

The effects of depth, temperature and food ingestion on the foraging energetics of a diving endotherm, the double-crested cormorant (*Phalacrocorax auritus*)

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Summary

Avian divers are confronted with a number of physiological challenges when foraging in cold water, especially at depth. Besides the obvious constraint imposed by the necessity to return to the surface for gas exchange, cold water temperatures and a reduction in body insulation due to the increase in pressure with dive depth will elevate the energetic costs of foraging in these endotherm divers. The complex effect that depth has on the diving energetics of aquatic birds has largely been ignored. To date, no study has assessed the impact of depth on diving energetics over a significant depth range, naturally encountered by the diver. We used open-circuit respirometry to study the energetic requirements of a foot-propelled pursuit diver, the double-crested cormorant (*Phalacrocorax auritus albociliatus*), when diving in a shallow (1 m) and deep (10 m) dive tank and when resting in air and water. We also investigated the modifying effects of air or water temperature and feeding status on the costs associated with diving and resting. Of all factors investigated, dive depth exercised the strongest

influence on diving metabolic rate. Diving to 10 m depth increased metabolic rate on average by 22% when compared with shallow diving. Declining temperatures in air and water significantly elevated metabolic rate of cormorants resting in air and water as well as during diving. Feeding before resting in water or diving increased metabolic rate by 5–8% for at least 2 h. Cormorants maintained an elevated stomach temperature (>42°C) when resting in water and during diving, even at cold temperatures. The elevated dive costs during deep diving, when compared with shallow diving, are most likely a consequence of the increased thermoregulatory costs associated with a greater heat loss to the water at depth. Nevertheless, our study shows that dive costs in double-crested cormorants are similar to those of other foot-propelled avian divers.

Key words: diving energetics, depth, double-crested cormorant, temperature, HIF, heat loss.

Introduction

Avian divers take advantage of rich food sources within a variety of productive aquatic ecosystems. However, when foraging underwater, they face a number of physiological challenges. As air breathers, they have to return to the water surface frequently to reload their oxygen stores and unload accumulated CO₂. Therefore, a central aspect of their diving behaviour is the economic use of finite oxygen stores during submergence to maximise underwater foraging time. Anaerobic metabolism routinely contributes very little to energy production during diving (Butler, 2004) and it appears that the majority of dives under natural conditions are predominantly aerobic in nature (Butler and Jones, 1997). Aerobic dive duration is governed by two factors: (1) the amount of oxygen stored in tissues and (2) the rate at which these stores are used. Modulation of aerobic metabolic rate will

influence diving performance, so that the higher the rate of aerobic metabolism, the shorter will be the aerobic dive duration (Butler and Jones, 1997). Biotic and abiotic factors influence the metabolic rate of endotherms foraging underwater, mostly by affecting the domains of hydrodynamics and thermoregulation. In pursuit divers, for example, required changes in swim speed when pursuing fast moving prey will affect mechanical costs. Since the physical work load to overcome hydrodynamic resistance increases with swim speed, so will the metabolic work performed by the diver (Fish, 2000). Decreasing water temperatures, on the other hand, will increase thermoregulatory costs (Stahel and Nicol, 1982; Bevan and Butler, 1992), while metabolic rate will be further increased after food ingestion *via* the heat increment of feeding (HIF) (Hawkins et al., 1997).

One factor, however, that has received little attention in most

studies investigating the energetic costs of diving in endotherms is dive depth. In avian divers, the increase in ambient pressure when diving to depth will decrease the amount of air trapped within the plumage. The consequences are twofold: (1) a decrease in buoyancy and, in turn, lower mechanical costs of underwater locomotion and (2) a reduction in thermal insulation. At 10 m depth, all air spaces will be reduced to half the surface volume, reducing insulation from air trapped within the plumage. The resulting increase in heat loss might outweigh any energetic advantages that might accrue from a decreased buoyancy at greater depth, especially if water temperature is low. We are only aware of one study to date that investigated the energetic consequences of diving to depth. De Leeuw found that dives to 2.2 and 5.5 m depth were equally costly in tufted ducks (*Aythya fuligula*) (De Leeuw, 1996). One possible explanation could be that, since the depth range covered in the study was rather small, the energetic savings in mechanical costs with an increase in depth (reduced buoyancy) were balanced by an increase in thermoregulatory costs. Hence, how depth shapes the energetic costs associated with diving still remains unclear.

Double-crested cormorants (*Phalacrocorax auritus* Lesson) are foot-propelled pursuit divers that forage on benthic and pelagic fish. They target their prey in the upper part of the water column [depth range observed = 1.5–7.9 m (Ross, 1974)] in coastal and freshwater ecosystems. Owing to a partially wettable plumage (Grémillet et al., 2005a), buoyancy is reduced in cormorants when compared with many other avian divers [2.7 N kg^{-1} at the surface for *P. auritus* (Lovvorn and Jones, 1991a)], lowering mechanical costs during diving. The accompanying reduction in thermal insulation makes cormorants susceptible to substantial heat loss, especially when diving in cold water. Hence, it is generally believed that diving is very costly in cormorants when compared with other avian divers (see table 1 in Enstipp et al., 2005). Grémillet et al. used a model integrating the effects of water temperature and dive depth on energy expenditure during diving to estimate the energetic costs of great cormorants (*Phalacrocorax carbo carbo*) during foraging (Grémillet et al., 2001). They calculated that dive costs will vary between 28 and 64 W kg^{-1} [i.e. 9–21 times resting metabolic rate (RMR) (Schmid et al., 1995)] when diving in shallow/warm water and deep/cold water, respectively. This has led to the suggestion that poor insulating properties of their plumage and the resulting high foraging costs might be a limiting factor for the geographic distribution of cormorants (Gaston, 2004). However, great cormorants winter in the Arctic and spend considerable time foraging in almost freezing water (Grémillet et al., 2005c). Similarly, European shags (*Phalacrocorax aristotelis*) spend up to 7 h per day foraging in water temperatures of $\sim 5\text{--}6^\circ\text{C}$ in Scotland (Daunt et al., 2006). If foraging costs in cormorants are as high as expected, then their required daily food intake (DFI) should be high compared with that of other seabirds. However, Grémillet et al. estimated the DFI for great cormorants to be similar to the required intake of other, well-insulated seabirds of comparable mass (Grémillet et al., 1999).

Moreover, dive costs of European shags diving in a shallow trench have recently been measured and were considerably lower than previous measurements for great cormorants. Dive costs of shags were, in fact, similar to those of other foot-propelled divers and this has led to the suggestion that dive costs might be overestimated in cormorants (Enstipp et al., 2005). The depth range exploited by cormorants in the wild and their unique morphological features (partially wettable plumage, which reduces buoyancy but also reduces plumage insulation) make them a very interesting model to investigate the energetic consequences of diving to depth within the constraints of a captive setting.

Beyond these physiological considerations, measurements of activity-specific metabolic rates and the evaluation of modifying factors are of great importance for the calculation of time–energy budgets. These allow detailed estimates of individual and population energetic requirements of seabirds, which are urgently needed for management purposes (Enstipp et al., 2006; Boyd, 2002). Hence, to gain a detailed understanding of cormorant energetics we studied the energetic costs of double-crested cormorants associated with (1) resting in air, (2) resting in water and (3) diving. To study the importance of modifying factors, we altered air temperature, water temperature, diving depth and feeding status. The hypothesis that diving to depth will increase dive costs in cormorants compared with shallow diving was tested. Also, since mechanical and thermoregulatory costs develop in opposite directions during deep diving, we predicted that the expected increase in dive costs during deep diving would be less than projected from heat loss considerations alone.

Materials and methods

Twelve adult or sub-adult double-crested cormorants (*Phalacrocorax auritus albociliatus*; minimum age 2 years) with a mean body mass of $2.10 \pm 0.16 \text{ kg}$ (mean \pm s.d., range 1.81–2.47 kg) were used in this study. Ten of the birds were captured as chicks (5–6 weeks of age) from the Mandarte Island breeding colony, British Columbia, Canada. Two birds were bred in our captive setting. All birds were well established in captivity and were housed communally in sheltered outdoor pens (8 m long \times 4 m wide \times 5 m high) with water-tank access at the South Campus Animal Care Facility of the University of British Columbia (UBC), Vancouver, Canada. Birds were fed approximately 10% of their body mass daily with a mixed diet consisting of Pacific herring (*Clupea pallasii*) and rainbow smelt (*Osmerus mordax*), supplemented with vitamin B1 tablets (thiamine hydrochloride; Stanley Pharmaceuticals Ltd, North Vancouver, Canada). Body mass was determined to the nearest 10 g when birds were post-absorptive and dry, usually every morning, using a digital spring balance (UWE HS-15K; Universal Weight Enterprise Co., Taipei Hsien, Taiwan). All birds maintained a stable body mass throughout the study. All experimental procedures were approved by the UBC Animal Care Committee (Animal Care Certificate # A02-0122) and were in compliance with the

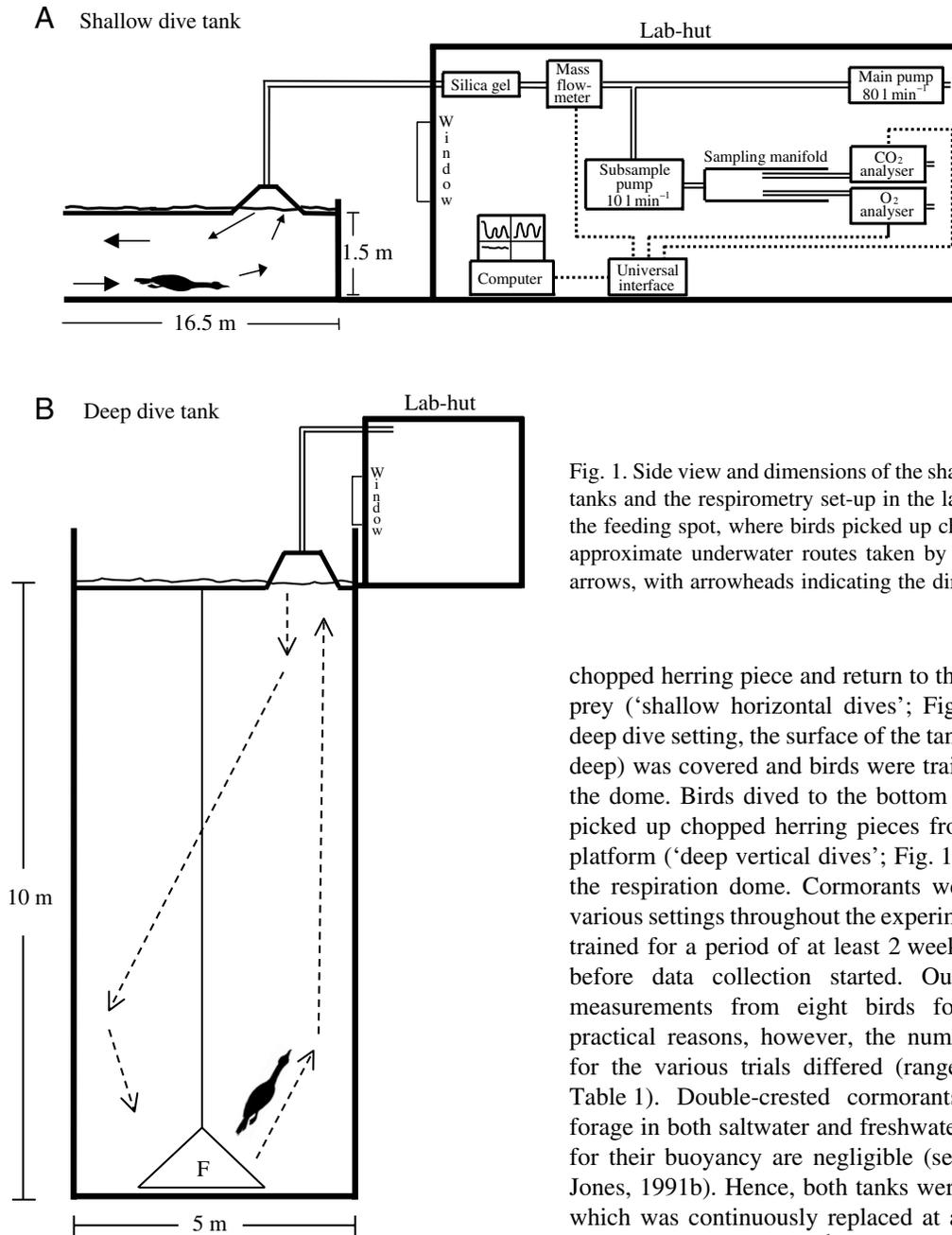


Fig. 1. Side view and dimensions of the shallow (A) and deep (B) dive tanks and the respirometry set-up in the laboratory hut. 'F' indicates the feeding spot, where birds picked up chopped herring pieces. The approximate underwater routes taken by the birds are indicated by arrows, with arrowheads indicating the direction of locomotion.

chopped herring piece and return to the dome to swallow their prey ('shallow horizontal dives'; Fig. 1A). Similarly, in the deep dive setting, the surface of the tank (5 m diameter \times 10 m deep) was covered and birds were trained to dive from within the dome. Birds dived to the bottom of the tank, where they picked up chopped herring pieces from a suspended feeding platform ('deep vertical dives'; Fig. 1B) before surfacing into the respiration dome. Cormorants were rotated between the various settings throughout the experimental period. They were trained for a period of at least 2 weeks in a particular setting before data collection started. Our aim was to collect measurements from eight birds for each condition. For practical reasons, however, the number of individuals used for the various trials differed (range 7–10 individuals; see Table 1). Double-crested cormorants in British Columbia forage in both saltwater and freshwater, and the consequences for their buoyancy are negligible (see fig. 7 in Lovvorn and Jones, 1991b). Hence, both tanks were filled with freshwater, which was continuously replaced at a water turnover rate of approximately 30 l min^{-1} for the shallow dive tank and 100 l min^{-1} for the deep dive tank. Because of this continuous mixing, water temperature in both tanks was homogenous (no stratification) and this was checked by running temperature profiles for both tanks throughout the seasons (max. temperature difference between top and bottom in the deep dive tank was $\pm 2^\circ\text{C}$).

Respirometry system

Oxygen consumption rates (\dot{V}_{O_2}) were measured using an open-circuit respirometry system (Sable Systems, Henderson, NV, USA). To measure the metabolic rate during diving, we used a transparent Plexiglas dome in the shape of a truncated pyramid as a respiration chamber (0.6 m long \times 0.6 m wide \times 0.4 m high; volume 50 litres), which was partially immersed

principles promulgated by the Canadian Council on Animal Care.

Diving facilities and training protocol

Birds were split into groups of four individuals, which were housed either within the shallow dive setting, the deep dive setting or in their outdoor pen. In the shallow dive setting, the entire surface of the tank (16.5 m long \times 2 m wide \times 1.5 m deep) was covered with flexible PVC mesh with the exception of a small area at the one end of the tank that held a Plexiglas dome inside a frame, which served as a respiration chamber. Birds were trained to submerge from within this Plexiglas dome, swim to the opposite end of the tank to pick up a

Table 1. Dive patterns observed in double-crested cormorants, and metabolic rate associated with different activities, temperatures and feeding status

	Temperature (°C)	Oxygen consumption (ml min ⁻¹ kg ⁻¹)	Energy expenditure (W kg ⁻¹)	Factor (×BMR)	Dive duration (s)	Surface duration (s)	Dive/pause ratio	No. dives per bout	N	n
BMR	22.1±1.0	13.97±1.57	4.59±0.51						10	27
Resting in water										
Post-absorptive										
Warm	15.0±0.7	32.98±5.32	10.83±1.75	2.4					8	24
Cold	7.9±0.0	44.54±6.81	14.62±2.23	3.2					8	21
Absorptive	14.6±1.1	33.16±5.02	11.11±1.68	2.4					7	36
Shallow diving										
Post-absorptive										
Warm	16.4±1.1	61.33±5.91	20.14±1.94	4.4	23.5±2.6	20.0±7.5	1.7±0.6	17.3±7.0	9	49
Cold	7.5±0.1	71.06±4.80	23.33±1.58	5.1	19.1±3.2	21.3±8.2	1.4±0.3	9.7±3.3	7	45
Absorptive										
Warm	15.5±1.2	61.34±6.34	20.55±2.12	4.5	23.9±2.9	24.9±9.8	1.5±0.6	14.9±5.2	9	62
Cold	7.3±0.1	74.05±6.19	24.81±2.07	5.4	23.1±0.9	26.8±9.8	1.3±0.5	8.9±3.4	7	23
Deep diving										
Post-absorptive										
Warm	13.9±1.2	76.08±2.61	24.98±0.86	5.4	21.2±3.6	14.4±4.3	1.7±0.4	3.8±0.7	7	55
Cold	7.6±1.4	83.65±4.00	27.46±1.31	6.0	19.1±1.3	13.3±2.7	1.7±0.4	3.9±0.4	7	77
Absorptive										
Warm	13.9±1.2	77.66±3.20	26.02±1.07	5.7	21.4±3.9	14.6±3.8	1.7±0.3	4.5±1.3	7	61
Cold	7.7±1.3	87.40±5.68	29.28±1.90	6.4	19.3±1.8	12.9±2.8	1.7±0.4	4.7±0.6	7	68

Values are grand means ± 1 s.d., which were established from individual bird means. Temperature refers to air temperature in the case of basal metabolic rate (BMR) and to water temperature in all other cases. Effects of body mass were removed from data following Packard and Boardman (Packard and Boardman, 1999); see Materials and methods for details. 'Factor' is the energy expenditure of the respective activity expressed in multiples of BMR. All behavioural parameters given for 'deep diving' exclude individual shallow dives that birds might have performed during a deep dive trial. *N* gives the number of birds, while *n* refers to the number of trials.

and received outside air through small holes on its four sides just above the waterline. Similarly, to measure metabolic rate during resting in air, we used a 55-litre bucket (0.35 m diameter × 0.65 m high) with an airtight Plexiglas lid, and air was drawn in *via* four small side holes near its bottom. Air from the respiration chambers was fed directly into the laboratory, which was set up inside a hut adjacent to the dive tanks (Fig. 1). The main airflow from the respiration chamber was dried using silica gel before being led into a mass-flowmeter (Sierra Instruments Inc., Monterrey, CA, USA), which automatically corrected the measured flow to STPD (273 K and 101.3 kPa). A sub-sample of 10 l min⁻¹ was bled into a manifold from which an oxygen (paramagnetic O₂-analyser PA-1B, Sable Systems; resolution 0.0001%) and CO₂ analyser (Beckman LB2 Medical CO₂-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.

Air flow through the respiration chamber was maintained at ~10 l min⁻¹ during the resting-in-air trials, at ~45 l min⁻¹ during the resting-in-water trials and at ~80 l min⁻¹ during the dive trials using a vacuum pump (Piston pump, Gast Manufacturing Inc., Benton Harbour, MI, USA). Oxygen concentration inside the respiration chamber was above 20.5%, and CO₂ concentration was below 0.4% during all trials. The gas analysers were calibrated before each trial using 99.995% pure N₂, 1.03% CO₂ (PraxAir, Richmond, BC, Canada) and outside air (set to 20.95% O₂ and 0.03% CO₂). 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 N₂ 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 18 s (resting in air) and 11 s (resting in water and diving) for the oxygen analyser and 21 s (resting in air) and 11 s (resting in water and diving) for the CO₂ analyser. These delay times were taken into account when calculating \dot{V}_{O_2} and \dot{V}_{CO_2} and relating them to diving events. The time constants of the respiration chambers were calculated to be 5.5 min for resting in air, 67 s for resting in water and 37.5 s for diving. 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 1 s (resting

in water and diving) or 5 s (resting in air) onto a desktop computer using Datacan (Sable Systems).

Resting metabolism

Basal metabolic rate (BMR) was measured during the day (08.00–18.00 h) while birds were resting, post-absorptive and within their thermo-neutral zone (mean air temperature was $22.1 \pm 1.7^\circ\text{C}$; range $18.3\text{--}25.4^\circ\text{C}$; lower critical temperature for our birds, calculated after the equation given by Ellis and Gabrielsen, 2002, should be $8\text{--}9^\circ\text{C}$). Birds were fasted overnight (for at least 15 h) before being placed inside the metabolic chamber. After the initial disturbance, birds calmed down quickly and sat quietly in the darkened chamber for the remainder of the trial. A stable \dot{V}_{O_2} was typically reached within the first hour of these 3–5 h-long trials. Air temperature in the respiration chamber was monitored using a digital thermometer (Oregon Scientific, Portland, OR, USA) and usually did not differ from outside air temperature by more than $\pm 2^\circ\text{C}$. Birds were familiarized with the procedure on at least three occasions before data collection began. BMR was determined from at least three trials per bird. To investigate the effect of air temperature on resting metabolism, measurements were also conducted at various temperatures ranging from 6 to 25°C .

Resting in water

The metabolic costs associated with resting in water were measured in separate trials during which birds floated calmly inside the dome, on the water surface of the shallow dive tank. A metal grid mounted about 30 cm below the base of the respiration chamber prevented birds from submerging. After the initial disturbance, when introduced into the chamber, birds calmed down quickly and a stable \dot{V}_{O_2} was typically established within 10 min. Trials lasted 30 min, during which undisturbed birds were observed from inside a hut through a tinted glass window (Fig. 1A). Birds were familiarized with the procedure on at least three occasions before data collection started. To investigate the effect of water temperature on metabolic rate when resting in water, we conducted trials in water temperatures ranging from 7.8 to 15.6°C . The effect of feeding (heat increment of feeding, HIF) on the metabolic rate during resting in water was investigated in specific trials when birds were fed a known amount of food (60 g of herring) at various times (30–120 min) before a trial. Mean water temperature during these trials was 14.6°C (range $13.5\text{--}15.8^\circ\text{C}$).

Diving metabolism

Diving metabolic rate was measured in all birds during shallow and deep diving at water temperatures ranging from 6.1 to 17.5°C . Water temperature was measured 10 cm below the surface after each set of trials. At the beginning of a trial a bird was captured, weighed and placed inside the respiration chamber. A trap door at the bottom of the dome prevented the bird from submerging directly. When the bird floated calmly and a stable \dot{V}_{O_2} was established (usually within 5–10 min) the

trap door was opened through a remote pulley system, and diving activity began. During a trial, birds dived continuously to the opposite end of the shallow dive tank or to the bottom of the deep dive tank. Through the tinted glass window in the laboratory hut (Fig. 1) it was possible to observe the surface behaviour of the birds without causing any disturbance. To monitor the behaviour of birds underwater, submersible cameras (Lorex, MBrands, Scarborough, ON, Canada) were positioned within the tanks and connected to a multiplexer (EverFocus Electronics Corp., Taipei, Taiwan) and a video monitor inside the hut. This was especially important in the deep dive setting, where dives were classified as deep (diving to the bottom) or shallow (diving to less than 3 m depth). Birds typically started a dive bout with an exploratory shallow dive before performing a series of deep dives to the bottom of the tank. Similarly, they terminated a deep diving bout by either remaining at the surface or by switching back to shallow diving. To avoid a mixture of shallow and deep dives within a deep dive trial as much as possible, the trap door was closed, preventing birds from submerging, as soon as it became apparent that birds would not return to depth. Deep dive trials during which birds spent more than 30% of the overall time spent submerged at shallow depths were excluded from the analysis. All relevant behaviour of the birds was marked onto the respirometry traces, so that behaviour as well as dive and surface events could be related to the respirometry recordings. The majority of shallow dive trials lasted $\sim 20\text{--}30$ min, during which birds dived voluntarily and without any interference. Here, a trial was terminated by remotely closing the trap door when a bird remained at the surface for more than 10 min. In the deep dive setting, birds were not motivated to dive to the bottom of the tank unless food was placed there. Hence, birds ingested small herring pieces during all deep dive trials. Preliminary results from experiments during which cormorants were fed a similar amount of herring while resting in air showed that metabolic rate was not increased during the first 10 min after ingestion (M. R. Enstipp, D. Grémillet and D. R. Jones, unpublished data). Consequently, the duration of deep dive trials was kept within this time frame. Therefore, 'post-absorptive deep dive trials' refers to trials where birds had been fasted overnight (at least 15 h) beforehand but ingested small amounts of herring during the trial.

To investigate the effect that feeding (HIF) might have on diving metabolic rate, dive trials were conducted in both the post-absorptive and absorptive state. For the absorptive trials, birds were fed a known amount of food (60 g of herring) at various times (30–150 min) before a trial. Trials were conducted in the mornings and afternoons with a maximum of two dive trials per bird per day.

Stomach temperature

In parallel with the respirometry measurements, temperature loggers (MiniTemp-xl, earth&OCEAN Technologies, Kiel, Germany; length 70 mm, diameter 16 mm, mass 25 g, resolution 0.03K) were employed for all birds to measure stomach temperature during the dive trials and when birds

rested in water. Stomach temperature should reflect abdominal body temperature during post-absorptive trials if no food is ingested. Temperature loggers were programmed to record stomach temperature every 10 s and were fed to the birds inside a herring. The loggers were equipped with a spring crown and were not regurgitated by the birds but retrieved when the memory was filled, after about 10 days (Wilson and Kierspel, 1998). After retrieval, the data were downloaded onto a laptop computer, and the logger was cleaned, re-programmed and re-fed to the bird.

Plumage air volume

To determine body insulation and allow comparison with other cormorant species, we measured the total plumage air volume in six bird carcasses (mean body mass 2.04 ± 0.35 kg) according to previously published methods (Grémillet et al., 2005a; Wilson et al., 1992b). The only difference in our assessment was that we collected the water displaced by the submerged carcass in a beaker and determined its volume using a precision balance, assuming a density of 0.9982 g ml⁻¹ for water at 20°C.

Stroke frequency and work against buoyancy

Underwater filming allowed the calculation of stroke frequencies (strokes s⁻¹) during diving as an indicator of locomotor effort. For deep (vertical) diving, we calculated stroke frequencies during descent (near top and near bottom) and bottom phase, while for shallow (horizontal) diving it was calculated for a position about halfway along the shallow dive tank. To this end, we recorded the video signal together with the signal of a video date time generator (RCA, resolution 0.1 s) onto VHS tape. Video analysis was performed on 10 sequences per bird for each category by counting the total number of strokes per sequence and dividing by the time elapsed. Duration of selected sequences ranged between 1 and 5 s. We also estimated the mechanical work conducted against buoyancy during deep (vertical) and shallow (horizontal) diving. To this end, we calculated the buoyancy of a 2.1 kg cormorant at the surface by adding the buoyancy of the respiratory system, the plumage and body tissues. We estimated the air volume in the respiratory system from the equation given by Lasiewski and Calder (Lasiewski and Calder, 1971) ($V_{\text{resp}} = 0.1608 M_b^{0.91}$), where M_b is body mass, and used our measurement of plumage air volume. The buoyancy of air was taken as 9.79 N l⁻¹, and the buoyancy of body tissues as -0.659 N kg⁻¹ (Lovvorn et al., 1999). During descent, hydrostatic pressure increases at a constant rate of 10 kPa m⁻¹ of depth, so that the air volume decreases by a factor of $10/(n+10)$, where n is depth in metres. By contrast, the buoyancy of body tissues does not change with water depth (Lovvorn et al., 1999). Mechanical work per second (W , in Joules) against buoyancy during descent in deep (vertical) dives was calculated as:

$$W = F_B D, \quad (1)$$

where F_B is the buoyant force at a given depth in Newtons and

D is the vertical distance moved in metres during 1 s. It took cormorants ~ 10 s to reach the tank bottom (10 m); hence, we assumed a vertical descent speed of 1 m s⁻¹ to model the changes in mechanical work against buoyancy during descent. To calculate the work against buoyancy during shallow (horizontal) diving, we assumed that birds swam at a depth of 1 m (which was typically observed, apart from the very short periods of descent and ascent). The distance (D_s) that a bird would float upward during the stroke cycle duration (t) and that has to be offset by the bird is given by:

$$D_s = U_0 t + \frac{1}{2} a_B t^2, \quad (2)$$

where U_0 is initial upward velocity (equal to zero) and a_B is acceleration due to buoyancy, given by F_B/M_b (Lovvorn et al., 1991). t was calculated as the inverse of observed stroke frequency. Substituting D in Eqn 1 by D_s yields the work against buoyancy per stroke cycle during horizontal swimming; this value was then multiplied by stroke frequency to arrive at the work against buoyancy per second underwater. To contrast the work conducted against buoyancy during deep (vertical) and shallow (horizontal) diving, we calculated the cumulative work against buoyancy during a 20 s dive for both situations. For deep (vertical) diving, a 20 s dive was split into a 10 s descent phase (Eqn 1), a 2 s bottom phase (Eqn 2) and a 8 s ascent phase (passive). For shallow (horizontal) dives, we only considered the bottom phase.

Data analysis and statistics

In a preliminary analysis, oxygen consumption rates (\dot{V}_{O_2}) were calculated using equation 3b given by Withers (Withers, 1977), which indicated a respiratory exchange ratio (RER) of 0.73 in post-absorptive birds resting in air. However, during some of the dive trials, these values seemed unreasonably low, probably because CO₂ was absorbed by the water or because of non-pulmonary CO₂ loss by our birds (Walsberg and Wolf, 1995). Hence, for our analysis, we assumed a RER of 0.71 for all post-absorptive trials and 0.8 for all absorptive trials and used equation 3a from Withers (Withers, 1977) to calculate \dot{V}_{O_2} .

Metabolic rate during resting in air was calculated from the lowest 15-min running average value of \dot{V}_{O_2} . Similarly, metabolic rate during resting in water was taken as the average from the lowest and stable 10 min section of \dot{V}_{O_2} from each 30 min trial. Our respirometry system was sufficiently fast to allow separation of individual dive and surface events. However, since we were interested in obtaining an estimate of the energetic costs associated with foraging activity, we decided to calculate diving metabolic rate (MR_d) as the average value of \dot{V}_{O_2} during a dive bout from its start until 30 s after the last dive in a bout:

$$\text{MR}_d (\dot{V}_{O_2 \text{ dive}}) = \dot{V}_{O_2 \text{ total dive bout}} / (\sum t_{\text{dive}} + \sum t_{\text{surface}}), \quad (3)$$

where $\sum t_{\text{dive}}$ and $\sum t_{\text{surface}}$ are the sum of all dive and surface durations in a dive bout, respectively. A dive bout was characterised by continuous diving activity and ended, by definition, when birds remained at the surface for longer than

Table 2. Equations for linear regressions of energy expenditure ($W\text{ kg}^{-1}$) against temperature for double-crested cormorants during resting and diving

	Temperature ($^{\circ}\text{C}$)	Intercept	Slope	N	n	r^2	F	P
Resting in air (post-absorptive)	16.6 (6.2–25.4)	7.223 ± 0.29	-0.127 ± 0.02	10	62	0.71	20.30	<0.001
Resting in water (post-absorptive)	11.7 (7.8–15.6)	19.484 ± 0.82	-0.581 ± 0.07	9	45	0.78	22.57	<0.001
Shallow diving (post-absorptive)	12.1 (7.1–17.5)	26.805 ± 0.46	-0.410 ± 0.03	9	94	0.79	34.09	<0.001
Shallow diving (absorptive)	13.1 (7.0–17.1)	28.732 ± 0.78	-0.521 ± 0.05	9	85	0.69	18.82	<0.001
Deep diving (post-absorptive)	10.2 (6.1–15.4)	30.844 ± 0.43	-0.413 ± 0.04	8	132	0.57	14.11	<0.001
Deep diving (absorptive)	10.7 (6.1–15.4)	32.554 ± 0.52	-0.473 ± 0.05	8	129	0.60	12.23	<0.001

Equations were determined by multiple linear regression that takes into account variability between subjects (Glantz and Slinker, 1990). Effects of body mass were removed from data following Packard and Boardman (Packard and Boardman, 1999); see Materials and methods for details. Values are means \pm s.e.m. Temperature (mean and range) refers to air temperature in case of 'resting in air' and to water temperature in all other cases. N gives the number of birds, while n refers to the number of trials.

100 s [using a log-survivorship plot as bout ending criterion (Slater and Lester, 1982)] or when the trap door was closed (deep diving). Birds typically started to dive from the moment the trap door was opened. Because of the intrinsic time constant of our system, however, it took approximately 1 min before our system stabilised at an equilibrium point. Dives performed during this time were excluded from analysis. Oxygen consumption rates ($\text{ml O}_2\text{ min}^{-1}$) were transformed to energy expenditure using the caloric equivalent corresponding to the assumed RER. We used a conversion factor of $19.7\text{ kJ l}^{-1}\text{ O}_2$ for post-absorptive trials (RER=0.71) and $20.1\text{ kJ l}^{-1}\text{ O}_2$ for absorptive trials (RER=0.8) (Schmidt-Nielsen, 1997) to transform these values to Watts. Mass-specific metabolic rate (MR in $W\text{ kg}^{-1}$) is given by $19.7\dot{V}_{\text{O}_2}/60M_b$ for post-absorptive trials and by $20.1\dot{V}_{\text{O}_2}/60M_b$ for absorptive trials, where M_b is in kg and \dot{V}_{O_2} is in $\text{ml O}_2\text{ min}^{-1}$. We removed the effects of body mass on metabolic rate following Packard and Boardman (Packard and Boardman, 1999). In brief, for all conditions investigated, we plotted the relationship between metabolic rate (whole animal) and body mass. We used the slope of the resulting relationship to adjust data to the mean body mass for our birds (2.1 kg), i.e. data were adjusted upwards and downwards for small and large birds, respectively. The adjusted data were then divided by mean body mass to arrive at the mass-specific metabolic rates reported.

Stomach temperatures were analysed using Multitrace (Jensen Software Systems, Laboe, Germany). Resting values during the night and day were established from periods when birds were calm. Temperature recordings were averaged over a period of 6 h during the night (between 23.00 h and 05.00 h) and over periods of at least 2 h during the day (between 08.00 h and 18.00 h). The average day temperature ('day avg') was taken as the mean stomach temperature during the hours of daylight (from sunrise to sunset). Stomach temperatures during the various phases of the dive and resting-in-water trials were taken as averages from the first and last minute of a trial ('start' and 'end', respectively), as the single highest value during a trial ('peak') and as the entire trial average. We included only stomach temperature recordings from birds that had not

ingested food for at least 3 h in our analysis (with the exception of 'day avg' temperature), to exclude periods of decreased stomach temperature after food ingestion.

Thermal conductance (TC) was calculated when cormorants rested in air and water and during shallow and deep diving (post-absorptive trials only) using the following equation:

$$TC = MR / [(T_b - T_a) SA], \quad (4)$$

where TC is in $W\text{ m}^{-2}\text{ }^{\circ}\text{C}^{-1}$ and MR (metabolic rate) in W ; T_b is the body temperature (mean stomach temperature during a trial) in $^{\circ}\text{C}$, T_a is the ambient temperature in $^{\circ}\text{C}$, and SA is the surface area in m^2 , which was estimated using Meeh's formula: $SA = 10M_b^{0.67}$ (Drent and Stonehouse, 1971), where M_b is in g and SA is in cm^2 .

Two-way repeated measures analysis of variance (ANOVA) with Tukey pairwise multiple comparisons was used for comparison of metabolic rate during different activities at various temperatures. To investigate the effect of depth, water temperature and feeding status on cormorant diving metabolic rate we used a repeated measure ANOVA on three factors. When single comparisons were made, Student's paired t -test was used. The relationships between energy expenditure/thermal conductance and air or water temperature for the various conditions (Table 2) that take into account variability between subjects were determined using repeated-measures multiple linear regression, with each bird being assigned a unique index variable (Glantz and Slinker, 1990). Analysis of covariance (ANCOVA) was used to compare these different relationships. Significance was accepted at $P < 0.05$. All mean values are presented with standard deviation (± 1 s.d.).

Results

BMR of double-crested cormorants was $13.97 \pm 1.57\text{ ml O}_2\text{ min}^{-1}\text{ kg}^{-1}$, which corresponds to an energy expenditure of $4.59 \pm 0.51\text{ W kg}^{-1}$ (Table 1, Fig. 2). Repeated-measure ANOVA comparisons revealed that activity (resting in air/water, shallow/deep diving, $P < 0.001$, $F = 402.65$) and temperature (warm vs cold; range 5–25 $^{\circ}\text{C}$ in air and

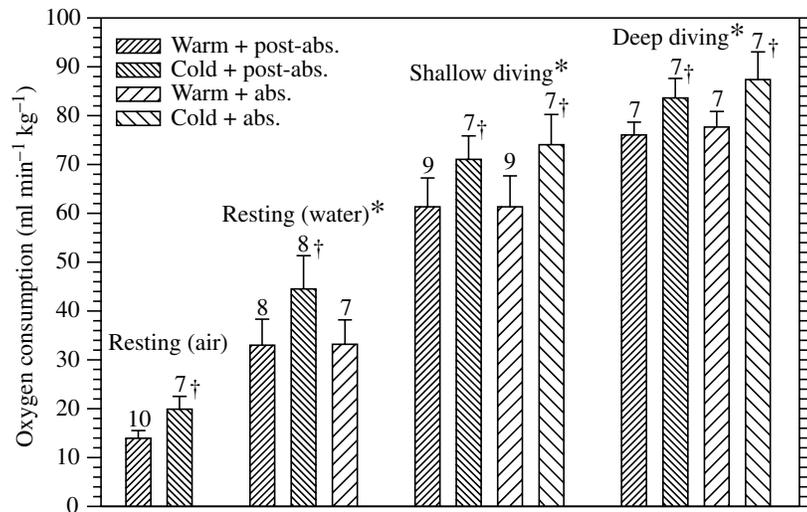


Fig. 2. Oxygen consumption rates ($\text{ml O}_2 \text{ min}^{-1} \text{ kg}^{-1}$) of double-crested cormorants during various activities at different temperatures ('warm' and 'cold') and feeding status ('post-absorptive' and 'absorptive'). Mean temperatures when resting in air were 22.1 and 8.6°C for 'warm' and 'cold' trials, respectively. For temperature values during all other trials, see Table 1. Oxygen consumption during resting in air ('warm') was taken as basal metabolic rate. Values are grand means ± 1 s.d., which were established from individual bird means. Values above the columns indicate the number of birds used. *Significantly different from resting (air) values. †Significantly different from respective 'warm' temperature values.

6.1 – 17.5°C in water; $P < 0.001$, $F = 96.24$) significantly affected metabolic rate (Fig. 2). Compared with the resting situation in air ('warm', which was taken as BMR), the metabolic rate of cormorants was significantly elevated when resting ($2.4 \times \text{BMR}$) or diving (shallow, $4.4 \times \text{BMR}$ and deep, $5.4 \times \text{BMR}$) in 'warm' water (Table 1).

Temperature had a significant effect on the metabolic rate of

cormorants during all activities ($P < 0.001$, $F = 96.24$). Resting in air at an air temperature around or below their lower critical temperature significantly elevated metabolic rate (Fig. 2; mean air temperature during 'cold' air trials: $8.6 \pm 1.1^\circ\text{C}$). When resting or diving in 'cold' water, the metabolic rate of cormorants was significantly increased when compared with the respective 'warm' water trials (Table 1; Figs 2, 3). In all cases, there was a significant negative relationship between metabolic rates of double-crested cormorants during different activities and temperature, which allowed the calculation of linear regression equations (Table 2; Fig. 3). ANCOVA comparisons revealed that the slope of these relationships for all activities in water were not significantly different from each other ($P = 0.43$, $F = 0.96$), while the intercepts were significantly different ($P < 0.001$, $F = 42.47$). However, slope and intercept for 'resting in air' were significantly different from those of all other activities ($P < 0.001$ for all comparisons, F -values between 25 and 1379).

Diving to depth was energetically more costly than conducting shallow (horizontal) dives (Figs 2, 3). Again, this difference was significant when comparing shallow (horizontal) and deep (vertical) diving while accounting for water temperature and feeding status ($P < 0.001$, $F = 83.36$).

While metabolic rate was increased after feeding, this increase was not significant when the effects of temperature and activity were accounted for ($P = 0.072$, $F = 5.15$). In other words, metabolic rates in absorptive and post-absorptive trials

during a particular activity (e.g. shallow diving) and at a particular temperature (e.g. 'warm') were not significantly different from each other (Fig. 2). Furthermore, ANCOVA comparisons showed that the relationship between energy expenditure and water temperature (Table 2) was not significantly different between post-absorptive and absorptive trials during shallow (intercept, $P = 0.055$; slope, $P = 0.18$) and deep diving (intercept, $P = 0.06$; slope, $P = 0.54$). However,

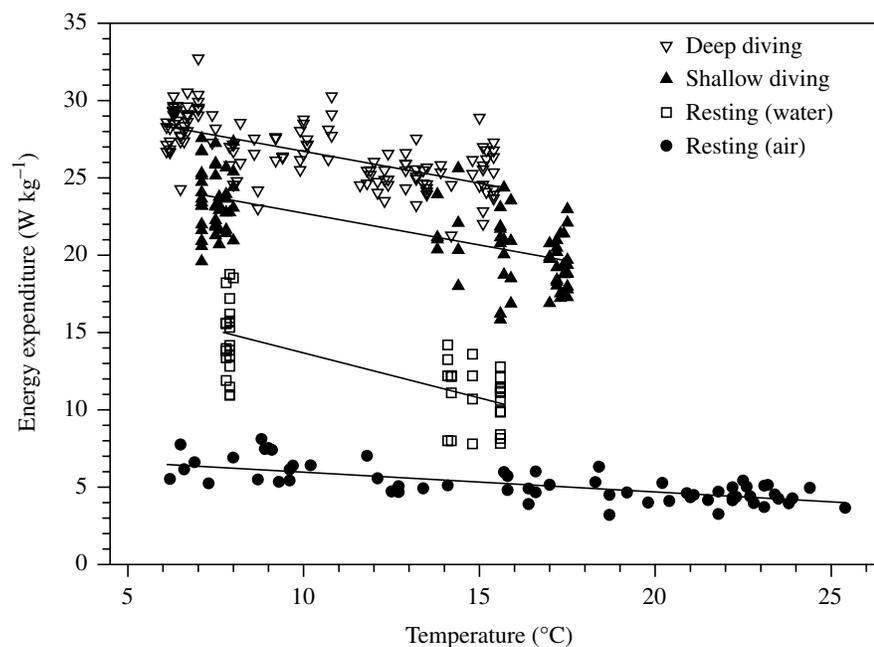


Fig. 3. Energy expenditures (W kg^{-1}) of double-crested cormorants during various activities in relation to temperature (post-absorptive trials only). Temperature refers to air temperature in the case of 'resting in air' and to water temperature in all other cases. See Table 2 for details about the regression equations and the number of trials and birds used for each category.

metabolic rates during all absorptive trials were increased by about 5–8% above the post-absorptive level within 30 min after feeding and remained elevated for at least 2 h.

The various factors investigated in our study (activity, temperature, depth, feeding status) were interactive and additive. While diving was more costly than resting (in water and air), descending to depth increased the energetic expenses even more. Ingesting food and decreasing water temperatures further increased the energy expenditure of cormorants. As can be seen in Table 1 and Fig. 2, the highest energy expenditure observed was during deep diving in cold water after food ingestion (absorptive), when metabolic rate increased by a factor of $6.4 \times \text{BMR}$.

Dive durations of birds were similar for shallow and deep diving. Surface duration, however, was significantly shorter during deep diving ($P=0.019$, $t=3.42$), resulting in a higher dive/pause ratio (Table 1). Similarly, the fraction of the dive cycle (dive and succeeding surface interval) spent underwater was higher during deep diving (60.4%) than during shallow diving (53.2%).

Stroke frequency during the early descent phase of deep dives (3.42 ± 0.25 strokes s^{-1}) was significantly elevated when compared with all other phases during deep and shallow diving ($P < 0.001$, $F=98.98$; Fig. 4A). Stroke frequency declined with increasing depth and reached values similar to the horizontal shallow dive situation at a depth of ~ 10 m (Fig. 4A). In accordance with the changes in stroke frequency, glide duration between strokes increased with increasing depth. Calculated mechanical work against buoyancy declined considerably with depth during the descent phase of deep (vertical) dives, ranging from 5.3 J s^{-1} near the surface to 2.2 J s^{-1} at 10 m (Fig. 4B). Assuming a vertical descent speed of 1 m s^{-1} , the cumulative mechanical work against buoyancy during descent (10 s) would be 35.1 J . During shallow (horizontal) diving, work against buoyancy was calculated to be 2.9 J s^{-1} , which would result in a total of 28.6 J during an equivalent 10 s period. However, while birds during shallow (horizontal) diving continued to stroke throughout a dive, birds within the deep dive tank ascended passively (after a brief bottom period) without further locomotor effort. As a consequence, total mechanical work against buoyancy during a 20 s dive was calculated to be 57.2 J during shallow (horizontal) diving and 36.4 J during deep (vertical) diving.

Stomach temperature showed a clear diurnal pattern in which resting temperature declined significantly from $40.5 \pm 0.2^\circ\text{C}$ during the day to $39.4 \pm 0.2^\circ\text{C}$ during the night (Fig. 5). During activity, temperature increased rapidly and remained at values significantly above those at resting. During all trials (resting in water, shallow diving), stomach temperature was elevated above 42°C and did not decline significantly throughout trials lasting up to 30 min in water temperatures ranging from 7.0 to 15.6°C (Fig. 5).

With a decrease in water temperature, thermal conductance (TC) decreased significantly during deep ($P < 0.001$, $F=19.62$) and shallow diving ($P < 0.001$, $F=24.07$), while it changed little when birds rested in water (Fig. 6). Similarly, TC decreased

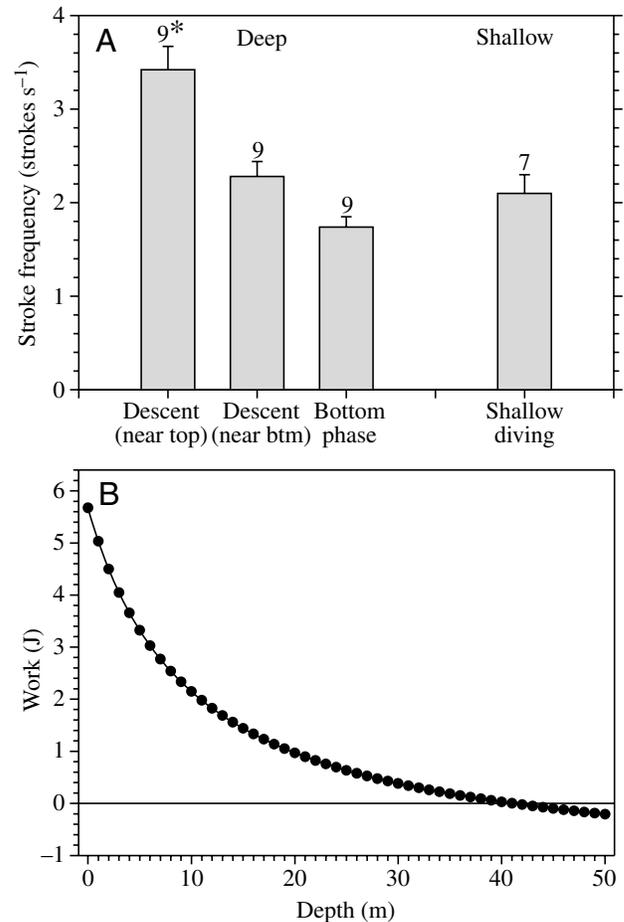


Fig. 4. (A) Stroke frequencies (strokes s^{-1}) during deep and shallow diving in double-crested cormorants. Values are grand means established from individual bird means ± 1 s.d. (the number of birds used is indicated above each column) and are based on 10 observations per bird and category. Birds ascended passively during deep diving. *Significantly different from shallow diving. (B) Modelled changes in mechanical work against buoyancy per s during descent for a 2.1 kg double-crested cormorant (assuming a vertical descent speed of 1 m s^{-1}). The horizontal line indicates the point of neutral buoyancy. Note that change is greatest within the first 10 m.

significantly with a decrease in air temperature when birds rested in air ($P < 0.001$, $F=5.91$).

Total plumage air volume of six double-crested cormorant carcasses was $0.13 \pm 0.04 \text{ l kg}^{-1}$.

Discussion

Our study is the first detailed investigation of the combined effects of dive depth, water temperature and feeding status on the energetic requirements of a diving endotherm. Activity-specific metabolic rates and the influence of modifying factors reported in this study will also be of great importance for estimating individual and population energetic requirements of diving seabirds (Boyd, 2002; Enstipp et al., 2006).

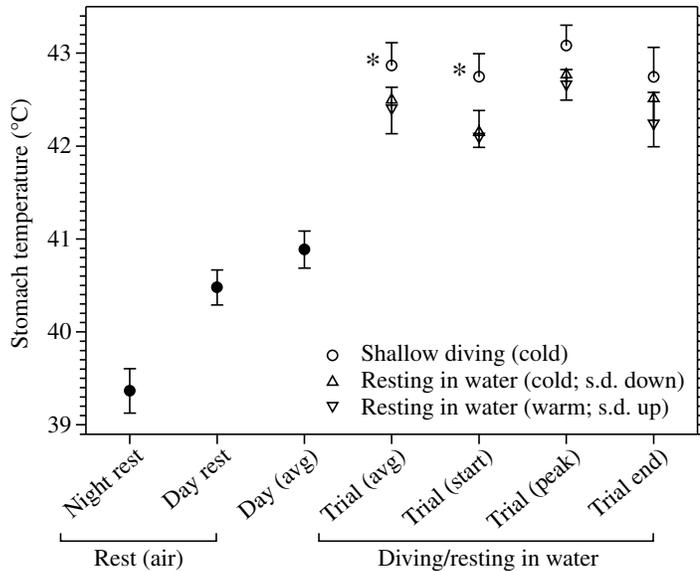


Fig. 5. Stomach temperatures ($^{\circ}\text{C}$) of double-crested cormorants during rest at night and during the day, during resting in 'warm' and 'cold' water (triangles) and during shallow diving in 'cold' water (open circles; values are grand means \pm 1 s.d.; $N=9$ birds). 'Day (avg)' is the mean stomach temperature during the hours of daylight (from sunrise to sunset), which includes periods of food ingestion. Air temperatures ranged from 10 to 26°C during the day and $0\text{--}11^{\circ}\text{C}$ during the night. All temperature values were significantly different from the 'day rest' value. *Significantly different from 'resting in water' values.

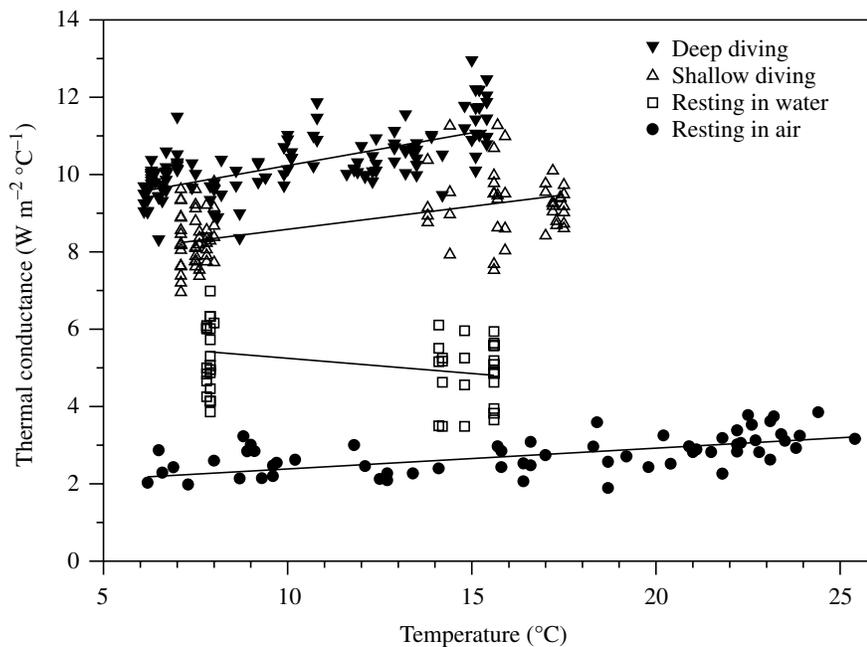


Fig. 6. Thermal conductance ($\text{W m}^{-2} \text{ }^{\circ}\text{C}^{-1}$) of double-crested cormorants at various air and water temperatures during deep and shallow diving, when resting in water and when resting in air (post-absorptive trials only). Temperature refers to air temperature in the case of 'resting in air' and to water temperature in all other cases. See Table 2 for details about the number of trials and birds used for each category. Deep diving: $r^2=0.56$, $P<0.001$; shallow diving: $r^2=0.68$, $P<0.001$; resting in water: $r^2=0.61$, $P<0.06$; resting in air: $r^2=0.54$, $P<0.001$.

Resting in air

Over the temperature range investigated in our study ($6\text{--}25^{\circ}\text{C}$), metabolic rate of cormorants increased slowly with a decline in air temperature (Fig. 3; Table 2). However, while metabolic rate was relatively stable between 25 and 10°C , it increased more rapidly at air temperatures below 10°C . Unfortunately, we were not able to manipulate air temperature and take measurements below 6°C , so that we could not unequivocally demonstrate the presence of a lower critical temperature (calculated to be $8\text{--}9^{\circ}\text{C}$).

Resting in water

Resting in water increased metabolic rate of double-crested cormorants by a factor of $2.4\times$ and $3.2\times$ BMR for 'warm' and 'cold' water, respectively (Table 1). This is considerably lower than that previously reported for great cormorants [$4.5\times$ RMR (Schmid et al., 1995)] and European shags [$4.1\times$ BMR (Enstipp et al., 2005)] but similar to Brandt's cormorants (*Phalacrocorax penicillatus*) resting in 20°C water during the day and at night [$2.5\times$ and $1.6\times$ BMR, respectively (Ancel et al., 2000); BMR predicted from Ellis and Gabrielsen (Ellis and Gabrielsen, 2002)]. Heat loss when resting at the water surface will be less than during diving, because the surface area exposed to water is smaller in the former. However, when resting in water, the temperature effect on metabolic rate was even stronger than during diving.

Metabolic rate increased by $\sim 35\%$ within the temperature range tested (Table 1), resulting in the steepest regression slope (Table 2; Fig. 3). This indicates that birds might be able to use some of the heat generated by muscle activity during diving to compensate for the heat loss in cold water. Inactive birds floating at the surface, however, will have to spend additional energy for thermoregulation. Similar patterns have been observed in other avian divers, like tufted ducks (Bevan and Butler, 1992) and macaroni penguins (*Eudyptes chrysolophus*) (Barré and Roussel, 1986). In 6°C water, body temperature of macaroni penguins dropped when birds remained inactive at the water surface but remained stable in birds that swam or dived (Barré and Roussel, 1986). While stomach temperature in our cormorants remained stable during both diving and resting in water, mean stomach temperature when resting in water was significantly lower than during diving (Fig. 5), indicating that overall heat production was greater during diving. A strong temperature dependence of metabolic rate in birds

floating on water was also observed in tufted ducks (Bevan and Butler, 1992; de Leeuw, 1996), common eiders (*Somateria mollissima*) (Jenssen et al., 1989), common murres (*Uria aalge*) and thick-billed murres (*Uria lomvia*) (Croll and McLaren, 1993) and little penguins (*Eudyptula minor*) (Stahel and Nicol, 1982).

Dive behaviour

Dive patterns displayed by the cormorants in our study (Table 1) were similar to patterns observed in double-crested cormorants foraging in the wild. Ross (1974) reported mean dive and surface durations for double-crested cormorants of 25.1 s and 10.3 s, respectively, when foraging in water 1.5–7.9 m deep. In our study, surface durations between consecutive shallow dives were longer than after deep diving, resulting in a higher dive/pause ratio during deep diving. This would indicate that birds dived more efficiently during deep diving since potential underwater foraging time was increased. However, surface duration after shallow diving was variable (see Table 1) and might be explained by a difference in motivation (birds were feeding on small herring pieces during deep diving but not during shallow diving). In support of this, Enstipp et al., working with the same individuals diving for food within both tanks, found that surface duration was greater after deep dives (Enstipp et al., 2001), as one might expect.

Diving metabolic rate and modifying factors

Our study clearly illustrates the importance of a variety of factors (depth, temperature, feeding status) on shaping the energetic costs associated with foraging in cormorants. Dive depth had the strongest influence on dive costs. While metabolic rate increased on average by 22% during deep diving (post-absorptive and absorptive trials) compared with shallow diving, a lower water temperature increased diving metabolic rate on average by 13% and 17% during post-absorptive and absorptive trials, respectively (Table 1). Feeding before a trial increased metabolic rate during diving or when resting in water. However, the effect was small (5–8%) when compared with the other factors investigated. Since the amount of food ingested before a trial was relatively small (60 g of herring), it is conceivable that the HIF for cormorants in the wild is greater, when birds might ingest up to a few hundred grams of fish in quick succession.

Our study also shows that dive costs in double-crested cormorants are similar to those of other avian divers (Fig. 7). While dive costs in cormorants as a group tend to lie above the average relationship relating diving metabolic rate to body mass in avian divers (Fig. 7), this is most noticeable in the great cormorant. To allow comparison between dive cost measurements for different *Phalacrocorax* species, we removed the effect of water temperature on diving metabolic rate by recalculating the dive costs during shallow diving for a water temperature of 12.6°C (the water temperature for *P. carbo sinensis* in Schmid et al., 1995). Our analysis revealed

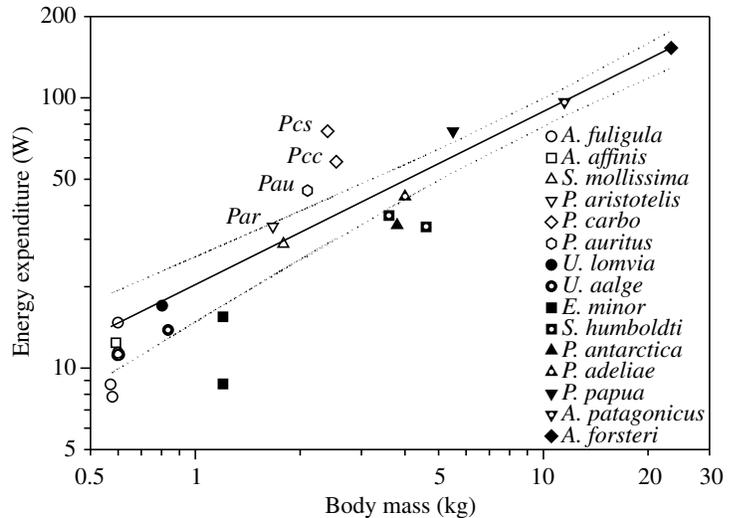


Fig. 7. Energy expenditure (W) of foot-propelled (open symbols) and wing-propelled (filled symbols) aquatic birds during diving. The relationship is best described by the following power function (two variables): energy expenditure = $20.36M_b^{0.64}$, where energy expenditure is in W and M_b is body mass in kg; $r^2=0.87$. Note that values are plotted along a \log_{10} scale. The dotted lines indicate the 95% confidence interval. Values are based on table 1 (from Enstipp et al., 2005) and include only respirometry studies. With the exception of two values for *A. fuligula* (De Leeuw, 1996) and the values for *U. lomvia* and *U. aalge* (Croll and McLaren, 1993), all values are based on studies of birds diving in shallow tanks (for references, see Enstipp et al., 2005). Values for the three cormorant species were recalculated for a water temperature of 12.6°C [the water temperature for *P. carbo sinensis* (Pcs) in Schmid et al., 1995] by using established regression equations [*P. aristotelis* (Par) (Enstipp et al., 2005); *P. auritus* (Pau) (present study); *P. carbo carbo* (Pcc) (data from Grémillet et al., 2001)].

that mass-specific metabolic rates for all three cormorant species are very similar [*P. auritus*, 21.64 W kg⁻¹ (present study, Table 2); *P. aristotelis*, 20.01 W kg⁻¹ (Enstipp et al., 2005); *P. carbo carbo*, 22.85 W kg⁻¹ (data from Grémillet et al., 2001)]. These mass-specific values are considerably below the dive costs previously reported for *P. carbo sinensis* by Schmid et al. (31.40 W kg⁻¹) (Schmid et al., 1995) and are similar to other foot-propelled divers (table 1 in Enstipp et al., 2005).

The effect of dive depth on diving metabolic rate

The observed increase in metabolic rate during deep diving, when compared with shallow diving (Table 1; Figs 2, 3), might be caused (1) by the distinct increase in locomotor effort during early descent (Fig. 4) or (2) by an increase in heat loss as a consequence of the reduced insulative properties of the plumage.

Mechanical costs

As indicated by stroke frequency, cormorants worked the hardest during the early descent phase of deep dives (Fig. 4A). It is important here to consider the different body orientation of birds when descending in the deep dive tank or when diving

in the shallow tank with no changes in depth. In the shallow tank, apart from the very short submergence and emergence to and from ~1 m depth, birds swam horizontally (with a body angle of ~0°, i.e. parallel to the surface), comparable to the bottom phase of a deep dive (albeit at a greater buoyancy). In the deep dive tank, however, birds swam almost vertically during descent and ascent, with a body angle of about -70° and 70°, respectively. The higher stroke frequency we observed during descent to depth might therefore be explained by the greater mechanical work required to overcome buoyancy during vertical descent to depth (5.3 J s⁻¹ near the surface; Fig. 4B), when compared with shallow (horizontal) diving (2.9 J s⁻¹) (see also Lovvorn et al., 1991; Lovvorn et al., 2004). The increased stroke frequency could also indicate that birds submerged with a greater air volume within their respiratory system during deep diving than during shallow diving. Respiratory movements were clearly visible in preparation for deep dives (brief period of hyperventilation), while this was not the case for shallow dives. Also, deep dives were typically initiated with a pre-dive leap (Wilson et al., 1992a), which rarely occurred at the onset of shallow dives. This could indicate that cormorants regulate their respiratory air volume in accordance with the anticipated dive depth, as has been suggested for other avian divers, namely penguins (Sato et al., 2002; Wilson, 2003).

In this context, it is interesting to note that Enstipp et al. reported heart rates during deep dives of double-crested cormorants (same individuals, identical set-up) that were significantly higher than during shallow (horizontal) dives of similar duration (Enstipp et al., 2001). Similarly, Froget et al. found that heart rate during the first 6 s of submersion in king penguins (*Aptenodytes patagonicus*) was higher in long (deep) dives than in short (shallow) dives (Froget et al., 2004). For the cormorants, it was argued that compression hyperoxia during descent and an assumed reduction in energetic costs associated with reduced buoyancy during deep dives would delay a chemoreceptor-mediated decline in heart rate. However, based on the suggestion by Sato et al. that king penguins inhale more air prior to deep dives (Sato et al., 2002), Froget et al. argued that higher heart rates during the beginning of deep dives might be explained by the greater effort necessary to overcome an increase in buoyancy (Froget et al., 2004). The high stroke frequencies we observed during the early phase of deep dives would point in the same direction and might have contributed to the significantly higher energetic costs we measured during deep diving. Seen in this light, the observed higher heart rates during deep diving in cormorants (Enstipp et al., 2001) might be explained by the necessity to maintain a high blood flow to the hard-working leg muscles, at least during early descent. On the other hand, submerging with a greater respiratory air volume and, hence, a larger oxygen store might enable cormorants to maintain a higher arterial oxygen tension (P_{aO_2}) in the deep diving situation despite a higher \dot{V}_{O_2} (when compared with shallow diving), delaying a chemoreceptor-mediated heart rate decline.

However, the propulsive effort of cormorants was greatest

during the initial descent phase of deep dives. Stroke frequency declined with increasing depth and was similar to the shallow dive frequency near the bottom of the 10 m tank (Fig. 4A). Furthermore, while birds had to continue stroking throughout shallow diving, they surfaced passively during deep diving, reducing overall locomotor costs. Watanuki et al. found that European shags foraging in the wild descend and ascend almost vertically (60–90° relative to sea surface) (Watanuki et al., 2005). Stroke frequency during descent decreased with depth, while ascent from dives to 40 m depth was passive. In our study we only recorded stroke frequency, while cormorants might have also altered stroke amplitude. However, in the shag study, Watanuki et al. found that birds maintained the duration and strength of the power stroke during descent but changed glide duration between strokes (Watanuki et al., 2005). Hence, stroke frequency might be a good indicator of locomotor effort during diving in cormorants. To get a better understanding of locomotor effort during deep and shallow diving, we estimated the average stroke frequency during both modes by adding the number of strokes during the different phases of a 20 s dive and dividing by dive duration. The resulting mean stroke frequency of 1.8 and 2.1 strokes s⁻¹ for deep and shallow diving, respectively, indicates that overall stroke frequency was reduced during deep diving. Similarly, calculating the cumulative work conducted against buoyancy during a 20 s dive showed that deep (vertical) diving required less work than shallow (horizontal) diving because of passive ascent during the former. Hence, overall locomotor effort was probably reduced during deep diving.

Thermoregulatory costs

Plumage air volume measured in double-crested cormorant carcasses was similar to that of great cormorants and well below the volume of other avian divers (Grémillet et al., 2005a; Wilson et al., 1992b), highlighting the great potential for heat loss during dives to depth in cormorants. However, as Wilson et al. pointed out (Wilson et al., 1992b), whether metabolic rate will be increased during deep diving as a direct consequence of a compromised insulative capacity will largely depend on peripheral heat conservation mechanisms such as vasoconstriction. In emperor penguins (*Aptenodytes forsteri*), among others, such a mechanism seems to be in place. During diving, birds maintain a high core temperature while the outer body shell cools (Ponganis et al., 2003). The latter is probably a consequence of peripheral vasoconstriction and decreased plumage insulation, which increases conductive and convective heat loss to the water. The reduced thermal gradient between core and peripheral tissues will decrease heat loss to the water. Whether cormorants use a similar mechanism to reduce heat loss during diving remains to be investigated. An indication that some mechanisms for heat conservation are in place in double-crested cormorants comes from the observation that *TC* declined significantly during both shallow and deep diving, when water temperature decreased (Fig. 6). The decrease in *TC* and, hence, increase in insulation did not completely prevent heat loss during diving, however, as can be

seen by the increase in heat production with falling water temperature (Fig. 3). The fact that abdominal temperature (as measured by stomach temperature loggers) did not change significantly during post-absorptive shallow diving in double-crested cormorants (Fig. 5) could indicate that peripheral vasoconstriction is restricted to the skin or adjacent tissues. Unfortunately, we did not deploy stomach temperature loggers during deep diving, so we have no information on abdominal temperature during these dives. If, as we will argue below, thermoregulatory costs are increased during deep diving and if stomach temperature loggers reflect whole-body temperature, we should have seen a temperature decline during diving that was, in turn, countered by thermogenesis. Previous recordings (using a thermistor positioned close to the heart alongside an electrocardiogram electrode; M. R. Enstipp, R. A. Andrews and D. R. Jones, unpublished data) showed that core temperature of double-crested cormorants remained stable during dives to a depth of 10 m (water temperature $\sim 15^{\circ}\text{C}$). However, this does not preclude regional heterothermy elsewhere in the body. Abdominal temperatures of South-Georgian shags (*Phalacrocorax georgianus*) have been shown to decrease during dive bouts in the wild (during which cold food was ingested) (Bevan et al., 1997). In a recent study, Grémillet et al. (Grémillet et al., 2005b) found that abdominal temperature declined progressively throughout dive bouts of great cormorants foraging off Greenland (up to 2°C). Mean dive depth for these birds ranged between 2 and 18 m. The greatest and smallest temperature drops coincided with deeper and shallower dives, respectively. Hence, this would support the notion of increased heat loss and, therefore, thermoregulatory costs during deep diving. In the current study, stomach temperature initially increased during shallow diving (even in cold water), which would imply that heat generated by the exercising muscles exceeded heat loss to the water. However, after reaching a peak, stomach temperature started to decline, implying that heat loss exceeded heat generation. At the end of a trial, stomach temperature typically reached the initial level (Fig. 5) and it is conceivable that during longer diving activity temperature would have fallen to lower levels. During deep diving, when insulation from air trapped within the plumage is greatly reduced, temperature decline should be accelerated, unless countered by thermogenesis.

When trying to investigate to what degree heat loss, and consequently thermoregulatory costs, might have contributed to the observed difference in diving metabolic rate between shallow and deep diving, we should also consider the following methodological concern. De Leeuw argued (De Leeuw, 1996) that diving metabolic rate (MR_d), the way it is usually calculated in respirometry studies, only reflects the mechanical costs but not the thermoregulatory costs, which are largely paid after the end of a dive bout and, hence, are excluded from analysis. While this raises some interesting points, Fig. 3 clearly illustrates that thermoregulatory costs associated with diving in double-crested cormorants were included in our analysis. Furthermore, the almost identical slopes of the

regression equations relating dive costs to water temperature (Table 2) suggest that, while absolute thermoregulatory costs are different, the relative increase with a decline in water temperature is similar during deep and shallow diving. However, to gain a better understanding of heat loss and potentially incurred thermoregulatory costs during diving, we conducted an analysis similar to that of De Leeuw (De Leeuw, 1996). To this end we calculated the 'excess diving costs' (EDC) of cormorants during deep and shallow diving in cold water (post-absorptive trials). EDC, the excess oxygen consumption over the resting rate, was calculated over the period from the first dive in a bout until metabolic rate returned to the resting level (see De Leeuw, 1996). Our analysis showed that EDC during shallow diving was about $1.5 \times \text{MR}_d$, whereas during deep diving it was about $2.6 \times \text{MR}_d$. This indicates that heat loss and thermoregulatory costs during deep diving are in fact substantially increased when compared with during shallow diving. However, to study the effect of dive depth on heat loss and to evaluate how heat loss might shape diving costs in cormorants, heat flux measurements during diving, as have been conducted in marine mammals (Willis and Horning, 2005), are urgently needed.

In the context of ecological energetics, one should also keep in mind that, in the wild, cormorants, like other avian divers, can potentially use a number of mechanisms to decrease thermoregulatory costs. For example, birds might be able to use the additional heat generated by the flight muscles when leaving the foraging area or they might be able to use heat generated from the HIF to substitute for thermoregulatory costs (Kaselloo and Lovvorn, 2003). These mechanisms might allow birds to make up for at least some of the incurred heat loss during diving without having to spend additional energy for thermoregulation by means of shivering or non-shivering thermogenesis. However, in our experimental set-up, capacity for these mechanisms was limited.

In the absence of direct measurements, thermodynamic modelling has been used to assess the impact of dive depth on the energetic costs of diving. Grémillet and Wilson (Grémillet and Wilson, 1999), for example, used a theoretical relationship between dive depth and heat flux to incorporate the increased heat loss experienced by great cormorants when diving to depth into their dive cost analysis. To compare our measurements with the model predictions, we adapted the depth–heat flux relationship from Grémillet et al. for double-crested cormorants (Grémillet et al., 1998) and used the output values to calculate their energetic costs of diving to 10 m depth, as predicted by equation 10 in Grémillet and Wilson (Grémillet and Wilson, 1999). This modelling approach predicts a metabolic rate for double-crested cormorants diving to 10 m depth of 33.87 and 37.37 W kg^{-1} when diving in 'warm' and 'cold' water, respectively. These values correspond to a 65% increase in dive costs during dives to 10 m depth when compared with shallow dives (1 m). Our own measurements, however, indicate an increase of $\sim 22\%$ (Table 1). The discrepancy between our measurements and the model predictions might be explained by the following

considerations. (1) The equation used by Grémillet and Wilson (Grémillet and Wilson, 1999) incorporates the theoretical heat flux experienced at a particular depth (max. depth reached) without considering descent and ascent phase, when heat flux will be lower. (2) The depth–heat flux relationship incorporated into the model is entirely based on physical properties and does not take into account any ability of birds to regulate heat flux to the environment. (3) Grémillet and Wilson (Grémillet and Wilson, 1999) did not consider any effect that changes in buoyancy with depth will have on the energetic costs during deep diving. Since the required work against buoyancy decreases with an increase in depth (Fig. 4B), this would tend to decrease overall mechanical costs during diving, especially if the time spent at the bottom and during passive ascent is great compared with the descent phase. Consequently, the model of Grémillet and Wilson (Grémillet and Wilson, 1999) greatly overestimates the effect that depth has on the energetic costs of deep diving cormorants within our set-up. Nevertheless, the discussion above confirms our prediction that the measured increase in dive costs during deep diving in cormorants reflects a composite of mechanical and thermoregulatory costs that develop in opposite directions.

Our study shows that depth is an important factor to consider when assessing the energetic costs associated with underwater foraging in a diving endotherm. By contrast, most respirometry studies to date, investigating the energetic costs of diving in endotherms, have been conducted in shallow dive tanks because of logistic difficulties. When diving to depth, work against buoyancy will be greatly reduced beyond the initial 10 m, while heat loss will be greatly increased as body insulation decreases. Divers might employ morphological (e.g. subcutaneous fat layer) and/or physiological means (e.g. peripheral vasoconstriction) to reduce heat loss at depth. They might also try to counter heat loss through increased heat production or they might allow certain tissues to cool as a potential mechanism to prolong aerobic dive duration. However, thermoregulation during diving is a complex issue and still awaits its full scientific appreciation. Only recently has it become possible to record temperatures of various tissues in avian divers foraging in the wild (Culik et al., 1996; Bevan et al., 1997; Handrich et al., 1997) and these studies have started to shed some light into the different strategies employed by endotherm divers to maximise underwater foraging time.

The elevated dive costs we measured during deep diving in our cormorants are probably a consequence of the increased thermoregulatory costs associated with a greater heat loss to the water at depth. While we found some evidence that heat loss during deep diving might be substantially higher than during shallow diving, mechanisms of peripheral heat conservation in cormorants and other avian divers await further study. To this end, heat flux measurements, which would allow quantification of heat loss during diving, would be an important first step. Investigating further to what degree heat generated as a by-product of locomotion (diving, flying) or the HIF is used to make up for the heat loss incurred during diving would allow the evaluation of how heat loss shapes diving costs in avian divers.

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