birds 9, 11 and 12 showed extremely low values throughout the fast with 94.4±1.7% of the mean hourly values lower than 10 (relative units), i.e. values clearly lower than the daily mean values observed in the control group during the days after feeding (range: 13.6±3.9–28.2±8.1 relative units; Table 1). On the other hand, for bird 14 a high proportion of values (63.2%) was above 30 (relative units), i.e. values higher than the daily mean values observed in the control group. Intermediate profiles of motility were also found between these two extremes (Fig. 6).

Compared to the mean value calculated for the control birds, the mean motility over the whole experimental period appeared to be significantly lower for birds 9–12 ($P<0.05$), while values were comparable for birds 8, 13 and 15, and higher for bird 14 (Fig. 5B).

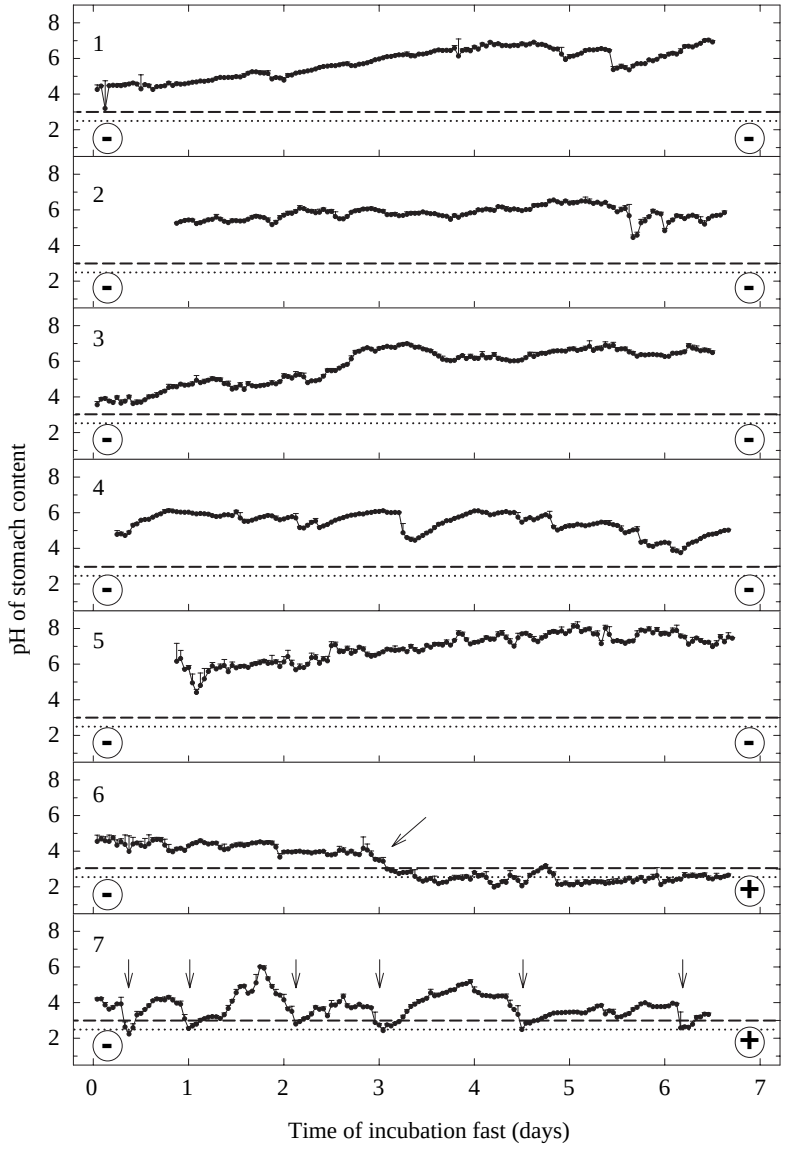


Fig. 4. Changes in mean hourly pH of the stomach contents of seven male king penguins (birds 1–7) during the first week of their incubation fast. Daily and minimum mean values found for control birds are indicated by broken and dotted lines, respectively. Absence (–) or presence (+) of bile pigment coloration in the food is indicated at the beginning and the end of the period studied. Values are means ± S.D.

*Gastric temperature evolution during the incubation fast*

The daily rhythmic fluctuations in stomach temperature were much less pronounced during the incubation fast (Fig. 3) than in the control group (Figs 1, 2). Except during the first day (38.1±0.4°C), daily temperature did not vary significantly ($P$>0.05) over the course of the experimental period, with a mean value of 37.9±0.3°C ($N$=14 birds).

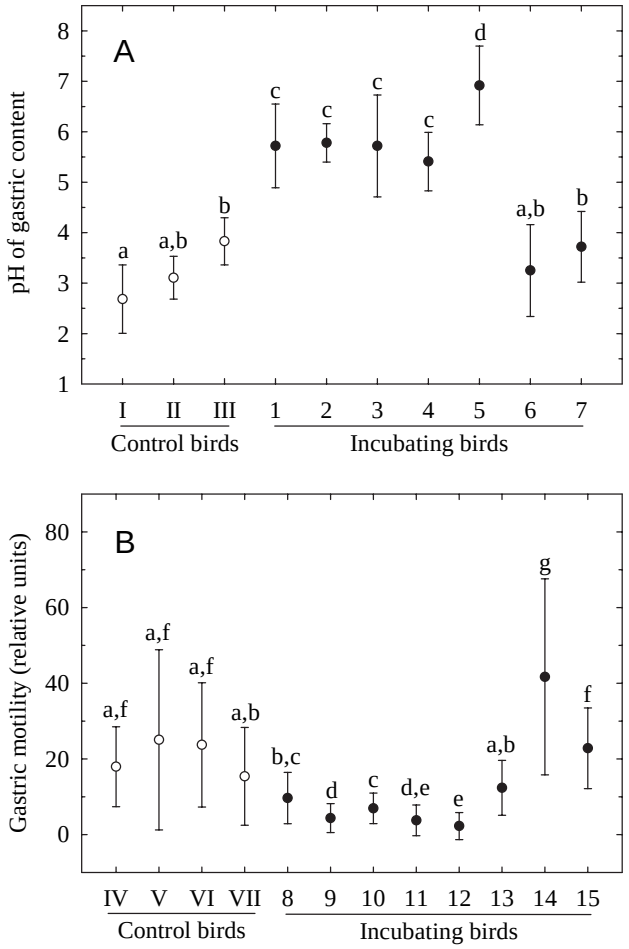


Fig. 5. Mean values of gastric pH (A) and motility (B) over the study period of fasting for incubating birds (1–15) and over the days after feeding for control birds (I–VII). Values in the same figure with different superscripts are significantly different ($P$<0.05). Values are means ± S.D. As the required conditions for applying a parametric procedure were not fulfilled, comparisons among birds were performed using a Kruskal–Wallis one-way ANOVA on ranks.

*Chemical composition of stomach contents during the incubation fast*

The chemical composition of stomach contents exhibited high variability, both between the onset and the end of the experimental period as reflected by the S.D. values and data ranges, and among individuals of the same fasting stage (Table 2).

On an individual basis, fresh lipid content was either elevated, constant or decreased between the onset and the end of the experiment, with amplitude ranging from –83.7% (bird 14) to +16.3% (bird 12). On average for the whole group of birds, however, fresh lipid content decreased significantly (–26.8±34.0%; paired $t$-test, $t$=2.858; $P$<0.02). Dry lipid content decreased between the beginning and the end of the experiment in all but one bird, for which lipid content was slightly increased, with amplitude ranging from –65.8% (bird 14) to +4.8% (bird 6). On average for the 15 birds, dry lipid content significantly decreased (–28.0±17.9%; paired $t$-test, $t$=5.221; $P$<0.001).

As was the case for lipids, fresh protein content was either elevated, constant or decreased between the onset and the end of the experiment, with amplitude ranging from –56.5% (bird 15) to +79.7% (bird 7). On average there was no significant variation in the fresh protein content of food ($P$>0.05). Dry protein content increased between the beginning and the end of the experiment in all but one bird, with an amplitude ranging from –0.6% (bird 15) to +46.6% (bird 2). On the average, for the whole group of 15 birds, dry protein content increased between the beginning and the end of the experiment (+15.1±11.4%; paired $t$-test, $t$=–5.909; $P$<0.001).

Notably, there was a significant ($P$=0.002) positive correlation between the decrease in dry food lipid content and the mean motility during fasting (Fig. 7).

None of the food samples at the beginning of the experiment showed bile pigment coloration (Figs 4, 6). At the end of the experiment, 33% of the birds had stomach contents coloured by yellow-green bile pigments (birds 6, 7, 13, 14 and 15).

Table 2. *Composition in water, lipid and protein for stomach contents of 15 incubating king penguins at the onset and end of the period studied*

| | | Fresh content (%) | | Dry content (%) | |
|---|---|---|---|---|---|
| Period of the trial | Water content (%) | Lipid | Protein | Lipid | Protein |
| Onset | | | | | |
| Mean±S.D. | 83.6±3.6 | 4.7±2.1 | 9.4±2.1 | 27.7±7.7 | 57.5±6.2 |
| Range | [77.8, 91.0] | [1.4, 8.5] | [4.9, 11.7] | [12.8, 39.6] | [43.0, 64.6] |
| End | | | | | |
| Mean±S.D. | 84.9±4.4 | 3.1±1.7 | 10.0±3.0 | 19.5±6.4 | 65.7±6.0 |
| Range | [78.6, 93.4] | [0.7, 6.8] | [3.8, 16.0] | [9.5, 31.9] | [57.3, 78.6] |

Comparisons between the onset and the end of the experiment were done using a paired $t$-test (see text for the results).

## Discussion

Here we provide the first *in situ* data on adjustments of key gastric digestion parameters accompanying prolonged food storage during the incubation fast of free-living king penguins. The pH and gastric motility were modified in a way that should inhibit gastric digestive processes. In contrast, the stomach temperature did not change relative to food conservation, which might be linked to temperature constraints on incubation. Finally, the large interindividual variability we observed in the extent of food conservation among birds highlights the importance of an intraindividual approach to the parameters studied throughout the incubation fast.

### *Gastric pH*

Our study demonstrates that a high modulation of gastric pH occurs in male king penguins at the end of the incubation fast. The maximum pH is similar to that observed for Magellanic penguins, i.e. around pH 6 (Peters, 1997a). However, there is a substantial difference among penguin species when comparing the duration of such an adjustment. While the smaller penguin species can maintain a high gastric pH for only a couple of hours or possibly up to a few days, we observed that a high pH can be maintained during the whole period of logger deployment in incubating male king penguins, i.e. for at least a week. In order not to threaten the reproductive success and also to ensure logger recovery, we did not extend our logger deployments until the female's return. From our data, however, it seems reasonable to suggest that the observed pH adjustment could be maintained during the whole 2–3 weeks of incubation until hatching.

Importantly, our study shows that pH profiles differ among the birds. This finding, in addition to a high variability in both chemical composition and visual aspect of the food, most probably means that the birds conserve food to a greater or a lesser degree. Thus, the pH for birds 6 and 7, that reached values as low as those observed during digestion in control birds and was associated with bile pigment coloration of the stored food at the end of the experiment, presumably reflects a lower efficiency in their food conservation during incubation. Why such variability exists remains to be elucidated. Gauthier-Clerc et al. (2000)

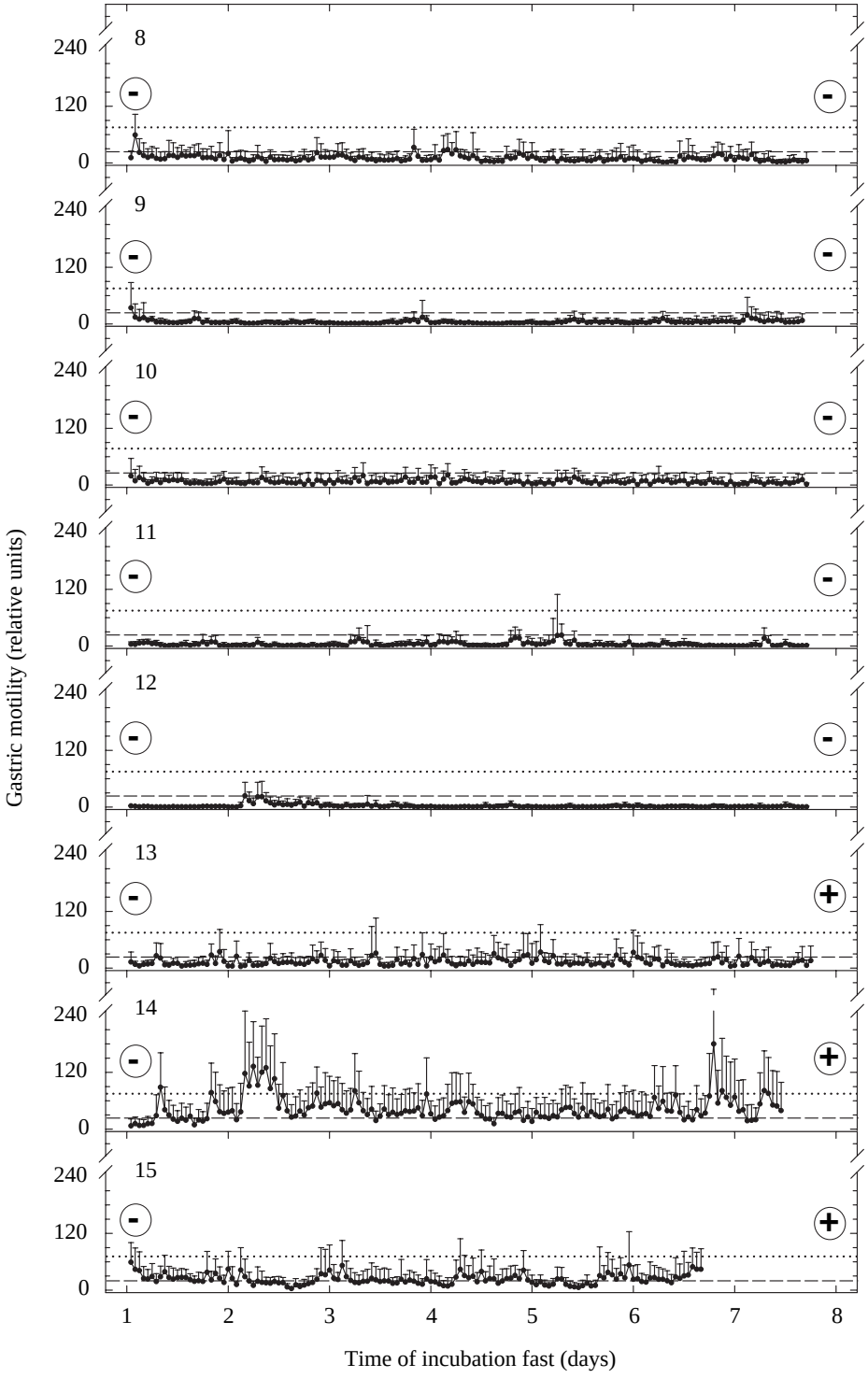


Fig. 6. Changes in mean hourly motility in eight male king penguins during the first week of their incubation fast. Daily and maximum mean values found for control birds are indicated by broken and dotted lines, respectively. Absence (–) or presence (+) of bile pigment coloration in the food is indicated at the beginning and the end of the period studied. Values are means ± S.D.

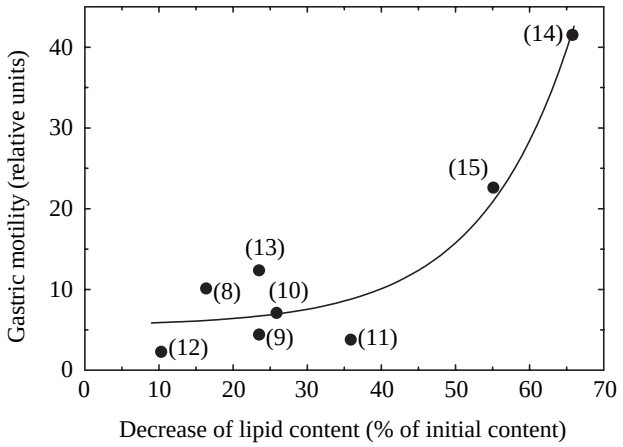


Fig. 7. Relationship between mean gastric motility and the decrease in lipid content of stomach content in male king penguins during the first week of their incubation fast. $y$=5.479+0.187exp(0.08$x$); $R^2$=0.92; $F_{2,7}$=30.0; $P$<0.002. The number of the bird at each point is given.

have suggested that in breeding king penguins some kind of internal clock could exist that would allow the male king penguins returning from the sea to bring back food in their stomach only if their arrival falls within the hatching schedule, i.e. if the probability that the bird will be on land at the hatching date is high. Such an internal clock could also influence the level of food conservation during incubation.

The pH variations observed most certainly reflect fundamental modulations in the gastric digestive function affecting enzymatic processes. Since avian gastric proteinases are adapted to acid conditions (Duke, 1986), the pH values found in the present study, usually higher than 4 and sometimes even as high as 6, are unfavourable for gastric protein breakdown. Thus, food protein is probably preserved not only in quantity (Gauthier-Clerc et al., 2002) but also in quality. It is more difficult to judge the extent of food lipid conservation. Indeed, regular refluxes from the duodenum into the stomach are a peculiarity of avian digestion and it is therefore likely that stomach contents are exposed to pancreatic and duodenal lipases (Duke, 1997). In the hypothesis of passage of some pancreatic enzymes from the duodenum to the stomach *via* refluxes, the pH of about 6 monitored in incubating penguins would allow these lipases to be active. Thus, some hydrolysis of food lipids could occur during storage, which would be consistent with the decrease in total lipid content observed during the period of food storage (Gauthier-Clerc et al., 2002; this study).

Even if the presence of food in the stomach results in itself in a short- to medium-term increase in pH (Rhoades and Duke, 1975), as we observed in the control experiment on the day of feeding, it does not explain the observed longer-term adjustments. The particular mechanisms responsible for these pH adjustments are not yet identified, as complex interactions among neural, hormonal and paracrine mechanisms are involved in the regulation of acid secretion in the stomach (Fanning et al., 1982). Factors leading to an inhibition of gastric acid secretions can originate from the animal's own physiology or from external stimuli. In fasting incubating king penguins, it seems unlikely that the high pH found in some birds would be linked to external factors, like ingesting food, since food quality seems to be the same among individuals irrespective of whether they conserved their stomach contents to a greater or a lesser extent. Furthermore, the positive correlation between the mass of the stomach contents brought back from sea and the probability of the bird to be on land at the time of hatching (Gauthier-Clerc et al., 2000) would indicate that the storage process is probably initiated by the bird itself and not by an external factor.

### *Gastric motility*

Gastric motility was markedly reduced for most of the incubating birds fasting with food in their stomach. In contrast, a small proportion of the incubating birds exhibited a gastric motility as high as that of control birds during digestion. For these incubating birds, a bile pigment coloration was found in the food at the end of the study period and their high gastric motility was associated with a large decrease in food lipid content compared to other birds. Thus, motility was variable, a lower motility probably being associated with a better conservation of stomach content. Such a correlation between inhibition of gastric digestion and stasis of gastric motility has been made in several amphibians (Fanning et al., 1982; Taylor et al., 1985).

Motor function is a major factor in the control of gastric emptying, and the conservation of the amount of food stored in the stomach, which was previously shown during incubation (Gauthier-Clerc et al., 2000), was probably correlated with an inhibition of the bird's gastric motility. In the present study, we did not determine the whole mass of the stomach contents, because flushing the birds would have threatened their reproductive success. However, the lower the mean stomach motility during the period of logger deployment, the lower was the decrease in lipid content of the gastric contents. Moreover, the stomach contents of most birds were in an almost unchanged state of preservation at the end of incubation compared to the beginning, still containing intact pieces of fish and squid (data not shown). This supports the hypothesis of a rather constant mass of stored food that has been efficiently preserved in the stomachs of our birds.

While the reduction of gastric motility can be important in penguins incubating with food in their stomach (see Fig. 6 for birds 11 and 12), it was never total. In fasting birds, the amplitude of motility was reduced but it still persisted, suggesting the influence of a 'pacemaker' (Duke and Evanson, 1976), which would initiate and regulate the gastric motility (Chaplin and Duke, 1988). The minimal gastric motility observed in incubating king penguins fasting with a full stomach might then be due to this gastric pacemaker.

Gizzard contraction frequency has sometimes been used as an index of overall gastrointestinal motility (Savory, 1987). In domestic fowl, gastric contraction is generally followed by

duodenal spikes, and a reduction in stomach activity induces an increase in antiperistalsis (Roche and Ruckebusch, 1978). These observations led the authors to suggest a permanent balance between intestinal and gastric activities in this species. However, their hypothesis may not be valid for king penguins, since an increase in antiperistalsis would have resulted in biliary pigmentation in the stomach content, which was not the case in most of our birds. This observation leads us to suggest a decrease in intestinal motility along with gastric motility in penguins, similar to reports in the gastric brooding frog (Fanning et al., 1982). Such a mechanism would be advantageous for food conservation in the stomach because an inhibition of duodenal motility would preclude the entry of pancreatic and intestinal enzymes into the stomach.

In penguins, the stomach constitutes the main retention organ of the solid component of ingested food. Its control is mainly under pyloric influence since this sphincter seems to prevent the gizzard from discharging material that has not been ground into small enough particles (Ferrando et al., 1987; Vergara et al., 1989). Thus, in penguins, it can be hypothesized that the mechanisms of gastric motility inhibition act principally on the pylorus. The high pH observed could also stimulate food retention in the stomach, in accordance with the delayed crop emptying caused by omeprazole-induced inhibition of gastric secretion of HCl in the chicken (Mabayo et al., 1995).

### *Temperature*

We found no difference in the stomach temperature of incubating king penguins whatever the extent of their gastric content preservation, excluding temperature adjustments from those factors involved in the mechanisms of stomach food preservation. No day–night stomach temperature cycle occurred in incubating birds, in contrast to our observations for non-incubating penguins. In the latter, the diel temperature pattern observed was probably the result of movements and thus of muscular heat generation (Aschoff, 1970). Whereas incubating king penguins remain active by day as well as by night (Challet et al., 1994; Le Maho et al., 1993), they spend most of their time resting in a motionless state during the course of fasting (Challet et al., 1994). This might contribute to the constancy of stomach temperature, but more importantly, this stability can be explained by the need to hold the temperature of the egg at an adequate level for embryo development, independent of the bird's digestive status. Indeed, the average stomach temperature (38.0°C; present study, or 38.2°C; Thouzeau et al., 2003) is identical to the brood patch temperature, which is maintained constant during incubation (Handrich, 1989). A similar absence of a distinct day–night cycle in core temperature has been observed in incubating blue petrels *Halobaena caerulea* (Ancel et al., 1998) and only reappeared in case of egg desertion, probably reflecting day–night bird movements. In some of our incubating birds, however, a positive correlation was observed between stomach temperature and motility (data not shown), which might correspond to a breeder being more active and engaged in territory defense behaviour.

### *Conclusions and perspectives*

The present study demonstrates the existence of substantial adjustments of pH and gastric motility in a way that contributes to the inhibition of digestive gastric processes in incubating male king penguins. Mechanisms underlying these adjustments are probably complex, including a combination of neuronal, humoral and/or hormonal factors. The body mass of our birds was always above the critical value that could jeopardize their survival in a prolonged fast (Cherel et al., 1988). Therefore, body condition was not involved in the decision of the bird to conserve its stomach contents to a greater or a lesser degree. The fact that incubating male penguins fast with a full stomach only when the probability of their being on land for hatching is high (Gauthier-Clerc et al., 2000) leads us to suggest the involvement of reproductive hormonal factors in gastric digestion inhibition during the last part of the incubation. It would therefore be interesting to determine whether different mechanisms are involved in the initiation of food storage during foraging at sea and the maintenance of this storage while on shore.

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