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Heat increment of feeding in double-crested cormorants (*Phalacrocorax auritus*) and its potential for thermal substitution

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SUMMARY

Diving endotherms inhabiting polar regions face potentially high thermoregulatory costs. Unless properly insulated, these animals will lose vast amounts of heat when diving in cold water, which has to be balanced by heat production. Heat generated as a by-product of digestion (heat increment of feeding, HIF) or from exercising muscles might be important in maintaining thermal balance under such conditions, as it would reduce the need for shivering thermogenesis. Recording the rate of oxygen consumption ($\dot{V}_{O_2}$), respiratory exchange ratio (RER), and stomach temperature, we studied the magnitude and duration of HIF in seven double-crested cormorants (*Phalacrocorax auritus*) following the voluntary ingestion of a single herring (*Clupea pallasi*) while birds rested in air. Conducting trials at thermoneutral (21.1±0.2°C) and sub-thermoneutral temperatures (5.5±0.7°C), we investigated the potential of HIF for thermal substitution. After the ingestion of a 100 g herring at thermoneutral conditions, $\dot{V}_{O_2}$ was elevated for an average of 328±28 min, during which time birds consumed 2697±294 ml O_2 in excess of the resting rate. At sub-thermoneutral conditions, duration (228±6 min) and magnitude (1391±271 ml O_2) of $\dot{V}_{O_2}$ elevation were significantly reduced. This indicates that cormorants are able to use the heat generated as by-product of digestion to substitute for regulatory thermogenesis, if heat loss is sufficiently high. Altering meal size during sub-thermoneutral trials, we also found that HIF in cormorants was significantly greater after larger food intake. Based on these experimental results, a simple calculation suggests that substitution from HIF might reduce the daily thermoregulatory costs of double-crested cormorants wintering in coastal British Columbia by ~38%. Magnitude of HIF and its potential for thermal substitution should be integrated into bioenergetic models to avoid overestimating energy expenditure in these top predators.

Key words: heat increment of feeding, thermal substitution, cormorant, thermoregulation, ecological energetics, respirometry, time–energy budget.

INTRODUCTION

Heat loss in diving endotherms is potentially high because water has a greater specific heat capacity and thermal conductivity than air. Avian divers rely to a large extent on the air layer trapped within their plumage for insulation. With increasing dive depth, however, the air layer becomes compressed, reducing insulation. Consequently, heat loss during foraging is greatly increased, especially when birds dive for extended periods and/or to great depth. If core temperature is to remain stable, heat loss has to be balanced by heat production. In birds such heat production is provided *via* facultative and obligatory thermogenesis. The former comprises heat production *via* shivering (Dawson and Whittow, 2000), which is specifically activated to maintain a stable body temperature during periods of increased heat loss (Hohtola, 2002). Besides shivering, other thermogenic reactions occur in birds and represent the obligatory component of thermogenesis. For example, heat is produced during the processes associated with the digestion and assimilation of food (heat increment of feeding, HIF) and during physical activity (muscular work). These processes underlie behavioural control to some extent and the heat produced could potentially be used to substitute for the facultative component of thermogenesis, thereby reducing thermoregulatory costs.

The heat increment of feeding (HIF), also referred to as specific dynamic action (SDA), might be especially important in supplementing heat production in resting endotherms exposed to sub-thermoneutral temperatures. In its essence, HIF is the increase in resting metabolic rate observed after ingestion of a meal, associated with heat production during the processes of digestion, assimilation and nutrient interconversion (Brody, 1945). Magnitude of HIF depends on meal size (Janes and Chappell, 1995; Kaseloo and Lovvorn, 2003; Green et al., 2006) and food composition (Blaxter, 1989). For example, because of differences in intermediary metabolism, high-protein foods tend to provoke a greater HIF than foods containing mainly lipid or carbohydrate (Blaxter, 1989). HIF has been investigated across a wide range of animal species (for a review, see McCue, 2006), although few studies considered avian divers. Early studies, in which birds were force-fed, produced conflicting results. Wilson and Culik found no evidence for HIF in Adélie penguins *Pygoscelis adeliae* (Wilson and Culik, 1991), and suggested that the increase in energy expenditure observed following ingestion was due to the physical costs of heating food to body temperature rather than to HIF. By contrast, Janes and Chappell found HIF to be present in Adélie penguin chicks, which accounted for ~10% of the gross energy (GE) intake (Janes and Chappell, 1995). More recent studies, in which birds ingested food voluntarily while resting in air, confirmed the presence of HIF in Brünnichs guillemots *Uria lomvia* (Hawkins et al., 1997) and little penguins *Eudyptula minor* (Green et al., 2006). Further confirmation is provided by studies of Kaseloo and Lovvorn, which most closely replicated ecologically relevant

conditions (Kaseloo and Lovvorn, 2003; Kaseloo and Lovvorn, 2005; Kaseloo and Lovvorn, 2006). They investigated HIF in mallard (*Anas platyrhynchos*) and lesser scaup ducks (*Aythya affinis*) which dabbled and dived for their food, respectively. Hence, just as for many other animal species, the presence of HIF in aquatic birds has been clearly demonstrated. Despite this demonstration and the recognition that HIF might account for a substantial portion of the energy of the ingested food, its exact nature and functional significance are still unclear (McCue, 2006). The latter point is illustrated by the equivocal findings of studies investigating the significance of HIF for thermoregulation. Results range from none to partial and even complete use of heat generated by HIF or exercising muscles for thermoregulation [see appendices 1 and 2 in Lovvorn (Lovvorn, 2007)].

Cormorants are foot-propelled pursuit divers that forage predominantly on fish (Johnsgard, 1993). They are unique among aquatic birds in having a partially wettable plumage (Grémillet et al., 2005a), so that the thickness of their plumage air layer is decreased. Consequently, their buoyancy is reduced and, therefore, the mechanical work required to counter buoyancy during diving (Lovvorn and Jones, 1991). However, a partially wettable plumage will also increase heat loss in water, especially during diving, and this effect will increase with dive depth. A wet plumage will also lead to a greater heat loss when resting in air. It is therefore no surprise that cormorants leave the water after a foraging bout and vigorously shake off water from their plumage. Some cormorant species inhabit thermally challenging environments. For example, a small population of great cormorants *Phalacrocorax carbo carbo* winters in West Greenland near the Artic Circle, encountering water temperatures below 0°C and air temperatures as low as –30°C (Grémillet et al., 2005b). Heat loss under these circumstances might be extremely high. This is illustrated by abdominal temperature decreases that have been observed throughout dive bouts of great cormorants [(Grémillet et al., 2005b) average decrease 1.9°C]. Nevertheless, Greenland cormorants continue to dive throughout the winter for up to several hours per day (Grémillet et al., 2005b). Consequently, thermoregulatory costs might account for a substantial part of their overall daily energy budget, unless birds are able to use heat produced through HIF or by exercising muscles (e.g. during flight when leaving the foraging area) to substitute for shivering thermogenesis.

In the present study, we used double-crested cormorants (*P. auritus*), which are closely related and morphologically similar to great cormorants, to investigate the magnitude and time course of the heat increment of feeding following a standard meal (100 g herring, *Clupea pallasi*) at thermoneutral conditions. In a second set of trials, conducted at sub-thermoneutral temperatures, we tested the hypothesis that cormorants use the heat generated by HIF to substitute for shivering thermogenesis. Finally, we studied the effect of meal size on HIF.

MATERIALS AND METHODS

Animals

Seven adult double-crested cormorants *Phalacrocorax auritus* Lesson, body mass 2.10±0.01 kg (mean ± s.e.m., range 1.81–2.21 kg), were used in this study. Six of the birds were captured as chicks (5–6 weeks of age) from the Mandarte Island breeding colony, BC, Canada, while one bird was bred in our captive setting. All birds were well established in captivity. Details of holding conditions and animal care are published elsewhere (Enstipp et al., 2006a). All experimental procedures were approved by the UBC Animal Care Committee (Animal Care Certificates no. A02-0122 and A06-0421) and were in compliance with the principles promulgated by the Canadian Council on Animal Care.

Respirometry measurements

Rates of oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) were measured during trials using an open-circuit respirometry system (Sable Systems, Henderson, NV, USA). A 55 l bucket (0.35 m diameter×0.65 m high) with an airtight Plexiglas™ lid served as a respirometry chamber, through which air was drawn *via* four small side holes near its bottom. Air flow during the trials was maintained at 10–11 l min^{-1} using a vacuum pump (Gast Manufacturing Inc., Benton Harbour, MI, USA). The main airflow from the respiration chamber was dried using silica gel before being led into a mass-flowmeter (Sierra Instruments Inc., Monterey, CA, USA), which automatically corrected the measured flow to STPD (273 K and 101.3 kPa). A sub-sample of 8 l min^{-1} was bled into a manifold from which an oxygen (paramagnetic O_2-analyser PA-1B, Sable Systems; resolution: 0.0001%) and CO_2 analyser (Beckman LB2 Medical CO_2-analyser, Schiller Park, IL, USA; resolution: 0.01%) sampled in parallel. All connections between the various components of the respirometry system were made with gas-impermeable Tygon™ tubing.

Oxygen concentration inside the respiration chamber was above 20.5% and CO_2 concentration was below 0.4% during all trials. The gas analysers were calibrated before each trial using 99.995% pure N_2, 0.95% CO_2 (PraxAir, Richmond, BC, Canada) and outside air (set to 20.95% O_2 and 0.03% CO_2). Analyser drift was minimal but, if any occurred, it was corrected for during data analysis. Before a trial the entire system was tested for leaks by infusing pure N_2 gas. Time delay between air leaving the respiration chamber and arriving at the gas-analysers was calculated by dividing the total volume of the tubing and drying columns by the flow rate. The delay was found to be 13 s for both analysers and was taken into account when calculating $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ and relating them to feeding events. The time constant of the respiration chamber was calculated to be 5.5 min. Data from the flowmeter and the gas analysers were fed into a universal interface (16 bits resolution, Sable Systems) and average values were recorded every 5 s onto a desktop computer using Datacan (Sable Systems).

Stomach temperature

In parallel with the respirometry measurements, temperature loggers (MiniTemp-xl; length 70 mm, diameter 16 mm, mass 25 g, resolution 0.03 K; earth&OCEAN Technologies, Kiel, Germany) were deployed in all birds to monitor temperature inside the proventriculus (hereafter 'stomach temperature') during feeding trials as a proxy for body temperature. Temperature loggers were programmed to record stomach temperature every 15 s and were fed to the birds inside a refrigerated herring before the start of experimentation. During HIF trials, temperature recordings enabled us to determine the time required to warm the ingested cold fish to body temperature. Furthermore, in separate trials five birds were fed various amounts of herring and smelt (*Osmerus mordax*) (refrigerated at ~5°C) when resting in their pen. The exact amounts (range: 19–118 g) and feeding times were noted to investigate the relationship between the amount of fish ingested and the required heating time. Loggers were equipped with a spring crown and were not regurgitated by the birds but retrieved through stomach flushing at the end of experimentation, after about 10 days (for details, see Wilson and Kierspel, 1998). Upon retrieval the data were downloaded to a laptop computer.

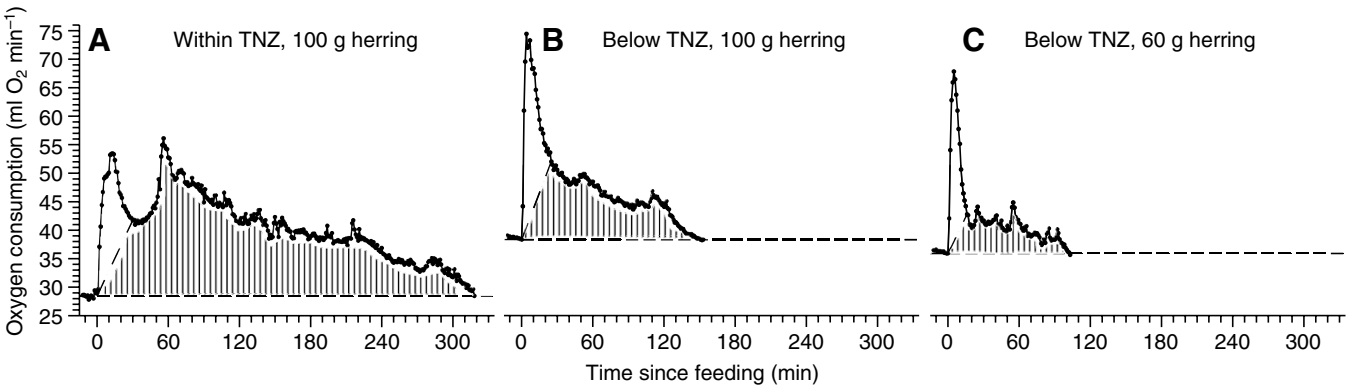

Fig. 1. Examples of changes in rates of oxygen consumption ($\dot{V}_{O_2}$) associated with the voluntary ingestion of a single herring. (A) The bird was resting within its thermoneutral zone (TNZ) and fed a 100 g herring at time zero. (B,C) Birds were resting at sub-thermoneutral conditions (below ~9°C) and fed a 100 g (B) or 60 g herring (C), respectively at time zero. The initial peak in $\dot{V}_{O_2}$ is related to movements during the feeding event and was excluded (using linear interpolation, see broken line) when calculating HIF (indicated by the shaded area). The horizontal broken line denotes resting $\dot{V}_{O_2}$, established from the stable period preceding feeding.

Experiments

We conducted two series of experiments. (1) In Nov–Dec 2003, three cormorants participated in feeding trials at environmental temperatures below their lower critical temperature [calculated to be ~9°C, eqn 11.9 (Ellis and Gabrielsen, 2002)]. During these trials the respirometer was positioned outside (mean air temperature: 6.6±0.4°C; range: 4.0–8.6°C) and birds were fed a single herring of mass either 60 g or 100 g. (2) In Nov 2006, we conducted feeding trials with five cormorants during which they were fed a single 100 g herring. The respirometer during these trials was kept inside a temperature-controlled room, with continuous ventilation from the outside. Mean air temperature during these trials was 21.1±0.2°C (range: 20.0–22.6°C) and was well within the thermoneutral zone (TNZ) of double-crested cormorants (Ellis and Gabrielsen, 2002). Air temperatures in the respiration chamber were monitored using a digital thermometer (Oregon Scientific, Portland, OR, USA) and did not fluctuate by more than ±2°C during all trials.

All birds were fasted for ~16 h (range: 13–29 h) before experiments. At the beginning of a trial a bird was captured in its holding pen, weighed and put into the respirometer. After the initial excitement, birds settled quickly inside the darkened chamber and typically sat on a piece of Styrofoam™ at the chamber bottom. When a stable level of O_2 and CO_2 was reached and maintained for at least 10 min (usually after ~1 h), the lid of the chamber was moved slightly to one side and the bird was offered a single herring. In preliminary trials birds would not ingest the offered fish voluntarily and were consequently force-fed. Since this caused considerable disturbance to the birds, we discarded these trials. Hence, only trials during which a bird readily took the herring offered and ingested it voluntarily are reported here. Once the bird had swallowed a fish, the lid was quickly closed again and the bird left undisturbed for the remainder of the trial. The feeding procedure usually took ~30 s. Fish offered during trials were refrigerated overnight to temperatures naturally encountered by the birds during that time of year (~5°C). Herring were fed immediately after removal from the refrigerator. A trial ended when the monitored O_2 and CO_2 levels appeared to return to the resting level (i.e. pre-feeding level). All trials were conducted during daylight hours and lasted on average between 3.5 h (60 g herring) and 6.5 h (100 g herring).

Data analysis and statistics

Rates of oxygen consumption [using eqn 3b from Withers (Withers, 1977)] and carbon dioxide production were calculated in Datacan (Sable Systems) for each minute of a trial. From this the respiratory exchange ratio was determined as $\mathrm{RER}=\dot{V}_{CO_2}/\dot{V}_{O_2}$. Resting $\dot{V}_{O_2}$ was established for each individual trial and served as a control measurement for each HIF determination. It was calculated as the mean $\dot{V}_{O_2}$ during a stable 10 min period immediately preceding feeding. Following ingestion of a herring, $\dot{V}_{O_2}$ increased to a peak value before declining linearly towards the resting value. HIF was calculated as the elevation in oxygen consumption over the resting rate during the course of digestion (see Fig. 1). Note, however, that HIF calculated in this way reflects the total amount of heat generated as a by-product of digestion only, when the animal is at thermoneutral conditions (see below). Ingestion was always accompanied by a brief spike in $\dot{V}_{O_2}$ (Fig. 1), most likely caused by movements associated with the feeding event. This initial spike was removed (using linear interpolation) before calculating the magnitude of HIF (indicated by the shaded area in Fig. 1A–C). In a few cases birds fed a 100 g herring did not reach their resting $\dot{V}_{O_2}$ by the end of a feeding trial. Since $\dot{V}_{O_2}$ declined in a linear fashion after reaching a peak, we calculated a linear regression between time and $\dot{V}_{O_2}$ and extrapolated this line to the resting value (see Green et al., 2006).

Thermal substitution was investigated by comparing magnitude of HIF at thermoneutral and sub-thermoneutral conditions. While at thermoneutral conditions the calculated HIF reflects the total amount of heat generated as a by-product of digestion, this is not necessarily the case at sub-thermoneutral conditions (i.e. if thermal substitution occurs). When resting at sub-thermoneutral conditions, the animal produces additional heat (thermogenesis) to offset increased heat loss. If substitution of some or all of the heat generated as a by-product of digestion for the thermogenesis during rest occurs, HIF will appear to be of lower magnitude and/or duration when compared with the thermoneutral condition. However, the total amount of heat generated as by-product of digestion does not depend on ambient temperature but on the size and constitution of the meal. We assumed that substitution occurred if the calculated HIF was lower at sub-thermoneutral conditions (Lovvorn, 2007).

Stomach temperatures were analysed using Multitrace (Jensen Software Systems, Laboe, Germany). For each HIF determination,

mean stomach temperature was calculated over 5 min intervals throughout a trial. When a bird was fed a cold fish, stomach temperature recorded by the logger declined precipitously. After reaching a minimum value, temperature started to rise gradually towards the pre-feeding value. The recorded stomach temperature should reflect body temperature of a bird before feeding and again from ~1 h after feeding, when pre-feeding temperature was re-established. From changes in stomach temperature (omitting the first hour after feeding) we calculated heat storage throughout a trial as $\Delta T_b M_b c$, where ΔT_b are the changes in body temperature (in °C), M_b is body mass (in kg), and c is the mean specific heat capacity of tissue [taken as 3.5 kJ kg^{-1} °C^{-1} (Dawson and Whittow, 2000)]. To calculate the time required for birds to warm up various amounts of ingested fish when resting in their pen, we first selected feeding events from the stomach temperature traces where the temperature returned to the pre-feeding value. This was not always the case, especially after feedings in the late afternoon, when the temperature started to decline towards the lower value maintained throughout the night (see Enstipp et al., 2006a). For each selected event we then calculated a baseline temperature before feeding, taken as the mean temperature during 5 min immediately before feeding. In a second step the time required to reach this pre-ingestion temperature was measured.

All statistical analysis was performed using SigmaStat software (Jandel Scientific, San Rafael, CA, USA). One-way analysis of variance (ANOVA) with Tukey pairwise multiple comparisons was used to compare the effects of environmental temperature and meal size on the magnitude and duration of HIF. When single comparisons were made, as in comparing resting and peak values of $\dot{V}_{O_2}$, Student's t-test was used. All percentage values were normalised by arcsine transformation beforehand. Significance was accepted at $P<0.05$. Values given are grand means established from individual bird means and are presented with standard error of the mean (± s.e.m.).

Calculation of thermoregulatory benefit

To explore the overall thermoregulatory benefit that double-crested cormorants wintering in British Columbia potentially accrue from thermal substitution *via* HIF, we estimated their maintenance costs, daily energy expenditure (DEE), and daily food intake (DFI). Air temperatures throughout coastal British Columbia during winter usually fluctuate between 0 and 10°C. Hence, we calculated maintenance costs of the cormorants (basal metabolic rate and thermoregulatory costs) for an ambient temperature of ~5°C (the mean temperature during our cold air trials) using values from Table 1 and an energy conversion factor of 19.7 kJ l^{-1} O_2 (Enstipp et al., 2006a). DEE was estimated from an algorithm established by Enstipp et al. (Enstipp et al., 2006b), modified for double-crested cormorants. This algorithm combines time–activity data with activity-specific metabolic rates to estimate DEE. We assumed the following time–activity budget (based on general patterns observed in great cormorants): birds fly for 1 h, dive for 2 h (mean dive depth and water temperature were taken as 10 m and 6°C, respectively), and rest on land for the remainder of the day. Activity-specific metabolic rates were taken from Enstipp et al. (Enstipp et al., 2006a), with the exception of flight costs, which were calculated using Pennycuick's aerodynamic model (Pennycuick, 1989). Cormorants forage on a variety of fish species of different nutritional composition and energy density. We assumed a composite energy density for the ingested fish of ~5 kJ g^{-1} wet mass, to convert DEE into DFI. We furthermore assumed that birds acquire their DFI during two foraging bouts, which are spread throughout the day, so as to make best use of HIF for thermoregulatory savings. From HIF_{net} measured in our cormorants digesting a 100 g herring (Table 2) and the estimated DFI, we calculated the daily HIF_{net}, assuming that the magnitude of HIF changes in proportion with food intake (Bech and Præsteng, 2004; Green et al., 2006) and is also comparable for fish species of varying energy density. The daily HIF_{net} represents the maximum amount of energy savings possible *via* HIF, if thermal substitution were complete.

RESULTS

Heat increment of feeding at thermoneutral conditions

When sitting calmly inside the respirometer at thermoneutral conditions, mean $\dot{V}_{O_2}$ of five cormorants was 26.13±1.37 ml min^{-1} (Table 1). Immediately after ingestion of a 100 g herring there was a brief spike in $\dot{V}_{O_2}$ (Fig. 1A), most likely caused by movements associated with the feeding event. However, within 20–25 min of feeding, $\dot{V}_{O_2}$ regained a stable, albeit elevated, level. This initial spike was removed and linear interpolation was used when calculating the magnitude of HIF (shaded area in Fig. 1A). After regaining a stable level, $\dot{V}_{O_2}$ rose gradually (Fig. 1A, Fig. 2) and reached a peak value at ~60 min, when it was significantly elevated over the resting rate ($P<0.001$, $t=-12.87$). Thereafter, $\dot{V}_{O_2}$ declined steadily towards the resting level (Fig. 1A, Fig. 2). Oxygen consumption was elevated for an average of 328±28 min after feeding, during which time birds consumed 2697±294 ml O_2 in excess of the resting rate (Table 1). Peak $\dot{V}_{O_2}$, taken as the maximum value reached during a trial

Table 1. Trial conditions and the effect of fish ingestion on oxygen consumption rates ($\dot{V}_{O_2}$) of double-crested cormorants

Condition	*N*	*n*	Body mass (kg)	Air temperature (°C)	Resting $\dot{V}_{O_2}$ (ml min^{-1})	Meal mass (g)	Peak $\dot{V}_{O_2}$ (ml min^{-1})	Increase (%)	HIF* (ml O_2)	HIF duration* (min)
Warm, 100 g fish	5	14	2.08±0.07	21.1±0.2	26.13±1.37	101.03±0.66	44.23±2.54	69.8±4.0	2697±294	328±28
Cold, 100 g fish	3	6	2.13±0.04	5.5±0.7	35.57±0.72[a]	100.84±0.65	49.74±1.42	39.8±4.0[a]	1391±271[a]	228±6[a]
Cold, 60 g fish	3	9	2.10±0.04	7.6±0.3	33.46±0.29[a]	60.12±0.57	43.31±0.48	29.7±2.0[a]	539±85[a,b]	100±10[a,b]

Values are grand means ± s.e.m., which were established from individual bird means. 'Peak $\dot{V}_{O_2}$' is the maximum average 1-min value during a trial, excluding the initial spike associated with feeding movements. 'Increase' depicts the percentage increase in $\dot{V}_{O_2}$ from resting to peak level reached after food ingestion.

*While HIF at thermoneutral conditions ('warm') represents the total amount of heat generated as a by-product of digestion, this is not the case for trials at sub-thermoneutral conditions ('cold'). Lower values at sub-thermoneutral conditions and the shorter duration of $\dot{V}_{O_2}$ elevation indicate thermal substitution. To calculate HIF, the initial brief spike in $\dot{V}_{O_2}$, associated with movements during the feeding event, was removed from all traces using linear interpolation (see Fig. 1).

N, number of birds; *n*, number of trials.

[a]Significant difference from trials at thermoneutral conditions.

[b]Significant difference from trials with larger meal size.

(excluding the initial spike), was on average ~1.7 times the resting rate (Table 1).

Effects of environmental temperature and meal size

Resting $\dot{V}_{O_2}$ was significantly elevated in cold temperature trials when compared with the trials at thermoneutral conditions (P<0.001, t=–6.00), confirming that birds were below their lower critical temperature (Table 1, Fig. 1A–C). After food ingestion, $\dot{V}_{O_2}$ increased rapidly and within ~30 min reached a more stable plateau (100 g herring) or started to decline (60 g herring) (Fig. 2). ANOVA comparisons revealed that both ambient temperature and meal size significantly affected the magnitude (P=0.001, F=16.74) and duration (P<0.001, F=22.55) of HIF (Table 1). At thermoneutral conditions $\dot{V}_{O_2}$ elevation following the ingestion of a 100 g herring lasted significantly longer (P=0.04, t=2.58) and was therefore significantly greater (P=0.02, t=2.97) than when birds were at sub-thermoneutral conditions (Table 1, Figs 1, 2). We only tested the effect of meal size on HIF at low ambient temperatures. Under these conditions, HIF was significantly greater (P=0.04, t=3.00) and lasted longer (P<0.001, t=11.07) when meal size was bigger (Table 1, Fig. 1B,C, Fig. 2).

Respiratory exchange ratio (RER)

The respiratory exchange ratio (RER) varied considerably throughout trials of individual birds and also between birds. At thermoneutral conditions, mean RER before feeding was 0.72±0.01 (Fig. 3). After food ingestion, RER briefly increased to 0.74, before declining to a value below resting. For the remainder of the trial, RER fluctuated between ~0.68 and 0.70 (Fig. 3). Mean RER before feeding at sub-thermoneutral conditions was 0.63±0.01. After ingestion of a 100 g herring, RER increased briefly to 0.73. In the following 80 min it varied between 0.68 and 0.73, before reaching a more stable level at 0.67 for the remainder of the trial.

Stomach temperatures

Stomach temperatures during HIF trials under various conditions were comparable and followed the same pattern. Fig. 4 shows the mean temperatures (± s.e.m.) observed during trials in warm air. Temperature before feeding was 41.2±0.1°C and declined rapidly upon ingestion of a cold herring. After reaching a minimum value at ~35°C, temperature rose again and reached the pre-ingestion level within 40–50 min after feeding. For the remainder of the trial temperature was relatively stable, slowly declining to 40.7±0.3°C at the end of a 6.5 h trial (Fig. 4). Mean stomach temperatures during cold air trials, when birds ate a 100 g herring were 41.5±0.1°C before feeding and 41.7±0.2°C at trial end. When fed 60 g herring in cold air, temperatures before feeding and at trial end were 41.6±0.1°C and 40.7±0.2°C, respectively. Heat storage, determined from changes in stomach temperature after food ingestion (omitting the first hour after feeding) was negligible. Assuming that all body tissues showed the same temperature variations as recorded by the proventricular probe, mean heat storage was –2.8±0.9 kJ in warm air trials, 2.0±1.4 kJ and –4.7±2.0 kJ in cold air trials when fed 100 g and 60 g, respectively.

When birds were fed a refrigerated herring containing a temperature logger, the temperature rise was sigmoidal, reaching a stable temperature within 40–50 min (Fig. 5, insert). Separate feeding trials, when birds rested in their pen, revealed a significant linear relationship between the fish mass ingested and the time required to warm this mass from ~5°C to body temperature (P<0.001, F=90.57; Fig. 5). As expected, the time required to warm the ingested fish increased with meal size.

Table 2. Estimates of the magnitude and duration of HIF in bird species at thermoneutral conditions

Species	Food	Meal mass (wet) as % M_b	Energy density (kJ g^{-1} wet mass)	GE intake (kJ)	HIF_{net} (kJ)	HIF_{net} as % GE intake	HIF duration (h)	Study
Double-crested cormorant (*Phalacrocorax auritus*)	Pacific herring (*Clupea pallasi*)	4.8	9.3	930.0	38.9	4.2	5.5	[1]Present study
Little penguin (*Eudyptula minor*)	West Australian sardines (*Sardinops neopilchardus*)	2.9 7.0	5.1 5.1	155.6 382.0	15.6 38.2	10.0 10.0	3.1 6.3	[2](Green et al., 2006)
Lesser scaup (*Aythya affinis*)	Blue mussels (*Mytilus edulis*)	–	20.2[a]	118.8	24.8	20.9	–	[3](Kaseloo and Lovvorn, 2006)
Tawny owl (*Strix aluco*)	Laboratory mice (*Mus musculus*)	5.3	8.9	200.0	14.2	8.0	10.0	[4](Bech and Præsteng, 2004)
Mallard (*Anas platyrhynchos*)	Mixed grain	–	17.5[a]	–	–	4.1	–	[5](Kaseloo and Lovvorn, 2003)
Brünnichs guillemot (*Uria lomvia*)	Arctic cod (*Boreogadus saida*)	2.3	4.9	92.6	6.5	7.0	1.4	[6](Hawkins et al., 1997)
House wren (chicks) (*Troglodytes aedon*)	Crickets (*Achaeta domestica*)	6.4	6.2	–	–	6.3	–	[7](Chappell et al., 1997)
Adélie penguin (chicks) (*Pygoscelis adeliae*)	Krill (*Euphausia* sp.)	26.4	22.6[a]	–	–	10.0	10.0	[8](Janes and Chappell, 1995)

In all studies, except 4, 7 and 8, birds ingested food voluntarily. In study 3, ducks dived voluntarily to a depth of 2 m, where they ingested blue mussels (shell removed), while ducks ingested food when floating at the surface in study 5.

HIF_{net} excludes the costs incurred when warming the ingested food from ambient to body temperature and therefore represents the amount of heat potentially available for thermoregulation. GE, gross energy; M_b, body mass; [a]energy densities reported in kJ g^{-1} dry mass.

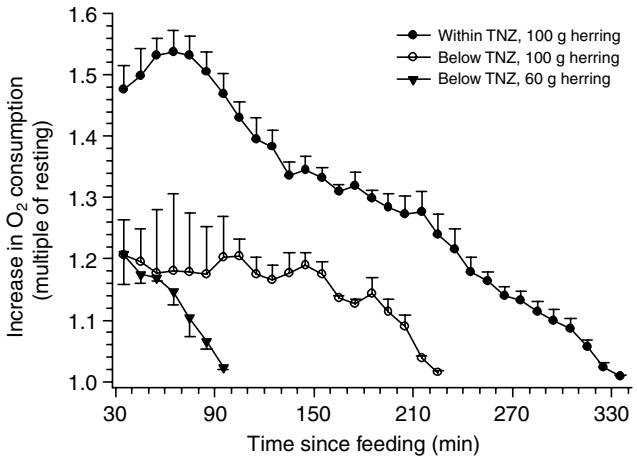

Fig. 2. Changes in oxygen consumption rate (means ± s.e.m., in multiples of resting value) of double-crested cormorants after voluntary ingestion of a single herring (mass 60 or 100 g) when resting in air at temperatures within or below their thermoneutral zone (TNZ) (lower critical temperature ~9°C). Sample sizes for the various conditions are indicated in Table 1.

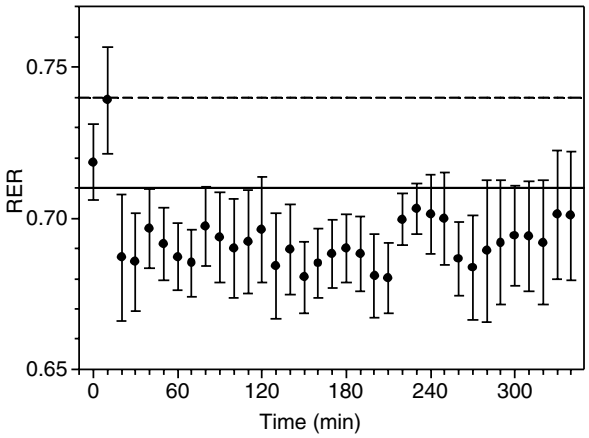

Fig. 3. Respiratory exchange ratio (RER=$\dot{V}_{CO_2}/\dot{V}_{O_2}$) of double-crested cormorants before (time zero) and after ingestion of a 100 g herring (means ± s.e.m., *N*=5 birds, *n*=14 trials), when resting at air temperatures within their thermoneutral zone. The solid and broken lines indicate the theoretically expected RER values when birds metabolise exclusively lipid or protein, respectively.

DISCUSSION

This study investigated magnitude and duration of HIF in double-crested cormorants following the ingestion of a single herring while resting in air. It furthermore examined the effects of meal size and the potential of HIF for thermal substitution. We found that HIF in cormorants was significantly greater after larger food intake. This was mainly the consequence of an increase in HIF duration rather than in the peak level of HIF (Table 1). In trials at sub-thermoneutral conditions, at identical food intake levels, magnitude and duration of $\dot{V}_{O_2}$ elevation were significantly reduced (by ~48% and ~30%, respectively), when compared with trials at thermoneutral conditions (Table 1). This strongly suggests that cormorants are able to use the heat generated by HIF to substitute for regulatory thermogenesis if heat loss is sufficiently high. Knowledge of HIF and its overall effect on the energy budget of cormorants is essential for the construction of bioenergetics models, in an attempt to better understand the energy and food requirements of these top predators. If thermal substitution (from HIF and muscular activity) is not taken into consideration, time–energy budgets that include an independent value for thermoregulation costs might greatly overestimate energy expenditure.

HIF at thermoneutral conditions

$\dot{V}_{O_2}$ of double-crested cormorants when resting at air temperatures within their TNZ was slightly lower than the mass-specific value reported in an earlier study [12.56 *vs* 13.97 ml min^{-1} kg^{-1} (Enstipp et al., 2006a)]. Magnitude of the peak $\dot{V}_{O_2}$ observed in cormorants after ingestion of a 100 g herring (~1.7 times resting, Table 1) was similar to that reported for other seabird species during digestion (Baudinette et al., 1986; Croll and McLaren, 1993; Janes and Chappell, 1995; Hawkins et al., 1997; Green et al., 2006). It was also similar to the increase observed in other fish eating endotherms, namely pinnipeds [see table 3 in Rosen and Trites (Rosen and Trites, 1997)]. The time course of the changes in oxygen consumption following ingestion in the cormorants was similar to that reported for little penguins (Green et al., 2006) and differed from that in Brünnichs guillemots (Hawkins et al., 1997). In the guillemots, oxygen consumption rate reached its peak within 7.5 min of fish ingestion, which was followed by a relatively stable plateau phase for ~50 min. Thereafter it declined slowly towards the pre-feeding value, which was reached within ~90 min of ingestion (Hawkins et al., 1997). By contrast, peak $\dot{V}_{O_2}$ was distinct and short-lived in the cormorants and oxygen consumption declined steeply towards the pre-feeding value thereafter (Fig. 1A, Fig. 2). Cormorants reached their peak $\dot{V}_{O_2}$ ~60 min after feeding (Fig. 2), which was in-between the 43 min and 73 min reported for little penguins (Green et al., 2006) and Adélie penguins (Janes and Chappell, 1995), respectively. $\dot{V}_{O_2}$ after ingestion of a 100 g herring was elevated for ~5.5 h in the cormorants (Table 1, Fig. 2). This is comparable to the duration reported for little penguins (Green et al., 2006), when relative meal size is taken into consideration (Table 2).

Warming the ingested food to body temperature might account for a substantial part of the increase observed in $\dot{V}_{O_2}$ after feeding (Wilson and Culik, 1991). In fact, Wilson and Culik attributed all of the increase observed in $\dot{V}_{O_2}$ in Adélie penguins after the ingestion of cold krill to this. However, warming of the ingested food (Arctic cod *Boreogadus saida* for Brünnichs guillemots and West Australian sardines *Sardinops neopilchardus* for little penguins) accounted only for a minor fraction of the observed increase in oxygen consumption of Brünnichs guillemots (~30%) (Hawkins et al., 1997) and little penguins (~17%) (Green et al., 2006). In our study it took birds on average ~36 min to warm a 100 g herring from 5°C to body temperature when they rested within their TNZ (Fig. 5). $\dot{V}_{O_2}$ after fish ingestion was elevated for a much longer duration (328±28 min), clearly indicating that HIF persisted beyond the time required to heat the meal.

The costs of heating a herring from ambient to body temperature can be calculated if we know the specific heat capacity of the herring. We used the proximate composition of macronutrients for Pacific herring (Zhao et al., 2006) (16.84% lipid, 15.15% protein, 65.74% water, 2.16% ash) and the specific heat capacities of these components (Kaseloo and Lovvorn, 2003), to calculate the specific heat capacity of our herring, estimated at ~3.33 J g^{-1} $°C^{-1}$. Hence, heating a 100 g herring from ambient (5°C) to body temperature (41.2°C, mean stomach temperature of birds before feeding) would require 12.05 kJ. We used an energy conversion factor of 19.08 kJ l^{-1} O_2 (based on the proximate composition of Pacific herring and the energy released from its components) (Zhao et al.,

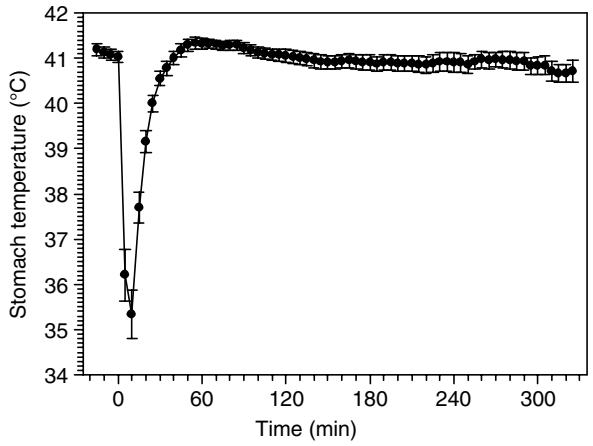

Fig. 4. Mean stomach temperature (± s.e.m.) of double-crested cormorants during HIF trials at air temperatures within their thermoneutral zone (*N*=5 birds, *n*=12 trials). The temperature drop at 0 min is caused by the ingestion of a 100 g herring. Note that temperature reaches pre-ingestion levels within ~40 min of eating the fish, after which temperature remains stable throughout the remainder of the trial.

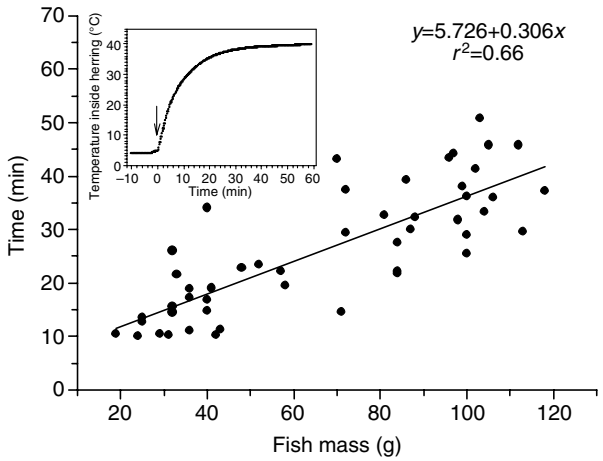

Fig. 5. Time required by resting cormorants to warm ingested fish of varying mass to body temperature. Temperature of fish ingested was ~5°C, while mean stomach temperature of birds before ingestion was 41.1±0.4°C (*N*=5 birds, *n*=49 trials). The insert shows a temperature trace recorded by a MiniTemp-xl-logger inside a 100 g herring. The fish was kept inside a refrigerator and fed to a cormorant (within 2 min of removal from the refrigerator) at time zero, as indicated by the arrow.

2006; Schmidt-Nielsen, 1997) to convert the mean oxygen consumption after fish ingestion at thermoneutral conditions attributable to HIF (26.7±3.0 ml O_2 g^{-1} fish) to metabolic rate. The total HIF for a 100 g herring was therefore calculated to be 50.9 kJ. Hence, fish warming in our study accounted for only ~23.7% of the measured increase in oxygen consumption. If we use the energy density for Pacific herring given by Zhao et al. [9.3 kJ g^{-1} wet mass (Zhao et al., 2006)], a 100 g herring would have a GE content of 930 kJ, and the 12.05 kJ warming costs would represent 1.3% of that. Excluding the costs incurred when warming the ingested food from ambient to body temperature, we arrive at a HIF_{net} of 38.9 kJ for a 100 g herring (Table 2).

Table 2 lists estimates of the magnitude and duration of HIF for various bird species investigated at thermoneutral conditions. Differences in experimental conditions (e.g. relative meal size and composition) have to be considered when comparing these values. The only studies to which our results can be directly compared are on Brünnichs guillemots (Hawkins et al., 1997) and little penguins (Green et al., 2006). If we take into account the relative meal size, then HIF_{net} observed in cormorants was greater than in these two species (Table 2). However, when expressing HIF_{net} observed in cormorants on the basis of the GE intake, it becomes clear that it falls into the lower range of what has been observed in aquatic birds (4.2%; Table 2). While values for the guillemots and penguins are about double the value observed in cormorants, the energy density of their ingested food was considerably lower (Table 2). Low HIF values were also reported for harbour seals *Phoca vitulina* when feeding on high-energy herring [5.1% of GE intake (Markussen et al., 1994)]. Hence, it is intriguing that these relatively low HIF values (when expressed as % GE intake) might be a consequence of the high energy density of the fish ingested. Markussen et al. (Markussen et al., 1994) took their results as evidence for a more efficient utilization of the ingested meal, when seals fed on energy-rich food (i.e. less 'wasted energy' in the form of HIF).

Mean RER in our birds before feeding (0.72) was similar to the value of 0.73 reported in an earlier study (Enstipp et al., 2006a) and identical to the value found in post-absorptive European shags *P. aristotelis* resting in air (Enstipp et al., 2005). However, after food ingestion, RER in our cormorants declined somewhat (apart from the brief initial increase) and fluctuated between 0.68 and 0.70 (Fig. 3). Although these values are lower than what is conventionally expected, they follow the same pattern observed in little penguins (Green et al., 2006), digesting similar meal masses. When resting at sub-thermoneutral conditions, RER before feeding was lower than expected at ~0.63. After feeding, however, it increased to levels typical for diet composition (for ~80 min), before declining again to lower levels. RER levels lower than conventionally expected have been reported throughout the bird literature and some of the potential mechanisms (e.g. non-pulmonary CO_2 loss) and implications for studies on avian energetics have been discussed (Walsberg and Wolf, 1995). However, more studies investigating these mechanisms, especially for piscivorous species, are desirable.

Effect of meal size

We only investigated the effect of meal size when birds were at sub-thermoneutral conditions. Nevertheless, under these conditions our results show that duration of HIF and, therefore, its magnitude, changed significantly with meal size (Table 1, Fig 1B,C, Fig 2). By contrast, peak $\dot{V}_{O_2}$ during digestion was not significantly affected by meal size, which was also true for HIF_{net}, when expressed either on a mass-specific basis (per g fish) or as percentage of the GE intake (0.14 kJ g^{-1} *vs* 0.12 kJ g^{-1} and 1.5% *vs* 1.3% for 100 g and 60 g herring, respectively). These findings are similar to previous studies that investigated the effect of meal size on HIF in birds and marine mammals (Masman et al., 1989; Markussen et al., 1994; Janes and Chappell, 1995; Chappell et al., 1997; Rosen and Trites, 1997; Bech and Præsteng, 2004; Green et al., 2006). However, in mallards, dabbling for grain, magnitude of HIF (% GE intake) increased with increasing food intake (Kaseloo and Lovvorn, 2003). Feeding conditions in the latter study differed, however, in that mallards fed on low protein grain at intake levels below maintenance requirements (for details, see Kaseloo and Lovvorn, 2003).

HIF, thermal substitution and eco-physiological relevance

The significantly shorter duration of $\dot{V}_{O_2}$ elevation and its smaller magnitude at sub-thermoneutral conditions, when compared with thermoneutral conditions (Table 1, Figs 1, 2), strongly suggest that

double-crested cormorants are able to use the excess heat to substitute for regulatory thermogenesis under these conditions. HIF_{net} at the lowest air temperature tested in our study (mean: 5.5°C) was reduced by 48%, indicating that thermal substitution at this temperature was only partial. However, it is conceivable that thermal substitution might be complete if heat loss is sufficiently high [e.g. at lower air temperatures or during/after foraging in cold water (see Kaseloo and Lovvorn, 2005)]. While the concept of using heat generated by HIF to offset thermoregulatory costs has been around for more than 100 years, experimental evidence for it has been equivocal. Results from studies in endotherms range from none to partial and complete substitution [see table 1 in Rosen and Trites (Rosen and Trites, 2003) and appendix 1 in Lovvorn (Lovvorn, 2007)]. For example, substitution from HIF accounted for >20% of the metabolizable energy intake in lesser scaups diving for blue mussels at a depth of 2 m (Kaseloo and Lovvorn, 2006). In kestrels *Falco tinnunculus* and tawny owls *Strix aluco*, substitution from HIF was ~50% and over 90%, respectively, when birds rested at sub-thermoneutral ambient temperatures (Masman et al., 1989; Bech and Præsteng, 2004). Similarly, substitution from HIF was clearly present in large house wren chicks *Troglodytes aedon* and was complete in many cases, when ambient temperature was sufficiently low (Chappell et al., 1997). By contrast, in arctic tern chicks *Sterna paradisaea*, no evidence for thermal substitution from HIF was found (Klaassen et al., 1989) and that was also the case for juvenile Steller sea lions *Eumetopias jubatus* (Rosen and Trites, 2003). In mallards dabbling for low protein grain and in lesser scaups diving for blue mussels to shallow depth (1.2 m), thermal substitution from HIF was also negligible (Kaseloo and Lovvorn, 2003; Kaseloo and Lovvorn, 2006). These last two studies illustrate some of the methodological problems when measuring thermal substitution (see Lovvorn, 2007). For example, the chances of detecting thermal substitution depend on the magnitude of HIF, which in turn depends on meal size and protein content. Hence, thermal substitution is more easily detected in animals eating large meals with high protein content (Kaseloo and Lovvorn, 2003). This contrasts with conditions in the mallard study, where birds ingested small amounts of low-protein grain. Also, in order for thermal substitution to occur, heat loss has to be sufficiently high, while excess heat (from HIF or exercising muscles) also has to be available to replace that heat loss (Kaseloo and Lovvorn, 2005). Due to the greater compression of the plumage, heat loss in lesser scaups must have been greater when diving to 2 m than when they dived to shallow depth (1.2 m), so that thermal substitution was detectable in the former situation but not in the latter (Kaseloo and Lovvorn, 2006).

Potential reasons for varying results in studies that investigate thermal substitution have recently been discussed (Rosen and Trites, 2003; Lovvorn, 2007). Besides problems related to differences in calculation and experimental conditions, one possible explanation could be real physiological differences between the species studied with respect to taxonomy, ecology or developmental stage (Rosen and Trites, 2003). However, as pointed out by the authors, there appears to be no clear pattern according to these criteria in the studies conducted so far. Clearly, more studies on a greater range of species, conducted at the most relevant ecological conditions, are needed before such a pattern might emerge.

The extra heat generated through HIF might serve cormorants at different times of their daily routine by reducing the need for shivering thermogenesis. For example, abdominal temperature decreases during diving have been observed in various avian divers such as South Georgian shags *P. georgianus* (Bevan et al., 1997), king penguins *Aptenodytes patagonicus* (Handrich et al., 1997) and great cormorants (Grémillet et al., 2005b). Heat produced during digestion might help to restore body temperature after a dive bout. During times of inactivity at low ambient temperatures (e.g. during roosting after foraging or during the night, when locomotor activity is minimal), excess heat from HIF might be important to maintain body temperature, thereby lowering the temperature for the onset of residual thermogenesis. In sea otters *Enhydra lutris*, HIF might allow animals to increase the time between activity bouts (Costa and Kooyman, 1984). These authors suggested that post-absorptive sea otters maintain their heat balance through periodic activity bouts, while post-ingestive otters decrease activity and use the heat produced from HIF to offset heat loss during rest. A similar scenario was proposed for guillemots that spend most of their life at sub-thermoneutral water temperatures (Croll and McLaren, 1993). Of course, to what extent muscular activity (for locomotion or shivering) can be reduced because of the extra heat from HIF depends on the timing. Ideally, bouts of activity and resting should be separated by the amount of time that metabolism is elevated following food ingestion. While this seems to be the case for sea otters (Costa and Kooyman, 1984), it is not clear if cormorants also structure their daily activity patterns in such a way.

When exploring the overall thermoregulatory benefit that double-crested cormorants wintering in British Columbia might accrue from thermal substitution *via* HIF, we calculated maintenance costs and DEE to be ~1009 kJ day^{-1} and ~2000 kJ day^{-1}, respectively. This would require a DFI of ~550 g of fish. When digesting a 100 g herring at thermoneutral conditions, HIF_{net} of the cormorants was 38.9 kJ (Table 2). Hence, 550 g of fish, taken over two foraging bouts, would produce a daily HIF_{net} of ~214 kJ. In our herring trials at sub-thermoneutral conditions (~5°C), the measured $\dot{V}_{O_2}$ elevation was reduced by ~48%, when compared with trials at thermoneutral conditions (Table 1), representing thermal substitution. If we extrapolate from these experimental trials to the wintering scenario, this would amount to energy savings through HIF of ~103 kJ per day. Such a saving represents ~38% of the daily thermoregulatory costs (calculated as the difference between RMR at 21.2 and 5.5°C, Table 1, ~268 kJ day^{-1}) and ~5% of the DEE. Hence, at the specific conditions investigated, the extra heat generated *via* HIF would reduce the need of cormorants to shiver by almost 40%. Thermoregulatory savings *via* the excess heat from exercising muscles during activity bouts are likely to reduce the need for shivering thermogenesis in cormorants even further (Kaseloo and Lovvorn, 2006). However, one should be aware that the above calculations are based on the combination of measurements under controlled laboratory conditions and a simple model. Furthermore, thermal substitution patterns might differ in birds resting in air or diving in cold water. Clearly, more information is needed before the fraction of thermoregulatory costs that is potentially met by substitution in free-ranging birds can be calculated with greater accuracy.

Diving and HIF – evidence for delayed food-processing?

$\dot{V}_{O_2}$ has been observed to increase within minutes of food ingestion in birds (Janes and Chappell, 1995; Hawkins et al., 1997; Green et al., 2006) and this was also the case in our study. However, such immediate onset of HIF would seem to conflict with the need of avian divers to effectively manage their finite oxygen stores during diving (Butler and Jones, 1997). Any increase in metabolic rate during a dive bout (i.e. HIF) would tend to reduce the aerobic dive capacity of these birds. Apart from the studies by Kaseloo and Lovvorn

(Kaseloo and Lovvorn, 2003; Kaseloo and Lovvorn, 2005; Kaseloo and Lovvorn, 2006), however, HIF measurements in avian divers were conducted while birds rested in air. It is possible that birds foraging in the wild are able to delay the onset of HIF to some degree. During diving, blood flow distribution changes as part of the overall oxygen saving 'dive response' (Butler and Jones, 1997). Hence, one possible mechanism to postpone digestion until after a dive bout would be peripheral vasoconstriction of the gastrointestinal tract, which seems to occur in shallow diving tufted ducks *Aythya fuligula* (Butler et al., 1988; Bevan and Butler, 1992). Peripheral vasoconstriction has also been indicated during deep dives of South Georgian shags [based on heart rate recordings (Bevan et al., 1997)] and emperor penguins *Aptenodytes forsteri* [based on temperature recordings (Ponganis et al., 2003)]. Furthermore, evidence for delayed food processing in diving animals has emerged from studies on king penguins (Gauthier-Clerc et al., 2000) and grey seals *Halichoerus grypus* (Sparling et al., 2007).

LIST OF ABBREVIATIONS

c	specific heat capacity
DEE	daily energy expenditure
DFI	daily food intake
GE	gross energy
HIF	heat increment of feeding
M_b	body mass
RER	respiratory exchange ratio
SDA	specific dynamic action
T_b	body temperature
TNZ	thermoneutral zone
$\dot{V}_{O_2}$, $\dot{V}_{CO_2}$	rate of oxygen consumption/CO_2 production

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