The behavioural physiology of diving animals, in particular tufted ducks (*Aythya fuligula*), and the implications for models of optimal diving

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Synopsis

Tufted ducks were trained to dive to and from a respirometer box on a 1.7 m dive tank, so that measurements of respiratory gas exchange could be measured, along with time budget data. These data were combined with power cost estimates of diving to show that the optimal breathing model quantitatively predicted surface duration and the oxygen metabolised during foraging for the mean of all subject ducks but not for individual birds. Respirometry data also showed that both the oxygen and carbon dioxide stores were close to full adjustment after mean surface duration suggesting they have a similar influence on surface duration in tufted ducks, while pre-dive hyperventilation caused hypocapnia suggesting carbon dioxide is more often a limiting factor on dive duration. Oxygen uptake was not affected by hypercapnic exposure between dives and minimally affected by hypoxia, however dive time budgeting changed in both cases. This confirmed an influence of carbon dioxide on diving behaviour while estimates of respiratory exchange ratios above one during dives from hypoxia suggested the employment of anaerobic metabolic pathways in hypoxic conditions. Allometric studies investigating relationships between body mass and diving parameters across and within taxonomic groups of divers highlighted a number of limitations in our current knowledge of diving animals and also questioned some of the mass associated correlations that are presently considered to exist across diving species.

Dedication

This thesis is dedicated to my family and in particular my Mum and Dad. Your love and support is so important to me and has been fundamental in guiding me through all my studies.

Frank Halsey (2002)

'Under carefully controlled circumstances, an animal will do exactly as it pleases, …'

Pyke (1984)

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Aythya fuligula

The common name for this species of diving waterfowl, 'Tufted duck', was coined by Gesner in 1555. The epithet refers to a tuft of feathers hanging down from the back of the head (Lockwood, 1984).

Tufted ducks are a small, short-necked diving duck. In eclipse, the male resembles the dark female, although the female is browner and has only a rudimentary crest (Peterson et al., 1974). The male summer plumage is strikingly black and white with a long, drooping crest. Close inspection reveals a purple sheen on the head.

Along with the mallard, tufted ducks are the most widespread duck in the British Isles. They frequent lakes and ponds of all sizes, often in towns and cities, where they readily breed and are often tame. Their ability to rapidly utilise new artificial water areas such as gravel pits caused the tufted duck population of Great Britain to treble between the early sixties and 1988 to 7000 pairs. Furthermore, the mid-winter peak is presently around 60 000 birds (Cady and Hume, 1988). Large numbers reach the British Isles from Iceland, northern Scandanavia and Russia, staying between September and March.

Tufted ducks prefer living in dense vegetation and thus generally have louder voices than those frequenting more open habitats (Welty, 1982) such as the Common Eider (*Somateria mollissima*). Tufted ducks will feed on a wide variety of food sources. Their preferred food is often freshwater bivalves, notably the zebra mussel (*Dreissena polymorpha*), although other benthos is also foraged such as insects, worms, seeds and aquatic plant life (e.g. Laughlin, 1972/73).

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Male Tufted Duck. Adapted from Cady and Hume (1988)

I. General Introduction

The aquatic descendants of terrestrial vertebrates, in returning to water, were provided with an ecological niche safe from predation and competition from land species (Crawford, 1978). Although they had to adapt to the considerable problems concerning gaseous exchange, there are an extremely diverse number of extant, air breathing, aquatic or semi-aquatic species. For instance, while numerous features of reptiles show a significant advance in respect to life on land compared to the amphibians, many are still aquatic, most notably the Chelonia and the Crocodilia. Several mammalian lineages have also returned to an aquatic existence, including the pinnipeds, which are one of the two suborders of the order Carnivora, and the entirely aquatic cetaceans (Carroll, 1997). An array of avian families spend considerable time foraging and locomoting in freshwater or saltwater environments, including alcids, penguins, albatrosses, cormorants and ducks.

The diving abilities of diving vertebrates

The diving behaviours of air breathing, aquatic vertebrates are most often considered in terms of the parameters of diving duration, diving depth and surface duration. To some degree, such parameters also indicate level of diving ability. These parameters vary considerably between species due to a multitude of behavioural and physiological differences. Environmental factors can also have a strong influence on diving behaviour.

The degree of specialisation to the aquatic environment clearly affects diving behaviour, for example the cetaceans are fully aquatic and thus highly specialised divers, seal species are only semi-aquatic and are adapted for certain terrestrial environments also, and otters are only marginally adapted for an aquatic existence.

Equally, penguin species are flightless and thus more specialised for diving than the pleustonic ducks, which require wings large enough to support their weight during flight but small enough to reduce drag during underwater swimming (Rayner, 1986). Modes of locomotion in aquatic vertebrates vary between the use of tails, forelimbs, hind limbs or combinations and some species, such as gannets, take advantage of their flying capability and plummet towards the water, which provides them with considerable downward momentum as they hit the surface (Garthe et al., 2000). The degree of adaptation of limbs to aquatic locomotion and buoyancy within the water are particularly influential on energy efficiency. For example, while ducks are relatively buoyant and use webbed feet for generating downwards thrust, penguins and dolphins have close to neutral buoyancy and use flippers or tails as hydrofoils (Butler and Woakes, 1984; Butler and Jones, 1997).

Some species are able to make very large cardiovascular and circulatory adjustments to reduce their metabolic rates. Tufted ducks may be able to reduce their oxygen metabolism by 75 % during extended dives (Bevan et al., 1992) while Webb et al. (1998) report the mean heart rate during diving in Northern elephant seals to be 36 % lower than resting rates. Regional hypothermia is also utilised to varying extents amongst diving species, reducing energy consumption (Butler and Jones, 1997). The ability to dive deeply is dependent upon adaptations to withstand the effects caused by high pressure such as nitrogen narcosis, high pressure neurological syndrome, shallow water blackout and the mechanical effects of compression (Kooyman, 1989; Butler, 2001; Phillips, 2001).

Environmental effects also heavily affect diving behaviour, for example a number of cormorant species have very variable mean diving depths and durations depending upon the depth of the water in which they fish (Cooper, 1986). Dovekies have dramatically different modal dive depths at night compared to daytime due to the change in water level of zooplankton in response to light levels (Bradstreet and Brown, 1985). Populations of Humboldt penguins have vastly different bounce dive

depths depending upon the depth of water they forage in (Wilson et al., 1989). Dive depths of ringed seals also differ according to the depth of the water column (Hyvarinen et al., 1995).

The use of aerobic and anaerobic metabolism

The ability to effectively metabolise anaerobically while diving can particularly enhance diving ability. While the maximum duration that an animal can stay submerged and still consume oxygen from its stores is called the aerobic dive limit (Kooyman, 1989), divers that are prepared to utilise anaerobic pathways during periods of submergence and tolerate lactate accumulation are able to dive for longer because their oxygen store levels are less restrictive. At present there is a lack of understanding about how regularly air breathing divers remain submerged long enough to develop a net increase in lactate. While Thompson and Fedak (2001) claim that many divers surface before all their oxygen stores are utilised, Croll et al. (2001) suggest that most species use anaerobic pathways as well as aerobic ones while diving.

However, there is growing evidence that anaerobic pathways are indeed employed by certain species (e.g. Weddell seals, Castellini et al., 1988, Kooyman et al., 1980; Brünnichs guillemots, Croll et al., 1992). Furthermore, diving models incorporating the potential for anaerobic metabolism during dives highlight the theoretical advantages that anaerobic dives can afford in certain ecological settings. Ydenberg and Clark (1989) considered optimal foraging approaches by western grebes and argued that in situations where a shoal of fish is within the vicinity of the diver and would be lost if the diver surfaced, then the predator would be advantaged by utilising anaerobic pathways in order to maintain pursuit. However, it is likely that certain taxonomic groups always dive aerobically, at least under the normal range of environmental conditions. For example, no studies on *Athyini* have suggested that natural dives ever exceed their calculated aerobic dive limit.

Studying and comparing diving species using allometry

In the pursuit to further our knowledge about the physiology and behaviour of diving species, we need to appreciate the adaptations of those species in relation to the environment in which they live. The presence of a behavioural trait in some, and its absence in other, maybe closely related species pursuing a different life strategy is very important evidence to consider as we develop an understanding of adaptation. Thus, the results of comparative studies are often used to complement those from observations and experiments (Harvey and Pagel, 1991), since they help unravel the fundamental principles on which animals are structurally and functionally organised (Heusner, 1984).

In biology, comparisons of interest often involve comparing characteristics that vary continuously across species, and because body mass is an excellent predictive tool for the interactions of animals with their environments, it is the trait most often compared against. Since diving capacity is fundamentally dependent upon two physiological factors; oxygen storage capacity (Butler and Jones, 1982; Butler and Jones, 1997) and metabolic rate (e.g. Scholander, 1940; Butler and Jones, 1997), both of which are associated with body mass, diving behaviour should also be related to body mass (Schreer and Kovacs, 1997). Larger animals generally have more blood because blood volume increases linearly with body mass and consequently more oxygen can be stored, while metabolic rate is not directly proportional to body mass but rather increases more slowly. Larger divers are therefore expected to be able to dive for longer, and thus potentially deeper, than smaller divers (Schreer and Kovacs, 1997; Watanuki and Burger, 1999), and as a result may surface for longer because the average rate that oxygen is gained after a longer dive is lower (Kramer, 1988).

Allometry and phylogenetic relatedness

Allometry is used to study the structural and functional consequences of changes in size or scale (Schmidt-Nielsen, 1984), since many relationships between experimental data are not linear. They are therefore transformed so that they approximate linearity and are statistically tractable by regression analysis (Heusner, 1984). Allometry has been used to test for correlations between body mass and parameters of diving behaviour. Correlations have been confirmed in intraspecific studies of diving animals (e.g. Irving, 1939) as well as in interspecific studies (e.g. Cooper, 1986; Burger, 1991). However, in analysing allometric relationships, the phylogenetic relationships between those species must be included (Harvey and Pagel, 1991). Put simply, this is because species, in particular closely related ones, share many similarities in addition to those of relevance to the comparative investigation. Such similarities can confound the comparative research.

Closely related species tend to resemble each other since they share many characters through common descent rather than through convergent or parallel evolution (Harvey and Pagel, 1991). Repeated convergence towards a particular association of traits, such as high body mass and deep diving, suggests that these characteristics may be fundamentally linked. However, if deep diving has evolved only a few times in these groups, then the number of evolutionary events is far less than previously assumed, and it is independent events that standard statistical tests assume. Therefore, strong correlations can simply be artefacts of the non-independence of species that share traits through descent from common ancestors. Consequently, phylogenetic relationships can show that there is little association between traits where 'traditional' approaches, not accounting for phylogeny, have recorded a correlation. Therefore, it is very important that phylogenetic relatedness is included in such analyses so that independent evolutionary events are considered.

The last 20 years have seen a gradual increase in the inclusion of phylogenetic information in comparative studies, thus avoiding spurious results rising from non-

independent data. Unfortunately, a large number of comparative studies looking at allometric relationships across species still do not include phylogenetic information and indeed no such studies investigating diving behaviour have done so. This may well mean that some relationships thought to be present are false, or indeed that certain relationships that are present have not been uncovered. Thus, allometric studies of diving animals across species aid our understanding of diving behaviour, by uncovering possible correlations and through the testing of model predictions, while allometric studies that also include phylogenetic information may well elucidate the true nature of relationships between body mass and diving behaviour.

Optimality models

Optimality models have often taken a central role in integrating comparative and experimental results (Harvey and Pagel, 1991). The reason for using optimality theory to study adaptations is that, subject to constraints, natural selection is expected to maximise the fitness of individuals through the optimisation of their behaviour and morphology. Thus, given certain constraints that they incorporate, optimality models produce testable predictions of behaviour or structure (McNeill Alexander, 1982). They also have a specific relevance to comparative physiology because they can uncover interesting and unexpected subtleties in the physiology being studied, for example Kramer (1988) developed an optimality model based around aspects of the respiratory physiology of diving animals.

Optimal foraging models

Optimal foraging models usually consider short term optimisation criteria, utilising models of resource gain maximisation to investigate patterns of energy attainment. One of the most prominent optimal foraging models is the marginal value theorem (Charnov, 1976). This model originally investigated the optimal load size, in terms of optimal rate of energy gain, of predators exploiting a patchy food source and

depressing the availability of the food for themselves over time from each patch visited (Fig. I-1). The distinctive 'loading curve' of the original model is an exponential decay, representing the progressively diminishing food resource. The highest rate of resource gain is shown from the line *AB*, which runs from t_T and is tangential to the loading curve. The optimal time spent at each food patch (t_f*) can be found by dropping a perpendicular from the point where *AB* touches the loading curve. The marginal value theorem has since been utilised to investigate multiple ethological traits concerning a variety of resource consumptions such as the exploitation of patchily distributed prey by great tits (Cowie, 1977), male dungflies searching for mates (Parker, 1978), central place foraging in a number of species (Orians and Pearson, 1979) including diving Antarctic fur seals (Staniland and Boyd, 2003), starlings collecting mealworms for their young (Kacelnik, 1984), the flight velocity of birds collecting or consuming food (Houston, 1986) and avian migration (Hedenström, 2000).

Optimal foraging underwater

Air breathing species that dive to forage must partition their time between periods underwater and periods at the surface (Ydenberg, 1989), and this partitioning is subject to their physiological constraints, with consequences for their foraging behaviour (Walton et al., 1998). Kramer (1988) presented a model predicting how long a diver should spend at the water surface replacing its oxygen stores, which is analogous to the marginal value theorem predicting how long a foraging animal should spend in a patch given decreasing returns over time (Fig. I-2). Kramer assumed that natural selection has favoured maximisation of the proportion (*P*) of time spent in the foraging area underwater and suggests that maximising *P* is equivalent to maximising the net rate of oxygen gained from each surface visit, or the net rate of oxygen 'delivery' to the bottom. He also assumed that the oxygen loading curve while at the surface equates to an exponential decay, arguing that it decreases as a function of time on the surface because the partial pressures of the animal's oxygen

stores decrease causing lower diffusion rates. As with the original form of the marginal value theorem, the optimal oxygen store may therefore be less than the maximum obtainable.

Houston and Carbone (1992) extended this model, still using P as the currency to be maximised, in order to predict both time at the surface and time underwater. For an animal spending time t_s on the surface, $x(t_s)$ is the oxygen acquired at the point that the individual dives, where x is the oxygen uptake rate.

Oxygen is metabolised at rate m_1 while travelling for a time t_T and at rate m_2 while foraging for time t_f. The animal is assumed to balance its oxygen gains and losses over the dive cycle (surface period plus diving period) in that

$$x(t_s) - m_1 t_T - m_2 t_f = 0$$

and thus never reaches a state of anaerobic metabolism but rather reaches the surface at the point its oxygen reserves are completely spent.

The above equation can be rearranged to discover the optimal foraging time, t_f^*, rather than the net rate of oxygen gained at the surface (Fig. I-3):

$$t_f^* = \left[x(t_s^*) - m_1 t_T\right] / m_2$$

where t_s^* is the optimal time on the surface i.e. will maximise the proportion of time spent foraging, and can also be found by constructing the tangent to the $x(t_s)$ curve. Thus, the same conclusions are reached as in Kramer (1988) but t_s^* and t_f^* have been estimated.

A number of predictions arise from the Houston and Carbone (1992) model of which most relevant to the present studies are:

i)   An increase in rate of oxygen utilisation during foraging, m_2, will not effect the optimal time spent at the surface (t_s*) but will decrease the optimal time spent foraging, t_f*.

ii)   An increase in oxygen concentration in air will increase dive duration with the magnitude of the effect depending upon depth.

Thus, the application of optimality theory to the foraging behaviour of air breathing divers has provided a holistic framework in which to order empirical findings (Kramer, 1988). These, through observations and experiments, will improve our understanding of the physiological and behavioural adaptations of these animals.

The debate on optimal foraging theory

While behaviourists originally welcomed optimal foraging theory because it generated testable predictions in an often subjective field, vehement arguments about the applicability of optimality models have abounded due to a number of criticisms of the theory. Since much of the work in this thesis is based upon optimality concepts and models, a synopsis of the criticisms and counter arguments of optimal foraging theory are presented here.

A number of criticisms of optimal foraging theory and of experimental testing of the theory are collated in a paper by Pierce and Ollason (1987). Perhaps the most important criticism they raise concerns the question of exactly what natural selection optimises since optimal foraging theory predicts that an organism will have perfected, in some currency, the behaviour of food assimilation, given its physiological limitations. However, the need to avoid predators, compete with conspecifics and find mates may constrain foragers to feed less than maximally (Dawkins, 1995; Perry and Pianka, 1997; Heithaus and Frid, 2003). Arguably then, it is impossible to identify independent activities to model building a priori and thus evidence that these activities exist is unobtainable. Dunstone and O'Connor (1979) rebut this claim, however, pointing out that most studies based upon optimality theory make implicitly, and often

explicitly, the assumption that organisms try to maximise their energy intake rate during foraging. This has certainly been the case with optimal diving models (e.g. Kramer, 1988; Houston and Carbone, 1992; Mori, 1998).

Optimal strategies may not exist in nature, even if natural selection tended to give rise to optimal behaviours and structures (Gould and Lewontin, 1979). Firstly, optimal strategies may not have had time to evolve yet, perhaps because populations have been tracking moving fitness optima, a theory known as the Red Queen effect (van Valen, 1973). Indeed, evidence is growing that physiological history dominates local behavioural adaptation (Perry and Pianka, 1997). Secondly, if foragers must learn about the environment, rather than have the behaviour hardwired, the optimal strategy may never be obtained (Pierce and Ollason, 1987). Thirdly, genetic variation in a gene pool may limit the progress towards optimality that can evolve (Dawkins, 1982), for example a limited accuracy of the perception of time spent at a foraging patch due to 'rate biased time perception' (Hills and Adler, 2002). Alternatively, the optimal strategy of one animal may depend on that of others (Dawkins, 1989).

Pierce and Ollason (1987) argue that many optimal foraging models simplify reality to the point of distorting it since many models of foraging behaviour assume that foragers optimise a single behavioural parameter only. Furthermore, the majority of models assume that the parameter is kept constant. For example, Houston and Carbone (1992) assumed tufted ducks foraged underwater with constant efficiency, however much evidence exists suggesting that predators can vary the rates with which they search for prey. For instance, Monaghan (1996) reported a significant difference between the duration of a dive and the subsequent surface pause in certain seabird species, between 1990, where food availability was low, and three other studied years. Therefore, the dive to pause relationship was affected by food availability and thus probably foraging effort.

However, many researchers have much faith in the evolutionary insights that optimal foraging theory can provide. Stearns and Schmid-Hempel (1987) argue that rather than the selective environment being too variable to produce well adapted traits, the selective environment usually remains fairly constant and provides ample opportunity for selection to refine design that may be subject to optimality testing. Optimal modellers argue that while Pierce and Ollason (1987) point out that there are a number of valid reasons for why optimal strategies may not exist, this is not problematical since the aim of the modeller is to identify those parts of the organism in which we can expect to see local adaptation (e.g. the blood physiology of diving animals) and those parts that are so historically constrained that it would be more fruitful to view them as unoptimisable traits (e.g. the feathered wings of birds versus the skin flap wings of bats; Dawkins, 1982).

Ollason (1980) criticises optimality modelling by arguing that because nobody can be certain a priori what function will be optimised, the definition of the optimal behaviour is simply refined with experience so that it approximates more and more closely to the observed behaviour. This is an ad hoc process, which is unfalsifiable because it is teleological. Certainly the lack of testing of alternative, non-optimal theories is problematical for optimality theory. Arguably, though, optimal foraging models show considerable success when modelling simplistic situations, such as certain instances of central place foraging (see earlier; Perry and Pianka, 1997). It is questionable, however, as to how far the results of these models can be extrapolated to the more complex choices faced in the wild. Ball (1994) developed a model examining diet selection of canvasback ducks (*Aythya valisineria*) and concluded that the experiment provided only minimal estimates of the problem solving abilities of the species. This was because the rules of prey selection that this species may use when given complex food choices can produce different diet compositions than that predicted by current foraging models. Dawkins (1995) argues that since true optimality is long term i.e. reproductive fitness, short term optimality is unlikely and thus the value of, for instance, optimal foraging theory then becomes a working

hypothesis to see how far one factor does explain what an organism does, rather than the last word in explaining behaviour. Indeed, Charnov (1976) has remarked that optimal foraging is often a concept to examine the behaviour of animals rather than a theory, although in a sophisticated version for a well studied animal, the marginal value theorem can provide quantitative descriptions of foraging.

Pierce and Ollason (1987) conclude that tests of optimality models have been mostly unimpressive. However, Stearns and Schmid-Hempel (1987) cite a number of studies that they consider have revealed the explanatory power of optimality models (e.g Kacelnik, 1984; Parker, 1978). They offer a number of directions that the optimisation approach should take, including the improved realism of models (e.g. by reducing the number of underlying assumptions and validating those assumptions) and the investigation of proximal links connecting general ideas (such as the marginal value theorem) to particular conditions (e.g. oxygen as a patchy resource accumulated by foraging divers; Kramer, 1988).

Quantifying and validating optimal diving models

Clearly, confidence in the robustness of optimality models is reduced when model assumptions are based on little evidence. Wariness about model validity is exacerbated because models may be good predictors even though they contain incorrect assumptions, often because the flawed assumption is irrelevant to the working of the model (Pierce and Ollason, 1987). Thus, despite a number of optimal diving models predicting qualitative behavioural trends, (e.g. Carbone et al., 1996; Mori, 1998; Jodice and Collopy, 1999), it is erroneous to accept such models as accurate when fundamental aspects, such as the oxygen uptake curve, have not been quantified. Instead, the trends can only be considered as guidelines to empirical research.

For example, Walton et al. (1998), in an attempt to apply the basic model of Houston and Carbone (1992) more specifically, and thus more accurately, to avian species, adapted the model by incorporating features of avian physiology. They claimed that the assumption by earlier models of a smooth curve of diminishing returns (Kramer, 1988) is unfounded because myoglobin and haemoglobin do not dominate the oxygen stores of avian divers since their respiratory tract and air sacs form on average about 50 % of their oxygen storage capacity (Keijer and Butler, 1982; Stephenson et al., 1989; Croll et al., 1992). They asserted that it is likely that oxygen is taken up into the respiratory tract before being taken up by haemoglobin and myoglobin since oxygen must enter the caudal air sacs before it becomes available for gaseous exchange. Walton et al. (1998) predicted that avian divers will produce a kinked oxygen uptake curve with the first, steeper portion representing oxygen gained in the air sacs and the second portion representing the reloading of the haemoglobin and myoglobin oxygen stores (Fig. I-4).

However, the assumption by Walton et al. (1998) that oxygen must be taken up into the caudal air sacs before becoming available for gaseous exchange is probably incorrect. Powell (2000) suggests that some inspired gas reaches all the way to the cranial sacs within one breath, thus reaching the lungs on the first inspiration (Parkes et al., 2002). The fine detail of the oxygen uptake curve has an important effect on the gross predictions of diving optimality models (Ruxton et al., 2000) and thus errors in the assumptions of the curve shape are likely to produce spurious predictions.

Quantifying the oxygen loading curve

However, as Stearns and Schmidt-Hempel (1987) suggest, the case for the use of optimal foraging models to investigate evolutionary adaptations is greatly strengthened by the validation of their assumptions and in particular the quantification of their predictions. Parkes et al. (2002) started this process for optimal diving models, quantifying the post-dive oxygen uptake curve of tufted ducks by measuring changes in the rate of oxygen uptake over time during surface periods between dives.

They found that there was always a particularly rapid phase of oxygen uptake for approximately the first three seconds after a dive, and then a slower phase. This created a biphasic oxygen uptake curve after longer duration dives, similar to that predicted by Walton et al. (1998), but probably due to an initially higher respiratory frequency rather than due to the anatomy of the avian lung. Parkes et al. (2002) suggest that the volume of oxygen consumed during the dive may be high enough that the tangent of the optimal breathing model (Kramer, 1988) routinely touches the curve beyond the inflection (Houston, 2000) and thus the presence of the inflection rarely affects the predictions of the models.

Furthermore, while optimal diving models have assumed that the oxygen uptake curve is the same after all dives, Parkes et al. (2002) found that increased dive durations were associated with an increased average rate of oxygen uptake during the succeeding surface period. This indicates that oxygen uptake rate varies depending upon energetic costs during submergence, which is likely to have important implications for the predictions of optimal diving models. Quantification of the other variables incorporated in optimal diving models would therefore allow the predictions of the models to be tested quantifiably, providing much stronger evidence for the viability of their assumptions and their predictions.

Oxygen loading and carbon dioxide unloading
An assumption made by the majority of optimal diving models that may be erroneous is that divers are most focused on budgeting their oxygen stores over the dive cycle (e.g. Kramer, 1988; Houston and Carbone, 1992; Carbone and Houston, 1994, 1996; Carbone et al., 1996, Walton et al., 1998; Acevedo-Gutierrez et al., 2002). Thompson and Fedak (2001) point out that if these 'oxygen balance' models realistically portray the optimal behaviour of air breathing divers then there is a paradox in that many diving mammals terminate their dives long before their oxygen reserves are exhausted. This has been inferred in a number of species that do not often get close to their estimated aerobic diving limit (Kooyman, 1989), including the California sea

lion (Feldkamp et al., 1988), harp seals (Lydersen and Kovacs, 1993), New Zealand fur seals (Harcourt et al., 1995) Northern elephant seals (DeLong and Stewart, 1991; Le Boeuf et al., 1988), ringed seals (Gjertz et al., 2000) and Ross seals (Bengston and Stewart, 1997).

Much of the evidence that carbon dioxide levels in the body, which affect the pH levels of the blood, may have considerable influence over the duration of the diving portion of the dive cycle has come from research into human diving. For instance, human divers are able to increase submergence time by hyperventilating prior to submergence, which functions to reduce carbon dioxide levels in the body rather than increase oxygen levels because the arterial blood is already close to being saturated with oxygen during normal ventilation (Ferrigno and Lundgren 1999). Gallivan (1980) suggested that carbon dioxide rather than oxygen is the important factor in ventilatory control and diving in the manatee while Påsche (1976) explained his observations that older harp seals and hooded seals dived for longer because of a decreased sensitivity to carbon dioxide with age. Boutilier et al. (2001) argue that harbour porpoises remain at the surface of the water after their oxygen stores have been replenished to eliminate built up levels of carbon dioxide.

An importance of carbon dioxide levels on diving behaviour also seems to be the case with many aquatic bird species (e.g. Adélie penguins, Watanuki et al., 1993; little penguins, Bethge et al., 1997; Heard Island shag, Green and Williams, 1997; shy albatross, Hedd et al., 1997; marbled murrelets, Jodice and Collopy, 1999). Tufted ducks follow this trend since they have sufficient oxygen stores to remain submerged during voluntary diving for over 40 s (Butler, 1991a), however their preferred dive duration is less than 20 s (e.g. Stephenson et al., 1986; Bevan and Butler, 1992; Parkes, 2002). Thus, the decision by tufted ducks to leave the foraging area and resurface may be influenced by factors other than oxygen store levels, suggestions include particle selection time (Draulans 1982) and the rate of food ingestion (Stephenson et al. 1986), but evidence is mounting that carbon dioxide is influential.

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Butler and Stephenson (1988) exposed freely diving tufted ducks to gases of varied composition and monitored their diving behaviour. The ducks would not dive for food when concentrations of oxygen or carbon dioxide were outside certain ranges, supporting the theory that blood and lung gases are important in influencing diving behaviour. Readhead ducks exhibited the same refusal to dive beyond similar ranges of gas composition (Furilla and Jones, 1986). More specifically, Butler and Stephenson (1988) found that both hypoxia and hypercapnia resulted in an increase in the proportion of total diving time spent breathing at the surface, and both were also associated with a reduction in dive duration. Measurements of the rates of carbon dioxide output during surface periods between dives in tufted ducks might help to elucidate how much this respiratory gas influences diving behaviour in tufted ducks and therefore whether it should be included into optimal diving models as an important variable improving their realism.

Tufted ducks in experimental research

A considerable amount of physiological research investigating adaptations to diving has studied tufted ducks. An increasing number of behavioural studies on diving have also chosen tufted ducks as the subject species, both in the field and for laboratory studies. This is because tufted ducks are abundant, are small and easy to keep, and adjust well to experimental environments. They are prolific divers that require only shallow bodies of water in which to perform natural diving behaviour and are thus an ideal diving species with which to test and explore optimal diving models. For these reasons, the majority of data presented in the experimental chapters of this thesis are measurements taken from tufted ducks. The anatomy and physiology of tufted ducks is, of course, intimately linked with their behaviour and thus with model predictions of their optimal diving behaviour. Our understanding of the anatomy of tufted ducks and their physiological adjustments during diving is considerable, although certainly not complete.

Anatomy of the avian lung

The anatomy of the avian respiratory system

The avian respiratory system is differentiated into two compartments; multiple air sacs and the parabronchial lung (Fig. I-5). Birds lack the presence of a diaphragm (McLelland, 1990). Instead, during normal respiration, muscular contractions of the abdomen cause the air sacs to act as bellows, increasing and decreasing in volume, thus pushing or pulling air through the rigid lung (Crank and Gallagher, 1978; for more details on the anatomy and physiology of the air sacs, see *Appendix I*). The trachea divides within the neck into the left and right main bronchi. The medioventral secondary bronchi, which occupy the ventral surface of the lung, constitute the first branches of the main bronchi within the lung. The avian lungs are present in the dorsal part of the thoracic cavity and are compact structures composed of parabronchi through which gas flows unidirectionally during inspiration and expiration. The direction of this flow is from the mediodorsal to the medioventral secondary bronchi. The three dimensional network of air and blood capillaries surrounding the parabronchi, known as the periparabronchial tissue, is the site for gaseous exchange during respiration, although a small proportion of respiratory gas exchange also occurs between the caudal thoracic air sac and the blood (Magnussen et al., 1976).

Unidirectional air flow in the avian lung

In the mammalian lung, air passes through multiple bronchiolar tubes to reach the alveoli that form the cul-de-sacs of a ventilated pool system, where air flows back and forth. However, the parabronchial lung is open at both ends such that air can potentially flow through it in either direction. A number of studies attempted to understand the airflow within the avian respiratory system (e.g. Bouverot and Dejours, 1971; Brackenbury, 1971; Bretz and Schmidt-Nielsen, 1971; Scheid and Piper, 1971; Scheid et al., 1972). These studies concluded the presence of a rectified

airflow where air travelled from the main bronchus into the mediodorsal secondary bronchi, and then through the paleopulmo parabronchi to the medioventral secondary bronchi, during both inspiration and expiration. Thus, in the avian lung, gas is known to flow through the major exchange area in the same direction during both respiratory phases, in sharp contrast to most air breathing vertebrates (Brown et al., 1995). There is no dead space in avian lungs, in contrast to the mammalian alveoli, thus the air is continually circulated past the flowing blood (Lawton, 1996). The mechanisms controlling the rectification of airflow have only recently been clearly understood. *Appendix I* includes a synopsis of the studies that have led to current understanding about airflow rectification.

Gaseous exchange in the avian lung

The parabronchial vascular system utilises a cross-current exchange system (Fig. I-6). Despite similar blood oxygen affinities to mammalian blood (Baumann and Baumann, 1977), such a system produces higher and more rapid levels of oxygen and carbon dioxide gaseous exchange than the ventilated pool system in mammals (Meyer et al., 1976) and the counter-current system in fish (Piiper and Scheid, 1975). This advantage is conferred due to gaseous exchange during both phases of respiration, and is particularly beneficial to those birds that dive, as well as those birds that fly (Woakes and Butler, 1983).

The periparabronchial tissue is entwined along its length with arterioles reaching from the pulmonary artery and branching into capillaries. These capillaries in turn run straight through the parabronchial wall towards the lumen. Counter-current diffusion drives gaseous exchange between air in the air capillaries and blood in the parabronchial lumen. Ventilated gas passes through the lumen, losing oxygen and collecting carbon dioxide. Oxygen diffuses into the air capillaries and carbon dioxide diffuses the other way (Crank and Gallagher, 1978). Blood flow within the capillaries occurs from the parabronchial periphery inwards towards the lumen (West et al.,

1977), with venous blood entering the capillaries peripherally and blood leaving the capillaries mixing to form arterial blood. Importantly, gaseous exchange between the lung and the blood may continue during periods underwater, enhanced by fluctuations in air sac pressures induced by synchronous foot paddling (Boggs et al., 1998; Boggs et al., 2001), and thus the lung could be utilised as an oxygen store during dives.

This general description of the arrangement of the avian lung with parabronchi extending exclusively between mediodorsal and medioventral secondary bronchi has been termed paleopulmo by Duncker (1974). However, the majority of bird species have, to a greater or lesser extent, an additional network of parabronchi known as the neopulmo, which is discussed in more detail in A*ppendix I*.

Avian respiratory adaptations

Avian lungs have an increased facility for gas exchange compared to mammalian lungs (James et al., 1976), which is likely to enhance the ability of diving birds to stay submerged. This is due to adaptational advantages in the avian respiratory system, which result in the removal of a larger fraction of oxygen from respired air than occurs in mammalian lungs (Schmidt-Nielsen, 1984). It is attributed mostly to the increased surface area of the air-blood interface (James et al., 1976) and the cross-current exchange system (McLelland, 1990). In addition, the air sacs of birds have very large volumes, averaging five times the size of the respiratory systems of most diving animals on a unit weight basis (Butler and Jones, 1997). Gaseous exchange is also enhanced by cardiogenic pressure oscillations (Torre-Bueno, 1980) that reduce air stratification in the respiratory system during ventilation. Furthermore, the concentration of myobglobin stores in the locomotory muscles is higher in many aquatic birds than in their non-diving relatives (Keijer and Butler, 1982; Butler, 1991b). In the mammalian lung, the presence of series ventilation and of incomplete mixing produces stratified inhomogeneity (Scheid et al., 1981), which reduces gas exchange efficiency. In contrast, the avian lung parabronchus, due to the serially

arranged blood-gas interaction rudiments along its length, generates considerable longitudinal oxygen and carbon dioxide diffusion gradients (Piiper and Scheid, 1978; Scheid, 1978), coupled with an increased surface area and decreased diffusion distance (Frappell et al., 2001).

Adaptive physiological adjustments during the dive cycle in tufted ducks

Pre-dive physiological adjustments

Before the first dive of a series, the heart rate and respiratory frequency of tufted ducks increases (Butler and Woakes, 1975; Woakes and Butler, 1983; Furilla and Jones, 1987) and hyperventilation ensues. Stephenson et al. (1986) found that when tufted ducks could anticipate a long duration dive, they showed an increased duration of pre-dive tachycardia. Since tachycardia is accompanied by hyperventilation (Butler and Woakes, 1979) this suggests a more complete loading of oxygen and / or unloading of carbon dioxide (hypocapnic alkalosis) prior to anticipated longer dives (Butler, 1982; Stephenson et al., 1986). While penguins often dive on inspiration, particularly when diving deeply (Sato et al., 2002), tufted ducks partially exhale before diving to reduce their buoyancy, in turn reducing the energy required to dive and stay submerged (Butler and Woakes, 1979).

Energetic costs of diving

Humboldt penguins are almost neutrally buoyant during dives, which enhances the efficiency of their underwater locomotion (Butler and Woakes, 1984). In contrast, tufted ducks are positively buoyant during all dives to normal depths and thus continual leg movements are required to stay underwater (Butler and Woakes, 1982). Because *Athyini* have a high positive buoyancy, foraging underwater is energetically very expensive, in particular during the descent phase of the dive where oxygen metabolism at mean dive duration is at a rate approximately equal to that during swimming at maximum speed (Butler, 1991b). Foraging generally requires the duck

only to maintain a depth in the water column and utilises about one third the amount of energy of descending (Lovvorn et al., 1991), however this still represents a considerable energy expenditure. The depth to which tufted ducks dive dramatically affects the energetic cost of that dive due to the changing effects of buoyancy (Lovvorn and Jones, 1991). Tufted ducks also adjust the amount of air in the lungs before a dive in anticipation of the dive depth (Wallace, 1998).

Physiological adjustments during dives

An instantaneous reduction in heart rate from the elevated pre-dive rate to below resting rate occurs after the dive starts, however this then rises over the next few seconds and then remains fairly constant for the rest of a dive of normal duration at a similar rate to when swimming fairly rigorously on water, or starts to decrease during longer dives (Butler and Woakes, 1979; Butler and Jones, 1982). However heart rate is lower than would be expected for the equivalent rate of oxygen consumption in air (Woakes and Butler, 1983). While decreases in heart rate are often associated with restricted blood flows, tufted ducks are the only species of diving animal where vasoconstriction during diving has been measured (Bevan and Butler, 1992). The active skeletal muscles, the brain and the heart receive elevated blood supplies through an increase in perfusion similar to that during surface swimming and there is a reduced blood supply to other parts of the body, including the inactive skeletal muscles (Butler et al., 1988). The decrease in flow to inactive parts of the body may be more intense than during an equivalent level of exercise in air (Woakes and Butler, 1983).

Woakes and Butler (1983) conclude that when tufted ducks dive, the cardiac responses to exercise, notably tachycardia and vasodilation in the active muscles, are tempered by the cardiovascular responses to submersion, notably bradycardia and selective vasoconstriction. The bradycardia is the result of a combination of sympathetic and parasympathetic stimulation on the heart's pacemaker (Butler, 1982).

The former responses have a greater effect producing a net mild inhibitory effect on heart rate during submersion (Butler and Woakes, 1982). This balance can be adjusted towards the forced submersion response if the bird is briefly unable to resurface, for instance when rising beneath ice (Stephenson et al., 1986).

Oxygen consumption during dives

For air breathing divers, the amount of oxygen that can be stored, the rate at which that oxygen is used underwater and the rate at which oxygen can be replenished at the surface are assumed to be very important influences on their diving behaviour (Kramer, 1988; Kanatous et al., 2002). During active dives, stored oxygen is used at a rate considerably higher than at rest in most species of diving animal. The average rate of oxygen consumption in tufted ducks is around 3 or 4 times that at rest (Woakes and Butler, 1983; Stephenson, 1994) and is similar to the rate during surface swimming at maximum velocity (Woakes and Butler, 1983), thus indicating that diving is a strenuous activity. However, the oxygen stores are rapidly replenished during surface periods and thus such dives can be performed in relatively quick succession i.e. tufted ducks have a high dive:pause ratio (1.7; Butler and Jones, 1997).

Carbon dioxide levels in the body are now also considered to be possibly associated with diving behaviour (e.g. Butler and Stephenson, 1988). Concentrations of the respiratory gas levels in the bodies of diving birds are monitored by chemoreceptors known to influence diving behaviour, which, among other stimuli, detect changes in partial pressure of oxygen in the arterial blood, partial pressures of carbon dioxide in the blood and pH levels (Butler and Stephenson, 1988; Enstipp et al., 2001).

Post-dive recovery

Following a dive, the surface period continues until a sufficient level of oxygen store reloading and carbon dioxide removal has occurred. A concurrent decrease in total peripheral resistance facilitates reperfusion of the organs and muscles starved of

oxygen during the dive (Butler and Jones, 1982). Rate of oxygen gain is likely to decrease as surface duration increases and subsequently the optimal level of oxygen store reloading may not equal total re-saturation if the animal wishes to minimise its time above the water surface, for instance during foraging (Kramer, 1988). Anyhow, Butler (1982) has argued that the level of carbon dioxide in the blood, rather than oxygen store levels, may be more influential in determining when the animal is ready to dive again. Furthermore, if tufted ducks are temporarily unable to surface from a dive, they exhibit a profound bradycardia likely to indicate anaerobiosis (Butler and Stephenson, 1986). In such cases, such disturbances in acid / base balance and the removal of anaerobic metabolites during the subsequent surface period are likely to prolong the stimulation of ventilation (Butler, 1982).

Ethical note

I feel that it is appropriate to justify here, in brief, the ethical acceptability of my work. Many difficulties emerge from attempts to define a robust moral standpoint from which all scientific work involving animal experimentation can be judged (Robinson and Garratt, 1996). Claims that animals have innate rights are rather hard to prove and while legal rights are much easier to defend because they are very specific and clear, there is a growing body of opinion that laws often do not provide enough protection for animals, in other words that they are not moral enough. I suggest that a utilitarian approach offers a better philosophy for considering animal ethics. This argues that we, the researchers, must convince the majority that the benefits of our research exceed the suffering caused to the animals. Here, I will list the benefits of my research and then discuss the welfare of my experimental birds.

Research benefits

As well as enhancing our knowledge of tufted ducks, the experiments I have conducted on tufted ducks can help us elucidate the factors that may limit the feeding

activity of aquatic birds. Combining respirometry data with foraging biomechanics, food assimilation costs and food intake measurements will improve our effectiveness at relating energy requirements of ducks to their food bases (Lovvorn, 1994). Profitability relations will yield important insights into the quality and extent of foraging habitat and thus the impact of the interactions of man with the natural environment involving habitat alteration. For example, a study commissioned by the EU entitled IMPRESS (Interactions between the Marine Environment, PREdators and prey: implications for Sustainable Sandeel fisheries) requires a differential equation model of a diving seabird and as such has incorporated data from Parkes et al. (2002). Generalisation of some of my findings to other semi-aquatic bird species, and even to other diving vertebrates, considerably extends the applicability of my work. Finally, the array of skills that I have developed through my Ph.D has greatly enhanced my ability to conduct scientific work in the future benefiting important environmental and conservation issues.

Animal welfare

Since I have studied the physiological responses to natural behaviour of a vertebrate species, it has been essential to ensure that the experimental procedures have caused as little stress to the animals as possible. Animals that did not become accustomed very rapidly to the conditions were removed from the trials.

Forty-eight hours after introduction to the dive tank, where the ducks were housed, they exhibited normal behaviour. Body masses dropped initially but this was partly due to prior mild obesity, and they quickly stabilised within natural ranges. The ducks had access to deep water, a dry area and multiple foods types including live foods. The housing area was kept clean and they were subjected to an 13h-11h day-night cycle. Only females were housed during the spring to limit stress from harassment from males. The ducks quickly learnt to move to the dry area prior to experimental sessions, ensuring minimal stress during their translocation from the dive tank, and

the subject duck on each occasion rapidly became calm in the respirometer and began diving for food.

Research objectives

The overarching aims of this thesis are to study the behaviour and physiology of tufted ducks, *Aythya fuligula*, to further understand their adaptations in the context of investigating optimal diving models. The scope of the work is extended by including a general study comparing the diving behaviours of a variety of diving species. As such, this thesis incorporates both experimental and theoretical investigations. The experimental sections (*Chapters II-IV*) focus on studying the tufted duck to investigate and test the predictive validity of relevant models, in order to facilitate improving the realism of these models. The theoretical sections (*Chapters V-VII*) study the diving behaviours of a range of air breathing vertebrates comparatively, through linear and allometric regressions of diving parameters and body mass.

Given the development of the methodology to quantify the oxygen uptake curve for tufted ducks in between dives (Parkes et al., 2002), quantification of the predictions of optimal diving models (Kramer, 1988) can now be achieved by combining measurements of oxygen uptake and metabolic rate with estimates of power costs during the dive. Empirical testing of optimal diving models will be undertaken in *Chapter II* by quantifying the variables of one particular model, the optimal breathing model, with these values for *Aythya fuligula*. The assumption of such models that divers primarily attempt to balance their oxygen stores through the dive cycle is investigated in *Chapter III* by studying the relative influences of oxygen and carbon dioxide on the diving behaviour of *Aythya fuligula*. Thus, the viability of present models that incorporate only the dynamics of body oxygen stores can be ascertained, and the likelihood of improved accuracy if the dynamics of carbon dioxide levels are included can be assessed. *Chapter IV* builds upon the previous chapter by measuring the effects of inspiring hypoxic or hypercapnic gas mixes on diving behaviour and

respiratory gas exchange in the same species. This will enable investigation of the extent to which the changes in the diving behaviour of tufted ducks in hypoxic and hypercapnic environments can be explained by changes in rates and volumes of oxygen uptake and carbon dioxide output. The theoretical chapters are designed to stimulate discussion about our present understanding of vertebrate diving behaviour and physiology. *Chapter V* compares the analyses of across-species relationships using traditional statistical methods with analyses that incorporate phylogenetic relationships to test if body mass really does correlate with diving capability. *Chapter VI* uses across-species relationships to investigate a very simple model predicting maximum diving capability to assess our understanding of vertebrate adaptations to diving, while *Chapter VII* uses across-species relationships to qualitatively explore a subtle prediction of the optimal breathing model (Kramer, 1988).

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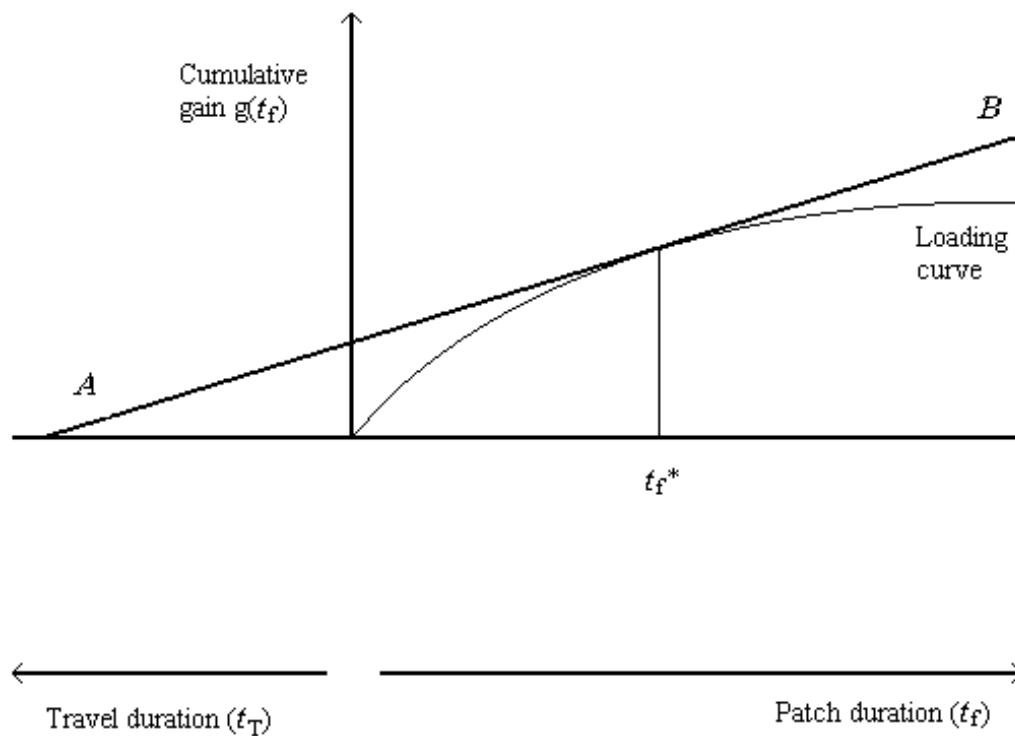
Figure I-1

Graphical representation of the marginal value theorem which finds the optimal duration, t_f*, to forage in a food patch (after Charnov, 1976). This is done by constructing a tangent (*AB*) from t_T, which touches the loading curve. To the left of the origin is the time taken to travel to the next patch (t_T), while to the right is the time duration at the patch. The *y* axis represents cumulative gain of the food resource, $g(t_f)$.
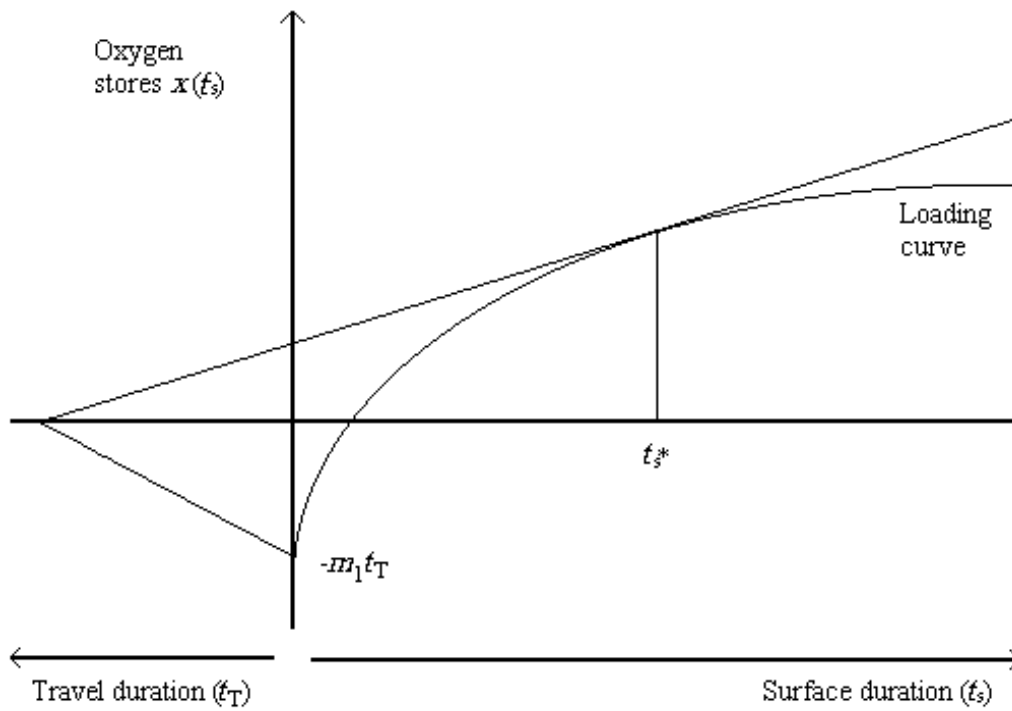
Figure I–2

Graphical representation of the marginal value theorem adapted to find the optimal surface duration (after Kramer, 1988). The ordinate represents oxygen stores, $x(t_s)$. The line from t_T to $-m_1 t_T$ represents a constant rate reduction of oxygen stores during travel time, t_T. The loading curve is the oxygen obtained over time at the surface (t_s) while t_s^*, found by constructing the tangent, represents the optimal surface duration for maximising the rate of delivery of oxygen to the foraging area.
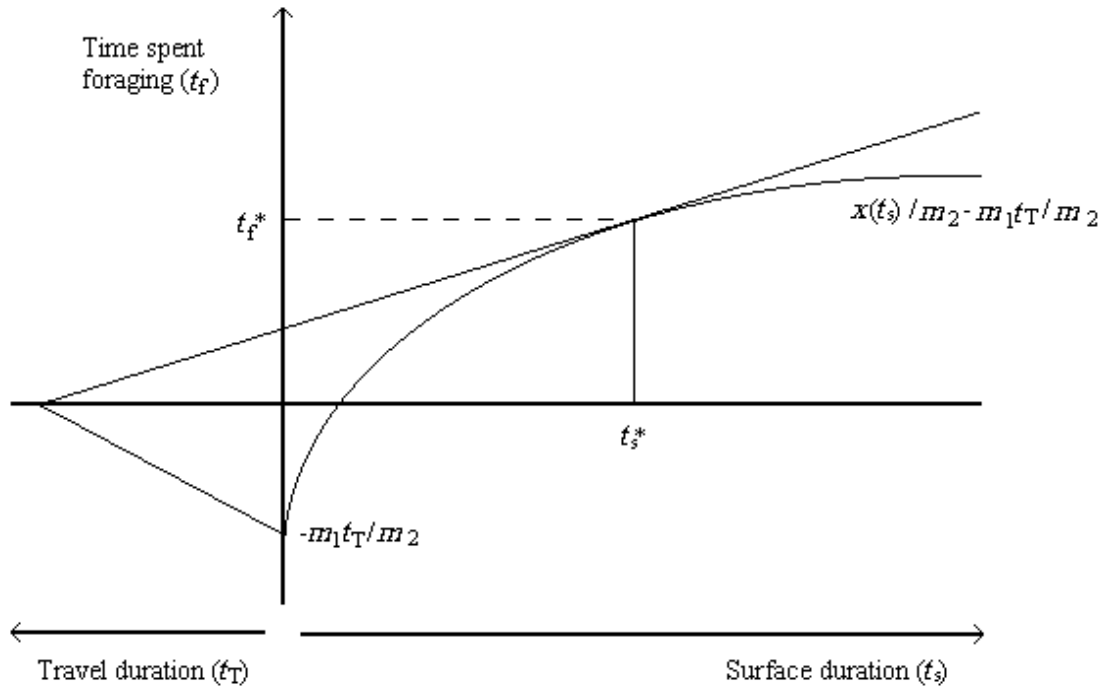
Figure I–3

Graphical representation of a modification to Fig. I-2, where the y axis is the time spent foraging (t_f; after Houston and Carbone, 1992). The line from t_T to $-m_1 t_T / m_2$ shows the oxygen used during t_T such that foraging duration is decreased by the duration $m_1 t_T / m_2$. The tangent construction represents the time at the surface, t_s^*, which maximises the proportion of time spent foraging.
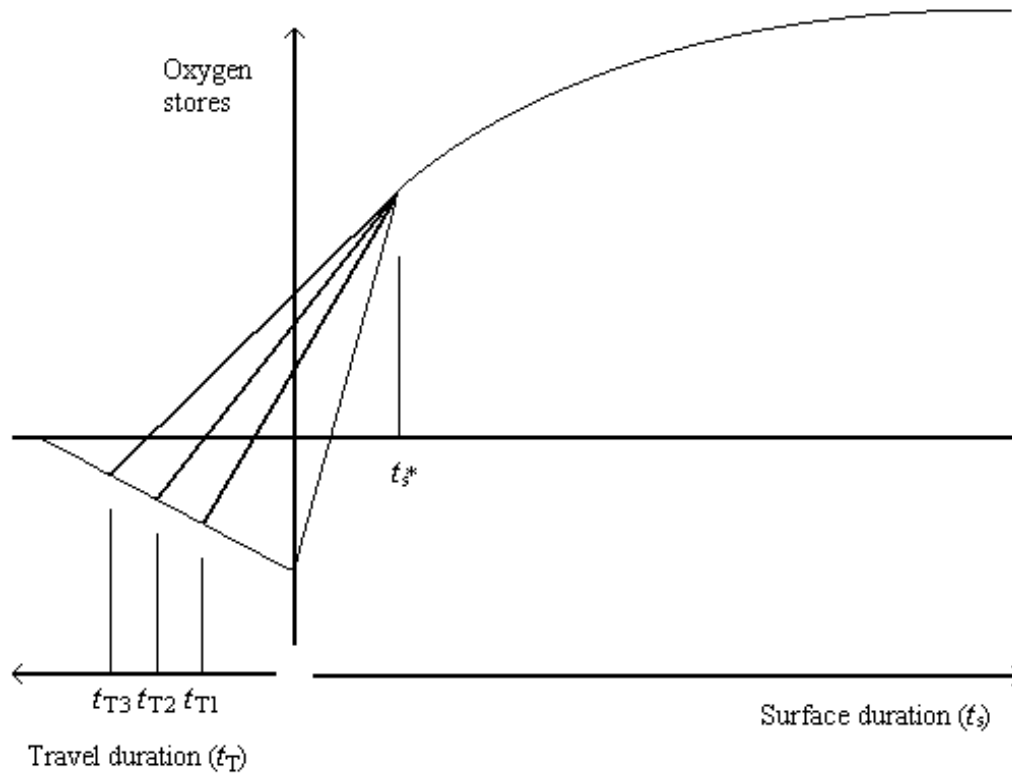
Figure I–4

Adaptation of Kramer's (1988) model specifically for birds (after Walton et al., 1998). When maximising the dive to surface ratio (t_s*) represents optimal surface duration. However, a range of short travel durations (t_{T1} to t_{T3}) have the same t_s* due to the prominent disfigurement of the oxygen loading curve.
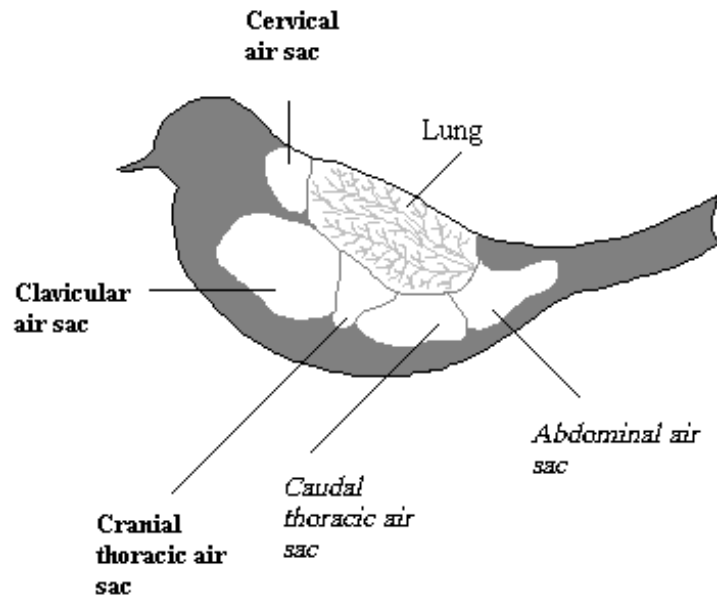
Figure I–5

Schematic of the differentiated avian respiratory system, comprising the lung and multiple air sacs. Cranial group air sacs depart from the medioventral secondary bronchi (bold) while caudal group air sacs are directly connected to the mesobronchus (italicised). (Adapted from Scheid, 1979).
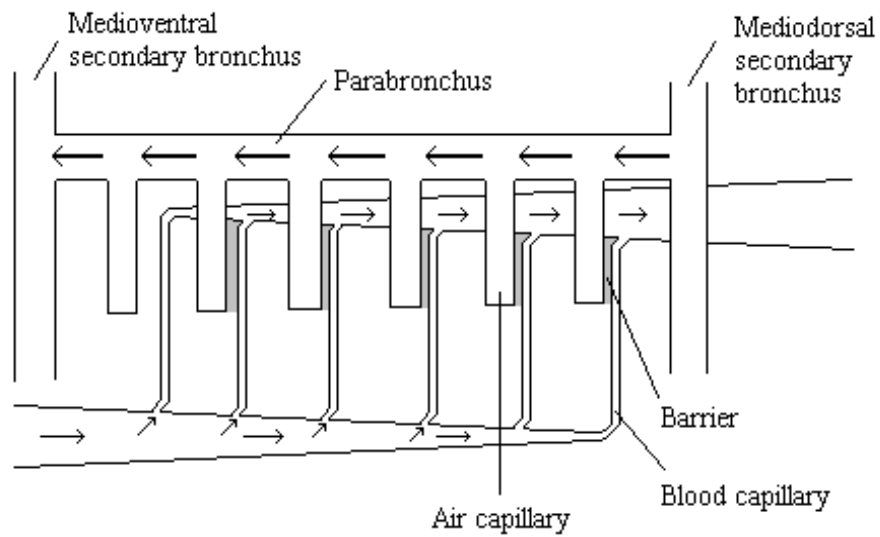
Figure I–6

The cross-current model of gas exchange. Thin arrows denote the direction of blood flow while thick arrows denote the direction of gas flow. (Adapted from Powell, 2000).

II. Testing Optimal Foraging Models for Air Breathing Divers

Lewis G. Halsey, Anthony J. Woakes & Patrick J. Butler

Models of diving optimality qualitatively predict diving behaviours of aquatic birds and mammals. However, none of them have been empirically tested. We examined the quantitative predictions of optimal diving models by combining cumulative oxygen uptake curves with estimates of power costs during the dives of six tufted ducks, *Aythya fuligula*. The effects of differing foraging costs on dive duration and rate of oxygen uptake ($\dot{V}_{O2up}$) at the surface were measured during bouts of voluntary dives to a food tray. The birds were trained to surface into a respirometer after each dive, so that changes in $\dot{V}_{O2up}$ over time could be measured. The tray held either just food or closely packed stones on top of the food to make foraging energetically more costly. In contrast to predictions from the Houston & Carbone model, foraging time (t_f) increased after dives incorporating higher foraging energy costs but surface time (t_s) remained the same. While optimal diving models have assumed that the cumulative oxygen uptake curve is fixed, $\dot{V}_{O2up}$ increased when the energy cost of the dive increased. The optimal breathing model quantitatively predicted t_s in both conditions and oxygen consumption during foraging (m_2t_f) in the control condition, for the mean of all ducks. This offers evidence that the ducks were diving optimally and supports the fundamentals of optimal diving theory. However, the model did not consistently predict t_s or m_2t_f for individual birds. We discuss the limits of optimal foraging models for air breathing divers caused by individual variation.

Introduction

An air breathing animal that is foraging underwater must decide when to leave the site of resource to surface and ventilate its lungs. This requirement to move away from the feeding site to obtain oxygen imposes a considerable limitation during periods of foraging. Air breathing divers are assumed to have evolved to apportion their time between the surface site and the feeding site to maximise the proportion of time spent foraging (Kramer 1988).

Popular models of diving optimality that have qualitatively predicted the optimal surface duration of a diver (e.g. Kramer, 1988; Thompson et al., 1993; Carbone and Houston, 1996; Mori, 1998) have assumed that the rate of oxygen reloading after a dive exponentially decreases with time, producing a smooth curve of diminishing oxygen gain. Kramer (1988) argued that this is because the partial pressures of the animal's oxygen stores increase with time at the surface, which causes a decrease in the difference in partial pressures between the stores and ambient air, thus decreasing the rate of oxygen diffusion.

Because the lung system of mammals collapses during descent (Kooyman and Ponganis, 1998), most of the oxygen stores in these animals are bound to haemoglobin and myoglobin and thus it is likely that this smooth curve would be seen. However, the respiratory tract and air sacs of birds form on average around half of their oxygen storage capacity (e.g. Keijer and Butler, 1982; Croll et al., 1992). Therefore, in contrast to mammalian divers, myoglobin and haemoglobin do not dominate the oxygen stores of most birds. Walton et al. (1998) suggested that oxygen must enter the caudal air sacs, via the primary bronchi and caudal secondary bronchi, before it becomes available for physiological gaseous exchange in the parabronchi. They predicted that avian divers will produce a biphasic oxygen uptake curve with the first phase representing oxygen taken into the air sacs and the second representing recovery of haemoglobin and myoglobin stores. Parkes et al. (2002) have shown that

the oxygen uptake curve in tufted ducks is biphasic during longer dives, although this is probably not due to the respiratory anatomy of the bird but rather to changes in respiratory frequency over time.

Both the smooth oxygen uptake curves of earlier models and the biphasic curve of Walton et al. (1998) predict a variety of optimal behaviour patterns. These concern adjustments to surface duration and foraging duration in response to changes in dive depth and energetic costs during the dive. It is likely that the details of the oxygen uptake curve have a critical effect on the gross predictions of diving optimality models (Ruxton et al., 2000). The predictions of present models reveal the importance of empirical studies on oxygen uptake curves so that further progress can be made in understanding observed diving behaviour.

Kramer (1988) developed the optimal breathing model to predict changes in surface duration in response to changes in the depth of foraging (Fig. II-1a). The basic model of Houston and Carbone (1992) is a modification of the optimal breathing model (Kramer, 1988) that allows prediction of foraging duration as well as surface duration (Fig. II-1b). One prediction of the model is that divers will spend less time foraging if the energetic costs of foraging increase, while optimal surface duration will not change. Carbone and Houston (1994) tested some of these predictions by manipulating the costs and benefits of foraging by pochard ducks, *Aythya ferina*. The trends from these experiments qualitatively agree with the model, however, it is erroneous to accept this model as accurate when fundamental aspects, such as the oxygen uptake curves, have not been quantified. Instead, these trends can be considered only as guidelines to empirical research (Pierce and Ollason, 1987).

Whereas the models have assumed that the oxygen gain curve is the same after all dives, Parkes et al. (2002) found that increased dive durations are associated with an initial increase in rate of oxygen uptake at the surface in tufted ducks, *Aythya fuligula*.

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This indicates that the rate of oxygen uptake varies depending upon energetic costs during submergence. This change in the oxygen gain curve in response to varying oxygen consumption is likely to have important implications for the predictions of optimal diving models. Furthermore, assuming aquatic birds and mammals have to balance the oxygen they consume during a dive with the oxygen they gain at the surface (Kramer, 1988), data on the volume of oxygen used to reload the stores would allow a quantitative comparison of the changes in energy expenditure during foraging dives, where the energy cost of foraging has been manipulated.

Our first main objective was to confirm, by quantifying changes in the uptake curve against changes in the energetic costs of foraging, that an increased rate of oxygen uptake during surfacing is associated with an increase in oxygen consumption during diving. Second, by incorporating power cost estimates for different phases of the dive taken from an earlier study (Lovvorn et al., 1991), we produced a graphical solution of the optimal breathing model (Kramer, 1988). This allowed us to determine whether the ducks were diving optimally according to the model and to test the validity of the model. Furthermore, time budget data allowed us to test a specific prediction of the basic Houston and Carbone (1992) model that, as foraging cost increases, foraging duration decreases without a change in surface duration.

Methods

We used five adult female and one adult male tufted ducks ($\bar{x} \pm$ SE weight = 692 ± 29 g) in this experiment. They were reared from eggs obtained (under an English Nature Licence) from Kingsbury Water Park, Sutton Coldfield, U.K., and, when adult, were kept in outdoor holding facilities at the University of Birmingham which included ponds and vegetated areas. For experiments we used an indoor dive tank (1.0 x 1.6 m and 1.7 m deep) that had access to an adjacent dry area (0.6 x 0.8 m). The ducks were housed on the tank for several weeks before the experiments, allowing them to become used to the noises and activities associated with the experiment and to the

concept of diving to a feeding platform for their food. During the experimental period, they were housed on the indoor tank for 6 months under a light:dark regime of 14:10 hours. Air temperature ranged between 12 and 22 º C and the water temperature between 10 and 18 º C. Food consisted of corn, pellets and a variety of live foods including maggots and mealworms. Food was not provided on the morning of an experimental day, encouraging the ducks to dive to the feeding tray during the experiment. All ducks maintained their weights during the experiments and received ad libitum food afterwards. The experiment was conducted under a Home Office licence.

For the experiment, we placed the subject bird on the water surface within the confines of a clear acrylic respirometer (35 x 25 x 25 cm) while the other birds were restricted to the dry area, out of view of the subject duck (Fig. II-2). The bottom edges of the respirometer were placed 10 cm below the water forming an airtight seal along its sides. This made the effective volume of the respirometer 13 125 ml. The surface of the tank was covered by mesh, apart from the entrance to the respirometer, and so the submerged duck always resurfaced into the respirometer. The duck was encouraged to dive by the availability of maggots on a tray (67 x 82 cm) suspended within the water at a depth of 1.1 m, ensuring the ducks could be easily observed when foraging. We had three experimental conditions. Food was dropped on to the tray either without a substratum present (control condition) or among gravel, or among stones (mean mass 65 g, placed on the tray one stone deep; substratum condition) in an attempt to affect the energetic demands on the duck while it foraged at the tray. Because the maggots remained active underwater for many minutes, they burrowed in between the gravel and stones when they were present, forcing the ducks to push them about to uncover the food.

Air was continuously pushed through the respirometer at a rate of 333 ml s^{-1} by a fixed flow pump such that the concentration of carbon dioxide within the respirometer was always kept below 0.2 %. Leak tests (Fedak et al., 1981) were regularly

conducted by bleeding a known amount of nitrogen into the respirometer and checking that the calculated decrease in oxygen content, according to the rate of air flow through it, equalled the recorded decrease. A further 11.7 ml s^{-1} was drawn as the sample gas, just beyond the outlet hole of the respirometer, with a second air pump (Ametek, model R1 Flow control). A 500 ml flask was placed in front of the sample pump to reduce any flow oscillations. A fan inside the respirometer ensured homogeneity of the gases and thus the measure of oxygen in the sample gas was an accurate measure of oxygen inside the respirometer (Woakes and Butler, 1983). Tubing 350 ml in volume was attached to the holes in the respirometer open to ambient air. This ensured that when the duck initially surfaced into the respirometer after the dive, air forced out of the respirometer did not escape the system and was subsequently sucked back in.

Differences between the concentration of oxygen in the gas entering and leaving the respirometer were measured by an oxygen gas analyser (Ametek, model S3A-1/N.22) such that the oxygen uptake of the duck (oxygen used to reload the oxygen stores and oxygen metabolised during the post-dive period) could be calculated (Fedak et al., 1981). Carbon dioxide levels were also measured, with an infrared carbon dioxide analyser (ADC Ltd, model SS-100), to ensure no build up occurred within the respirometer. Temperature and humidity readings were taken (Vaisala, Helsinki) to check that the variations in water vapour and gas temperature were sufficiently small to have only a negligible effect on the oxygen concentration of the airflow through the respirometer. The connecting tubing was impermeable to oxygen and was as small a bore (3 mm diameter) and as short as possible to limit dead space within the system. The response time of the oxygen sensor was less than 0.2 s and the lag time of the respirometer and tubing was 3.0 s. The residual time constant of the system after deconvolution (the conversion of oxygen concentration in the respirometer to rate of oxygen uptake, see below) was 0.4 s and was determined by nitrogen injections at various points within the respirometer. The effect of the residual time constant is a

slight filtering of the V_{O_2} data, which results in a slight blurring of the cumulative oxygen uptake curves.

The absolute values and gains of the sensors were recalibrated before and after experimental sessions with a precision gas-mixing pump (Wösthoff Pump, type 2M301/a-F, Bochum) and gain drift was found to be negligible. To allow compensation for the inherent drift in the oxygen analyser, a desktop computer (DLS, P166MMX) controlled a rotary valve, which switched the analyser's gas input from air leaving the respirometer to ambient air each time the duck was diving for food.

Output signals from the oxygen analyser, carbon dioxide analyser and humidity and temperature probes were sent to a terminal block connected to an analogue-to-digital converter unit (AT MIO-16L, National Instruments) in the computer. Every 0.25 s, 180 scans of each sensor device input were made and the mean recorded; these data points were stored on the hard drive by means of a custom made program written with a software package for automating data collection and manipulation (LabVIEW, National Instruments), which also automated control of the rotary valve.

Each experimental session lasted on average 1 h and recordings were collected when a dive bout commenced. As well as total dive duration, we recorded the times of the descent, foraging and ascent phases of each dive when foraging occurred. At the end of longer periods of diving activity, the gas concentrations in the respirometer reached a maximum of 0.18 % for carbon dioxide, and oxygen was reduced by a maximum of 0.35% from its ambient level. The range of humidity levels in the respirometer was 90 to 93 % across the duration of all the experimental sessions (and this range was usually much smaller within an experimental session) and the temperature was constantly 21 ° C.

Analysis

To convert oxygen concentration into rate of oxygen uptake we used a modification of the formula proposed by Woakes and Butler (1983) that allows measurement of fast changes in oxygen uptake from an open circuit respirometer system:

$$V_{O_2} = (FO2_{(t_2)} - FO2_{(t_1)})V + \frac{(FO2_{(t_1)} + FO2_{(t_2)} - 2FO2_{(amb)})}{2}(t_2 - t_1)\dot{Q}$$

where,

V_{O_2} = Total oxygen consumption between times t_1 and t_2 (ml),

$FO2_{(t_1)}$, $FO2_{(t_2)}$ = fractional concentrations of oxygen at times t_1, t_2 leaving the chamber,

V = respirometer volume (ml),

$FO2_{(amb)}$ = fractional concentration of (ambient) oxygen entering the respirometer,

t_1, t_2 = start and finish of a period of time where variation of oxygen concentration in the respirometer is recorded and

$\dot{Q}$ = flow rate out of the respirometer (ml s^{-1})

All oxygen volumes are corrected to STPD

By treating the respirometer as both an open and closed system, oxygen uptake could be determined for 0.25 s intervals, despite the system having a much longer response time. The changes in oxygen concentration in the respirometer between t_1 and t_2 were very small and often within the error of the oxygen analyser creating a low ratio of measurement signal against signal noise. However, the noise component of each measurement was random and therefore equally likely to be negative or positive. Thus, recording multiple data points for each value of t allowed signal averaging to recover the measurement signal at each 0.25 s by reducing the magnitude of the mean noise value (Bentley, 1983). I.e. averaging maintained the root-mean-square (r.m.s.) value of the measurement signal while reducing the r.m.s. value of the noise. Because a very large number of data points (n = 890 and 1218) were collected and averaged,

the signal to noise ratio was greatly increased. A change of 1 ml oxygen in the respirometer could then be measured with an error of approximately ± 0.03 ml.

We analysed a dive whenever the duck visited the foraging tray. Bout criterion interval analysis (Slater and Lester, 1982) was used to eliminate all surface durations greater than 28 s. Mean ± SE values are given, usually for six ducks, but sometimes for less than six. To avoid animal bias we obtained mean values for each bird and used these means to obtain the final mean. A significant difference between means was tested with paired *t* tests unless stated otherwise. Where we used one-tailed *t* tests, we stated this in the text.

The mean rate of oxygen consumption (oxygen metabolised) over the dive cycle ($\dot{V}_{O2c}$) was calculated by dividing the mean total oxygen uptake during the surface interval, V_{O2up}, by mean dive duration plus mean surface duration, $t_d + t_s$. This assumes that the birds are recovering from the previous dive during the surface interval, rather than preparing for the next dive. We calculated the mean total oxygen consumption over a dive cycle of mean duration, V_{O2d+s}, by combining the mean oxygen consumption rate during submersion of mean dive duration ($\dot{V}_{O2d}$), multiplied by t_d, with the mean oxygen consumption rate during surface intervals of mean duration ($\dot{V}_{O2s}$) multiplied by t_s. $\dot{V}_{O2d}$ and $\dot{V}_{O2s}$ were estimated with multiple linear regressions (Woakes and Butler, 1983) between V_{O2up}, t_d and t_s.

Results

The oxygen uptake data for the gravel condition (1087 dives) were not significantly different to the control condition, probably because they did not significantly increase the energetic demands on the ducks during foraging. These data have therefore not been included in the results section, for clarity.

Diving time budgets

Table II-1 contains time budget data from six ducks all diving in the two conditions (control and substratum conditions). We recorded a total of 2108 dives for these two conditions. The time budget data were normally distributed about the mean for all but one individual bird in both conditions (Anderson-Darling normality test; the exception showed mildly bimodal diving behaviour) thus the mean was the most effective measure of the average. The oxygen uptake data for birds in both conditions were also normally distributed about the mean (Anderson-Darling normality test; NS). For all variables, the means of the six ducks were assumed to be a normally distributed, representative sample of the population.

In the control condition, with the foraging tray devoid of substratum, mean foraging duration (t_f) was significantly lower than that when the stones were present (substratum: 5.3-11.0 s; control: 4.4-7.0 s; Table II-1). The same trend in terms of mean total dive duration (t_d) was also significant (substratum: 11.3-16.1 s; control: 9.1-14.1 s; Table II-1). This is due to the difference in mean foraging duration, since there was no significant difference in total travelling time (t_T; substratum: 4.3-7.6 s; control: 4.6-7.1 s) because the distance to the foraging site was fixed. The percentage of the dive spent foraging ($t_{\%f}$) was also significantly lower in the control condition than in the presence of stones (substratum: 44.7-65.0 %; control: 43.2-56.1 %; values arcsine transformed for statistical analysis; Table II-1). Surface durations (t_s) were not significantly different between the two conditions (substratum: 9.8-18.6 s; control: 8.8-16.7 s; Table II-1).

Oxygen consumption during activity

The multiple regressions between V_{O2up}, t_d and t_s were significant for each duck. Table II-2 shows the mean values for calculated rates of oxygen consumption during mean dives and mean surface intervals, along with the relevant mean partial correlation coefficients. The partial correlation coefficients for the dives were low. Calculated

oxygen consumption during the dives was 0.48-0.90 ml s^{-1} when stones were present and 0.53-0.79 ml s^{-1} in the control condition, with partial correlation coefficients of 0.47-0.76 when stones were present and 0.46-0.83 for the control. The corresponding values of $\dot{V}_{O2s}$ were 0.49-0.82 ml s^{-1} in the presence of stones and 0.39-0.71 ml s^{-1} in the control condition, with partial correlation coefficients of 0.66-0.93 in the presence of stones and 0.73-0.89 in the control condition.

The mean total oxygen uptake during the surface interval (V_{O2up}) and the mean rate of oxygen uptake during the surface interval ($\dot{V}_{O2up}$) were significantly higher when stones were present. $\dot{V}_{O2c}$ was significantly higher in the presence of stones. In both conditions, V_{O2d+s} did not differ significantly from V_{O2up}.

Calculation of oxygen reload curves

The oxygen uptake of the ducks at the surface includes not only the reloading of the lung, blood and muscle oxygen stores but also the oxygen used for post-dive metabolism. The optimal breathing model (Kramer, 1988) is based only on the curve of the oxygen used to reload the stores. The rate of oxygen uptake decreases with surface duration to an almost constant value somewhere between 10 and 15 s (Parkes et al., 2002). This constant oxygen uptake can be used as an estimate of the post-dive metabolic rate, which is ongoing during the surface period. To remove post-dive surface metabolism from the oxygen uptake curves and be left with the oxygen reload curves, we calculated the gradient of the slope between 15 and 20 s. This slope represents the post-dive metabolic rate in ml oxygen s^{-1}, which can be removed from the entire curve (Fig. II-3).

Oxygen uptake at the surface

The shape of the oxygen uptake curve and oxygen reload curve against surface duration changes with the duration of the dive and the foraging conditions of the dive (Figure II-4). To analyse the changes in the shape of the uptake curve after dives of

different periods, we placed dives from the substratum condition in to duration bins of 5-9.75 s, 10-14.75 s and 15-19.75 s (Table II-3). We used one-tailed tests because the increased foraging costs of the stones were predicted to be associated with an increased rate in oxygen uptake, supporting the conclusions of Parkes et al. (2002). To analyse the changes in the shape of the uptake curve after dives in the two foraging conditions, we controlled for dive duration by comparing means of dives from the same duration bins (Table II-3). Again, one-tailed tests were used because these results were predicted to confirm the trend found by Parkes et al. (2002). In all cases, we compared the uptake curves achieved by testing for a significant difference between the cumulative oxygen values of the curves at 5, 10 and 15 s. When we compared uptake curves between the dive duration bins, all the values at 5, 10 and 15 s were significantly different. When we compared uptake curves between the two foraging conditions, again all the values were significantly different.

The decrease in rate of oxygen uptake into the stores over time may be caused by the decrease in the difference in partial pressure of oxygen between the ambient air and the cardio-respiratory system as the stores become reloaded. If this is the case then the shape of the oxygen reload curve in the control condition should be the same as the shape of the curve in the substratum group, accounting for dive duration, after a certain period at the surface. This assumes that the metabolic rate of surface activities during the interval after the dive is the same in both conditions and that this rate is unchanging over the surface period.

To test this, we compared the oxygen reload curve for all control dives, from 0 to 14 s, with the reload curve for all substratum condition dives, from 1 to 15 s (Fig. II-5). In a comparison of $\dot{V}_{O2d}$ and t_d for both conditions, the ducks consumed on average 2 ml of oxygen more per dive when stones were present. If the gradient of the curves is due purely to the difference in partial pressure of oxygen then the shape of the oxygen reload curve in the control condition should be the same as the shape of the reload curve in the substratum condition after 2 ml of oxygen have been added to the oxygen

reserves, which takes 1 s. These two curves were fitted to a model determined by non-linear regression. *F* ratios were run to determine that a third order polynomial equation was most appropriate for both curves ($r^2 > 0.99$ in each case). These models were then compared with an ANCOVA with factors of individual duck, condition and the cubic relation with respect to time. There was a significant difference between the two curves ($F_{1,674} = 193.1$, $P < 0.001$) suggesting that the ducks increase $\dot{V}_{O2up}$ by their own volition during surface periods in between energetically more costly dives.

The optimal breathing model

Time budget data and oxygen reload curves from the two conditions were combined to construct and test Kramer's optimal breathing model (1988). By using values of power output during different phases of the dive derived from other studies, and converting these values to oxygen consumption (Table II-4), we could test whether the experimental ducks were diving optimally according to the model.

Lovvorn et al. (1991) calculated the power requirement per kg of body mass during descent and 'staying at the bottom' of a water column 1.2 m deep, for three *Aythya* species. This is similar to the present study where the foraging tray was suspended at 1.1 m in the water. We used values for the lesser scaup, (*A. affinis*), because this species is similar morphologically and behaviourally to *A. fuligula* (Lovvorn et al., 1991; Stephenson, 1994). Taking into account an aerobic efficiency (mechanical power output / diving aerobic power input) of 12.6 % for *A. affinis* (Stephenson, 1994), and then converting power output to rate of oxygen consumption, where 1 W = 20.1 ml oxygen s^{-1}, provides rates of oxygen consumption for the descending and foraging phases of the dive. The ascent phase of the dive is deemed to be passive (Lovvorn et al., 1991), and thus no oxygen consumption for locomotion is attributed to it. Oxygen consumption during this phase is assumed to be equal to resting metabolic rate while the bird is on the water surface (Lovvorn et al., 1991). Using the values in the second part of Table II-4 and the oxygen reload curves, we can construct the optimal breathing model (Kramer, 1988; Fig. II-6 and Fig. II-7).

We calculated the exact point of intersection of the tangent and oxygen reload curve for each duck and for the mean of all ducks, for both conditions. The oxygen reload curves were fitted to a model (third order polynomial; equation 1). For the control condition curves, r^2 of the models was 0.99-1.00, with a mean of 1.00 ± 0.00. For the substratum condition curves, r^2 was 0.99-1.00, with a mean of 1.00 ± 0.00. Since the gradient of the tangent and the curve fit were known, their interception could be calculated:

The model used to describe the oxygen reload curve is given by the equation

$$V_{O2up} = at_s *^3 + bt_s *^2 + ct_s * + d \,. \tag{1}$$

Differentiating with respect to V_{O2up},

$$dV_{O2up} / dt_s * = 3at_s *^2 + 2bt_s * + c \,. \tag{2}$$

Since the gradient of the tangent is given by

$$V_{O2up} /(t_T + t_s *),$$

from equation 2, it follows that

$$V_{O2up} /(t_T + t_s *) = 3at_s *^2 + 2bt_s * + c \tag{3}$$

$$V_{O2up} = (t_T + t_s *)(3at_s *^2 + 2bt_s * + c) \tag{4}$$

From equations (1) and (4)

$$at_s *^3 + bt_s *^2 + ct_s * + d = (t_T + t_s *)(3at_s *^2 + 2bt_s * + c) \,. \tag{5}$$

$$2at_s *^3 + bt_s *^2 + t_T (3at_s *^2 + 2bt_s * + c) = d \,, \tag{6}$$

and

$$d = -m_1 t_T \quad \text{(Fig. II-1a)} \tag{7}$$

From equation (6), t_s* can be found, and $m_2 t_f* = V_{O2up}$

For each curve, the model predictions were statistically compared to the observed values for t_s and m_2t_f with a single sample t test. Table II-5 shows the model predictions and observed values of surface duration and oxygen consumed at the foraging site for the means of all ducks, in both conditions. The model predictions t_s^* and $m_2t_f^*$ for the mean of all the ducks in both conditions were not significantly different from the observed values, t_s and m_2t_f. At the level of the individual ducks, four of the model predictions for t_s^* in the control condition were significantly different from the observed values of t_s ($P < 0.01$ and < 0.001). All six predictions of $m_2t_f^*$ from the same models were significantly different from the observed values of m_2t_f ($P < 0.001$). In the substratum condition, all six of the model predictions of t_s^* for individual ducks were significantly different from the observed values of t_s ($P < 0.001$), and four of the six predictions of $m_2t_f^*$ were significantly different from m_2t_f ($P < 0.001$).

Discussion

This study was designed to investigate the behavioural adjustments of tufted ducks to changes in the energy costs of foraging (m_2), and to test whether these could be predicted by optimal foraging models of air breathing divers. No other study has attempted to test the quantitative predictions of these models by combining data of cumulative oxygen uptake at the surface with estimates of rates of oxygen consumption during the descent, foraging and ascent phases underwater.

Time budgets and foraging costs

The dive duration and surface duration values for the present study are comparable to time budget data recorded from previous studies on the same genus diving to similar depths (Table II-6). The mean surface duration of the ducks did not differ significantly whether or not stones were present on the foraging tray. This result agrees with the predictions of Houston and Carbone (1992). However, in

contradiction to their model, the foraging duration of the ducks was significantly higher when stones were present. The basic Houston and Carbone model (1992) predicts that the diver balances its oxygen gains and losses over a dive cycle, for a given time at the surface. Therefore, if the energy used during travelling does not vary, for instance because the depth of the foraging site is constant, then an increase in the energy costs of foraging forces a decrease in time spent at the foraging site. The model assumes that the curve of oxygen gain with surface duration is fixed. However, our findings show a significant increase in $\dot{V}_{O2up}$ (mean rate of oxygen uptake during the surface interval) in response to foraging among stones. The presence of the stones was presumed to create more energetically demanding conditions because the ducks had to force their bills between and under the stones to obtain the maggots. This was confirmed by the significantly higher values of $\dot{V}_{O2s}$ (estimated mean rate of oxygen consumption during surface intervals of mean duration) in the substratum condition for each dive duration bin ($P < 0.05$). This supports the work of Parkes et al. (2002) who found that V_{O2up} (total oxygen uptake during the surface interval) increased after longer dives and concluded that $\dot{V}_{O2up}$ therefore increased after dives where more oxygen had been consumed. In both conditions, V_{O2up} did not differ significantly from V_{O2d+s} (mean total oxygen consumption over a dive cycle of mean duration), indicating that the increase in oxygen consumption in the presence of stones, caused by an increase in m_2, is fully compensated for by the increase in oxygen uptake at the surface.

Despite t_s (mean surface duration) not differing between the two conditions, V_{O2up} was significantly higher in the presence of stones because $\dot{V}_{O2up}$ was significantly higher. However, $\dot{V}_{O2d}$ (estimated mean rate of oxygen consumption during dives of mean duration) and $\dot{V}_{O2s}$ were not significantly different between the two conditions. There are two possible reasons for this. First, the higher rate of oxygen consumption during foraging in the presence of stones was partially offset by the increased time that the ducks spent underwater because of the increased time at the foraging site. This meant that there was more time for air bubbles to escape from the duck's feathers, causing a

greater reduction in buoyancy, and therefore a reduction in the energy required to remain submerged. Secondly, and probably more importantly, the descent phase of the dive, which is several times energetically more costly than the foraging phase (e.g. Lovvorn et al., 1991), was a smaller proportion of the dive when the stones were present, because the foraging duration, and hence the total dive duration, was longer. Nevertheless, $\dot{V}_{O_{2c}}$ (mean rate of oxygen consumption over the dive cycle) was significantly higher when the stones were present, suggesting that these factors did not entirely mask the increased foraging costs imposed by the stones on the average rate of oxygen consumption over the whole dive cycle.

Quantification of the optimal breathing model

Estimates of power costs of each phase of the dive cycle (e.g. Stephenson et al., 1989; Lovvorn et al., 1991; Stephenson, 1994) can be used to produce estimates of oxygen consumption during a dive. These values, along with the oxygen reload curves of the present study, are incorporated into the optimal breathing model (Kramer, 1988). This model makes two important assumptions: (1) the ducks are diving optimally and (2) on average, oxygen reload at the surface equals oxygen consumption during the dive.

The optimal breathing model (Kramer, 1988) successfully predicted both t_s and m_2t_f (mean oxygen consumption during mean foraging duration) in the control condition for all ducks (Fig. II-7a, Table II-5). The model also successfully predicted t_s for all ducks in the substratum condition (Fig. II-7b, Table II-5). Although the model also successfully predicted m_2t_f, m_2t_f* was lower than m_2t_f, while the power cost estimate for foraging was derived from calculations of the energy required only to equal buoyancy at a certain depth. It does not account for the energy required to manipulate the stones to uncover and gain access to the maggots and therefore m_2t_f is an underestimate of foraging costs in the substratum condition. The difference between m_2t_f* and the true cost of foraging is thus larger than calculated.

Therefore, according to the model predictions using the mean values of six ducks for each condition, the model is a successful predictor of surface duration. It is also a successful predictor of oxygen consumed during the foraging phase of the dive in the control condition. This provides evidence that the ducks were diving optimally in that they were maximising the proportion of time spent at the foraging site during each dive. However, quantification of the model using data for individual ducks does not support its validity. The model was not consistent at successfully predicting t_s or $m_2 t_f$ in either condition.

Quantification of the optimal breathing model (Kramer, 1988) has therefore produced uncertainty concerning its predictive validity. The model appears to be fairly successful at predicting the average diving behaviour of a number of tufted ducks but was unsuccessful at doing so for individual birds. For a single animal, the model may not always incorporate all the parameters that are influential in determining its diving behaviour. This is because an explanation of the differences between the model predictions and observed values is that diving optimally, in terms of the model parameters, would entail costs (Houston et al., 1980; Stephens and Krebs, 1986; Johnstone and Norris, 2000). For example, some individuals may choose to surface for longer than t_s* to increase observation time if they are more wary of predators approaching. Other individuals may surface for less time than t_s* if they perceive that conspecifics may start to compete with them for the available food. The model may sometimes be attempting to predict foraging behaviour at the wrong scale, for example, some divers may attempt to maximise time spent at the foraging site at the unit of a diving bout rather than that of a single dive. Some of the ducks in the present study showed significant negative correlations, albeit weak ones, between the number of preceding dives within a dive bout and the length of time spent at the feeding site. The ducks may have decreased foraging time in response to the decreased density of maggots, which is predicted by the marginal value theorem (Charnov, 1976) assuming that the rate of food intake decreases as the number of maggots decreases. Other

factors such as decisions to explore for new food patches and the onset of fatigue could also be influential.

The lack of consistent predictive validity of the model at the individual level for oxygen consumed at the feeding tray suggests that the power output estimates used in the present study may not be accurate. Indeed, there are large variations in power cost values from different studies. For example, Stephenson (1994) estimated considerably higher power costs than did Lovvorn et al. (1991), such as 0.84 W kg^{-1} more to descend and 1.11 W kg^{-1} more to maintain a depth (at 1.5 m). These power cost estimates often leave the birds with little or no oxygen for consumption at the foraging tray according to the optimal breathing model (Kramer, 1988). Methodologies, duck species and water depth appear to be the main factors influencing these estimations. In the present study we used power requirements estimated by Lovvorn et al. (1991), where power output needed for descent was calculated to a depth of 1.2 m, which was similar to the depth of the feeding tray (1.1 m) in our study. Power output needed to maintain a depth, which is a requirement during foraging at a tray suspended within the water, was also calculated at 1.2 m. The power costs of diving probably varied between ducks because of physiological differences other than just body mass. Unfortunately, we have no indication of variance for the power cost estimates of Lovvorn et al. (1991) and so we used the same values for all ducks in the optimal breathing model (Kramer, 1988), which might also explain the lack of predictive validity at the individual level.

At present there is no technique available to record the baseline metabolic rate of a duck during the dive. The only sensible estimate available for our study was metabolic rate while the ducks were at rest on the surface, which we used to represent oxygen consumption during the ascent phase of the dive. This value is unlikely to be entirely accurate. A baseline metabolic cost is difficult to ascertain since it does not remain the same during exercise as during rest (Stainsby et al., 1980). At the risk of adding a poorly known baseline value to the locomotory costs of descent and

foraging, it is perhaps preferable to ignore this small energy cost (J.R. Lovvorn, personal communication).

Changes to the oxygen reload curve

There are two possible explanations for the change in shape of the oxygen reload curve after different dives. More rapid oxygen reloading after longer dives or in the presence of the stones could be caused by an increased effort by the bird to load its stores more quickly, for instance by increasing respiratory frequency and / or tidal volume. An alternative explanation is that the average rate of oxygen reloading is higher (i.e. that the curve is steeper) when the oxygen stores are lower, at the start of the surface period. A greater difference in the partial pressures of oxygen between the cardio-respiratory system and ambient air would allow a more rapid uptake of oxygen at this time. If changes to the shape of the uptake curve are governed by partial pressure differentials alone, then we would expect the reload curve after a less energetic dive, for example a shorter dive or one involving energetically less costly foraging, to be the same shape as the reload curve after a more energetic dive, after a certain portion of the surface period. In other words, once the partial pressure differentials at the surface after a more energetic dive have decreased to the same level as those after a less energetic dive, the rate of oxygen uptake over time should be the same.

The shape of the oxygen reload curve in the control condition from 0 s was statistically different from the shape of the oxygen reload curve in the stones condition after 1 s. This suggests that the rate of oxygen uptake at the surface is not controlled by partial pressure differentials alone. Rather, the ducks are actively increasing $\dot{V}_{O2up}$ in between energetically more costly dives, perhaps by an increase in respiratory frequency and / or tidal volume. This agrees with the findings of Butler and Woakes (1979) who reported tachycardia in tufted ducks before dives, serving to load their oxygen stores, and tachycardia after dives related to the duration of the dive. Webb et al. (1998) reported very similar behaviour in Northern elephant seals

(*Mirounga angustirostris*), which increased $\dot{V}_{O2up}$ after longer dives without adjusting surface duration. Because increased tachycardia and respiratory frequency increases surface costs while decreasing recovery time and increasing time at the foraging site, optimal foraging in air breathing divers appears to be more complex than has been previously appreciated.

Variation within a species

Assessing the qualitative validity of optimal foraging models is entirely viable because trends predicted by the model can be tested. However, it is not possible satisfactorily to test whether empirical data support the model if the model lacks confidence intervals. In the present study, we had to compare observed values for each duck with the model predictions using single sample *t* tests since the model prediction was a fixed value. This statistical analysis accounts for the confidence limits around only one of the two values and so is less thorough than a standard *t* test. It is more likely to indicate a significant difference between observed and predicted values and so the model is more likely to be deemed inaccurate. Models need to include a measure of variability around the solution so that data collected to test the model can be more robustly compared with the predictions.

Houston and McNamara (1985) discussed the problem that optimality models require the behaviour of an animal under a given condition to be regular, whereas it is usually variable. A similar problem is that individual animals can behave quite differently from each other under a given condition (Krebs et al., 1977; Maynard Smith, 1978; Kacelnik and Houston, 1984; Ball, 1994). This variation within a species creates a second difficulty in demonstrating quantitative validity in the present study. Figure II-6 represents a graphical solution to the optimal breathing model (Kramer, 1988). The solution implies the representation of an entire species through a single data set. However, the process of averaging data to represent a data set can also serve to remove information about that data set. The large variation in diving strategy and rate of oxygen reloading within the group of six ducks cannot be fairly represented by a

single reload curve and single values of diving energy costs. In comparing the time budget data and reload curves of the six birds in the present study, there are arguably four foraging strategies present (Fig. II-8, Table II-7).

From the oxygen uptake curves and time budget data, we placed the six tufted ducks we used into four strategy types (I-IV). Duck *lbdg* had the highest t_f (mean foraging duration) and significantly the highest t_d (mean dive duration; $P < 0.001$) as well as significantly the steepest oxygen uptake curve (quantified by the highest oxygen uptake after 15 s; $P < 0.001$). In contrast, duck *op* had the shortest t_f and significantly the shortest t_d values ($P < 0.01$) as well as significantly the lowest oxygen uptake after 15 s ($P < 0.001$). These represent two contrasting strategies of foraging behaviour. Ducks *bdg* and *pr* had very similar values for t_f (NS) and t_d (NS), as well as similar oxygen uptake rates (NS) and t_s (NS). They therefore appear to have used similar foraging strategies. Their t_d and t_s values were significantly different from those of *lbdg* and *op* ($P < 0.001$ and $P < 0.01$, respectively), and their oxygen uptake values were significantly different from those of both *lbdg* and *op* ($P < 0.001$) and so probably represent a third strategy. Ducks *pinr* and *bblu* also had similar values to each other in terms of all three time budget values (NS) and oxygen uptake rate (NS). Furthermore, all these values were mostly different to those for the other strategies, with oxygen uptake rate and t_s being significantly different to all other strategies ($P < 0.01$ and $P < 0.001$, respectively), suggesting a fourth type.

Ducks *pinr* and *bblu* dived fewer times in a bout than the other ducks. However, they spent longer than the average time ($\bar{x} \pm$ SE = 5.6 ± 0.5 s) foraging per dive and also appeared to work particularly hard to consume the food while at the foraging tray. Thus, they may have ingested relatively large numbers of maggots per dive. These observations are supported by the two highest $\dot{V}_{O_{2c}}$ values of all the ducks. In contrast, *op* foraged for less time than all the other ducks and observations suggest that it also foraged less energetically. This corresponds to the short time it spent at the surface. However, *op* tended to dive more times within a foraging bout than *pinr* or

bblu ($P < 0.05$). Duck *lbdg* spent longest at the tray and also took up oxygen most quickly at the surface to compensate for particularly large amounts of oxygen consumed each dive because of long dive durations. Observations do not suggest that it worked as hard as *pinr* or *bblu* when foraging, supported by the low $\dot{V}_{O_2c}$ ($P < 0.001$), but *lbdg* did tend to dive the most times in a diving bout.

Although the categorisation of foraging strategies in the present study is somewhat arbitrary, it demonstrates the wide variation in behaviour within a species. Mean values derived from varied individuals, generated to represent the behavioural strategy of a species, are consequently misleading. Tufted ducks may have different optimal diving strategies because of their individual physiologies, or their strategies may be optimal under particular remembered conditions.

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Table II-1. Time budget data from six tufted ducks in the control condition and in the substratum condition, where stones were present on the food tray

	Control ($n = 890$)	Substratum ($n = 1218$)
Dive duration, t_d (s)	11.2 ± 0.7	$13.6 \pm 0.8**$
Foraging duration, t_f (s)	5.6 ± 0.5	$7.6 \pm 0.8*$
% time spent foraging, $t_{\%f}$	$55.26 \pm 1.9^{\dagger}$	$63.62 \pm 3.3*^{\dagger}$
Surface duration, t_s (s)	12.3 ± 1.4	12.5 ± 1.4

Values given are means ± SE.

$*P < 0.05$; $**P < 0.01$; t test comparing control and substratum conditions.

† Values are arcsine transformed.

Table II-2. Mean values of gas exchange from six tufted ducks in the control condition and in the substratum condition, where stones were present on the food tray

	Control ($n = 890$)	Substratum ($n = 1218$)
Mean estimated oxygen consumption rates during dives of mean duration, $\dot{V}_{O2d}$ (ml s^{-1})	0.63 ± 0.04 (0.67 ± 0.06)	0.61 ± 0.06 (0.66 ± 0.02)
Mean estimated oxygen consumption rates during surface intervals of mean duration, $\dot{V}_{O2s}$ (ml s^{-1})	0.57 ± 0.05 (0.81 ± 0.03)	0.66 ± 0.05 (0.80 ± 0.02)
Mean total oxygen consumption over a dive cycle of mean duration, V_{O2d+s} (ml)	13.82 ± 1.13	16.48 ± 1.85
Mean total oxygen uptake during surface interval, V_{O2up} (ml)	14.56 ± 1.72	17.44 ± 2.10**
Mean rate of oxygen uptake during surface interval, $\dot{V}_{O2up}$ (ml s^{-1})	1.19 ± 0.05	1.39 ± 0.04**
Mean rate of oxygen consumption over the dive cycle, $\dot{V}_{O2c}$ (ml s^{-1})	0.61 ± 0.04	0.66 ± 0.03*

Values given are means ± SE.

Values in parentheses are partial correlation coefficients.

*$P < 0.05$; **$P < 0.01$; t test comparing control and substratum conditions.

Table II-3. Statistical comparison between oxygen uptake curves after dives of six tufted ducks in the control condition and in the substratum condition, where stones were present on the food tray, and between three dive duration bins in the substratum condition

Dive duration bins	5 s (substratum v. *control*, ml oxygen)	10 s (substratum v. *control*, ml oxygen)	15 s (substratum v. *control*, ml oxygen)
5-9.75 s	6.00 ± 0.42 *5.21 ± 0.40**	9.54 ± 0.62 *8.66 ± 0.52***	12.90 ± 0.83 *11.45 ± 0.65***
10-14.75 s	***8.33 ± 0.20 *7.37 ± 0.36***	***12.68 ± 0.38 *11.30 ± 0.40***	**15.87 ± 0.60 *14.12 ± 0.35**
15-19.75 s	**10.44 ± 0.41 *8.89 ± 0.28**	***15.45 ± 0.46 *13.49 ± 0.45**	***18.40 ± 0.60 *16.50 ± 0.38***

Values given are means ± SE.

*P < 0.05; **P < 0.01; ***P < 0.001; *t* test comparing conditions and duration bins. Asterisks to the left of the substratum values represent a significant difference between that duration bin and the duration bin one range smaller (e.g. 10-14.75 s and 5-9.75 s).

Asterisks to the right of the control values represent a significant difference between the two conditions in that duration bin.

Table II-4. Predicted power costs and oxygen consumption rates during the dives of six tufted ducks in the control condition and the substratum condition where stones were present on the food tray

	Control ($n = 890$)	Substratum ($n = 1218$)
Power requirement to descend to 1.2 m (W kg^{-1})	5.46 (Lovvorn et al. 1991)	
Power requirement to maintain position at 1.2 m (W kg^{-1})	1.69 (Lovvorn et al. 1991)	
Mean body mass of experimental ducks (kg)	0.692 ± 0.029	
Aerobic efficiency (%)	12.6 (Stephenson 1994)	
Conversion factor of W to ml oxygen s^{-1}	20.1 (Stephenson et al. 1989)	
Mean descent time (s; t_{desc})	3.07 ± 0.30	3.17 ± 0.28
Mean ascent time (s; t_{asc})	2.54 ± 0.15	2.83 ± 0.23
Mean total travel time (s; t_T)	5.62 ± 0.43	6.01 ± 0.49
Mean foraging duration (s; t_f)	5.62 ± 0.45	7.80 ± 0.31
Mean resting metabolic rate (ml s^{-1})	0.189 ± 0.022 ml	
Estimated oxygen metabolised during travel phases of dive ($m_1 t_T$)	4.59 ± 0.42	4.78 ± 0.41
Estimated oxygen metabolised during mean foraging duration (ml; $m_2 t_f$)	2.32 ± 0.19	3.14 ± 0.31

| Mean surface duration (s; t_s) | 12.30 ± 1.42 | 12.50 ± 0.56 |

Values given are means ± SE where available.

Table II-5. Comparison of predictions of t_s and m_2t_f from the optimal breathing model with direct measurements of these variables during diving for six tufted ducks in the control condition and the substratum condition where stones were present on the food tray

	Control ($n = 890$)	Substratum ($n = 1218$)
t_s*, optimal surface duration according to optimal breathing model (s)	12.56	10.26
t_s, mean surface duration of six tufted ducks (s)	12.3 ± 1.4	12.5 ± 1.4
m_2t_f*, oxygen consumed at tray according to optimal breathing model (ml oxygen)	2.46	2.98
m_2t_f, mean oxygen consumption at the feeding tray of six tufted ducks according to power cost estimates (ml oxygen)	2.59 ± 0.21	3.51 ± 0.35

Values given are means ± SE, where available.

Table II-6. Dive budget data from previous studies on *Aythya* species

	Species	Depth (m)	Dive duration (s)	Surface duration (s)	Mean total oxygen uptake during surface interval (ml)
Present study[†]	*fuligula*	1.1	12.4	12.4	16.0
Woakes and Butler (1983)	*fuligula*	1.7	14.4	16.1	16.2
Bevan and Butler (1992a)*[a]	*fuligula*	0.6	18.9	11.6	12.1
Bevan and Butler (1992a)*[b]	*fuligula*	0.6	16.2	12.8	16.4
Bevan and Butler (1992b)*	*fuligula*	0.6	14.9	-	-
Stephenson (1994)	*affinis*	1.5	13.5	16.3	18.6
Parkes et al. (2002)	*fuligula*	1.7	15.6	12.3	17.2
S Wallace (unpublished data)	*fuligula*	1.5	15.9	17.9	-

[†] Means of data from both conditions.

* Ducks were trained to dive for certain durations using a system of computer-controlled lights.

[a] Summer acclimated birds.

[b] Winter acclimated birds.

Table II-7. Data for individual tufted ducks in the control condition, where no substratum was present on the food tray

| | Strategy type | Oyxgen uptake after 15 s (ml) | Dive duration budget data | | | No. dives / bout | $\dot{V}_{O2c}$ (ml) |
			Foraing time, t_f (s)	Dive duration, t_d (s)	Surface duration, t_s (s)		
lbdg	I	18.1 ± 0.2	7.0 ± 0.3	14.1 ± 0.3	11.4 ± 0.3	23 ± 7	0.54 ± 0.01
op	II	12.7 ± 0.2	4.4 ± 0.2	9.1 ± 0.2	11.7 ± 0.4	12 ± 2	0.56 ± 0.01
bdg	III	15.9 ± 0.5	5.0 ± 0.3	11.7 ± 0.4	8.9 ± 0.6	7 ± 3	0.51 ± 0.01
pr	III	15.4 ± 0.2	4.6 ± 0.1	9.7 ± 0.1	8.8 ± 0.2	11 ± 2	0.65 ± 0.01
pinr	IV	14.3 ± 0.3	6.7 ± 0.3	11.3 ± 0.3	16.7 ± 0.5	4 ± 1	0.67 ± 0.01
bblu	IV	14.3 ± 0.4	6.0 ± 0.4	11.5 ± 0.4	16.3 ± 0.9	3 ± 1	0.75 ± 0.01

Values given are means ± SE.

Significant differences between means were tested with a paired *t* test.

For further details on strategy types see Discussion.

(a)

Oxygen
stores

$m_2t_f^*$

t_s^*

$-m_1t_T$

Travel duration (t_T) Surface duration (t_s)

(b)

Time spent foraging (t_f)

t_f^*

t_s^*

$-m_1 t_T / m_2$

Travel duration (t_T) Surface duration (t_s)
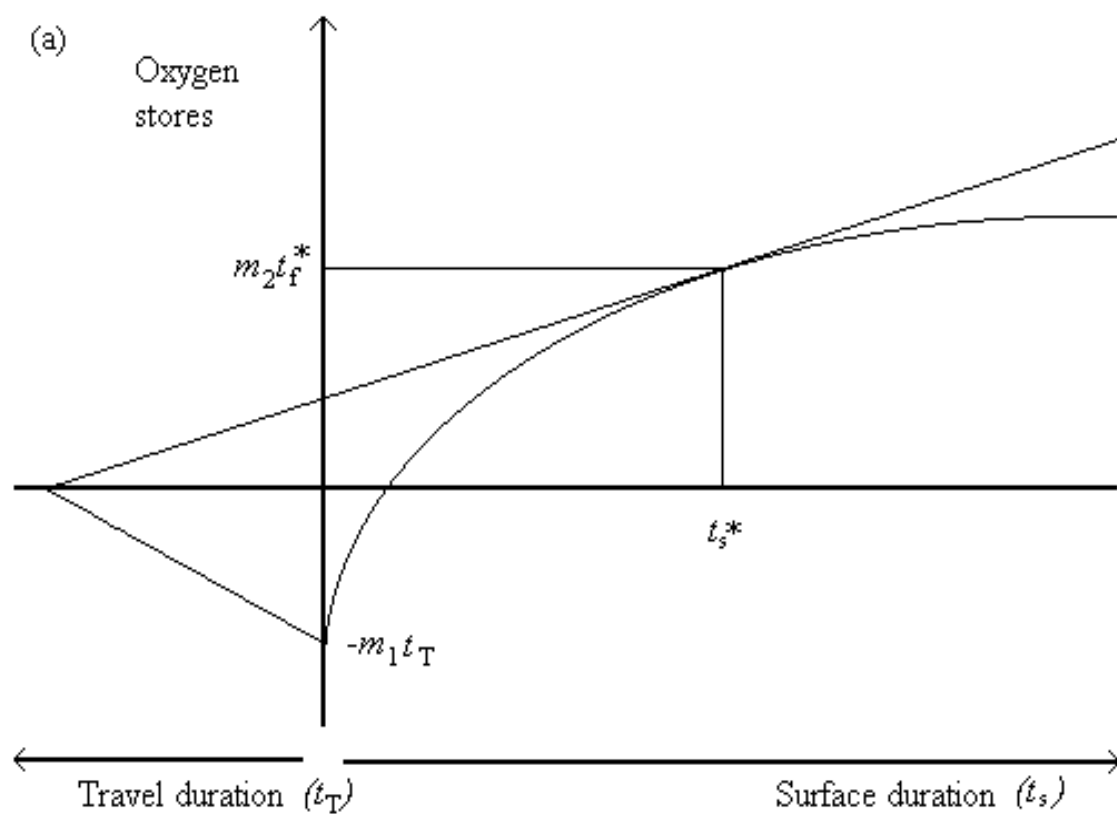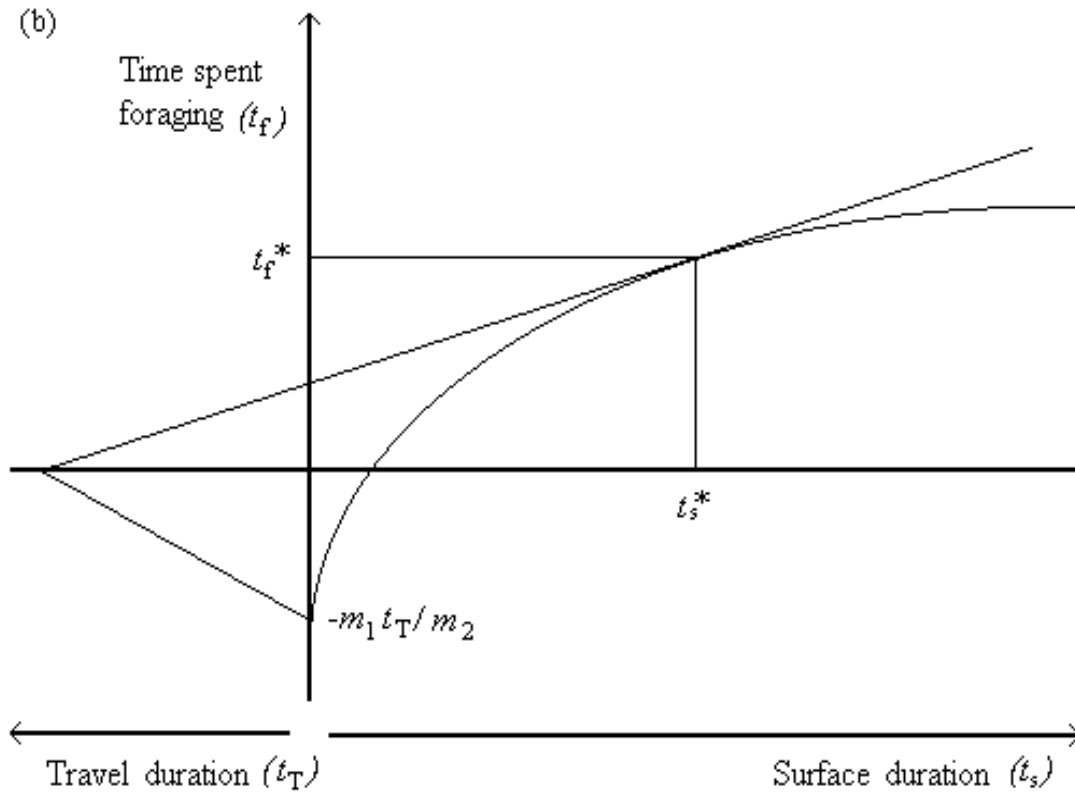
Figure II-1

(a) Adaptation of the optimal breathing model (Kramer, 1988). The abscissa shows time spent travelling to and from the foraging site to the left of the ordinate, and time at the surface to the right. The ordinate shows the amount of oxygen consumed during travel and gained during surface periods. t_s^* denotes the optimal surface duration for the diver in terms of maximising the proportion of time at the foraging site. $m_1 t_T$ is the amount of oxygen consumed during travel for time t_T. $m_2 t_f^*$ represents the amount of oxygen consumed at the foraging site for time t_f, when the duck is diving optimally.

(b) Adaptation of the basic model of Houston and Carbone (1992). The ordinate above the abscissa shows time spent at the foraging site, t_f^*. Because some oxygen is consumed during travelling, $m_1 t_T$, foraging duration is decreased (by $-m_1 t_T / m_2$).
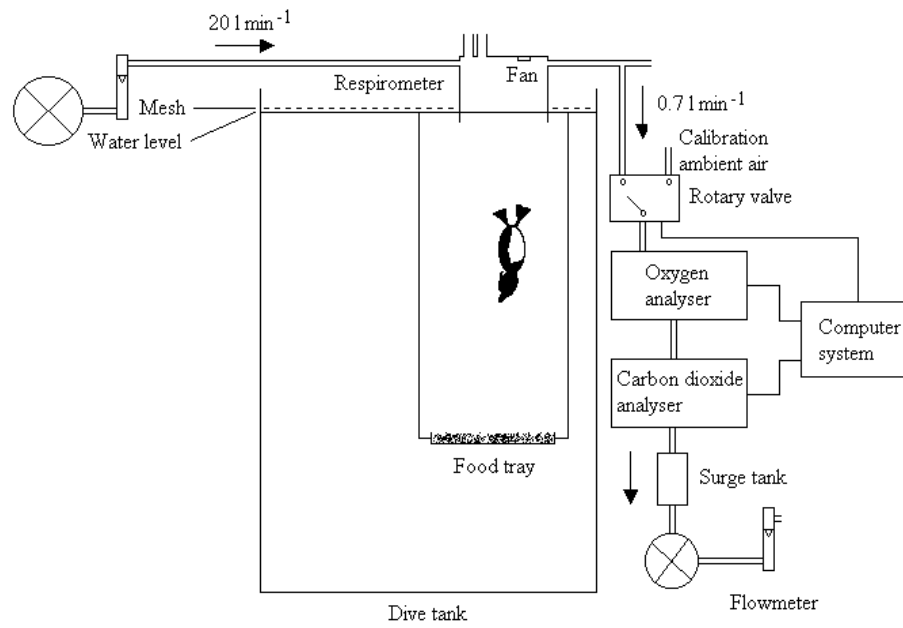
Figure II-2

Diagram of experimental apparatus showing a tufted duck diving from the respirometer. For further details see Methods.
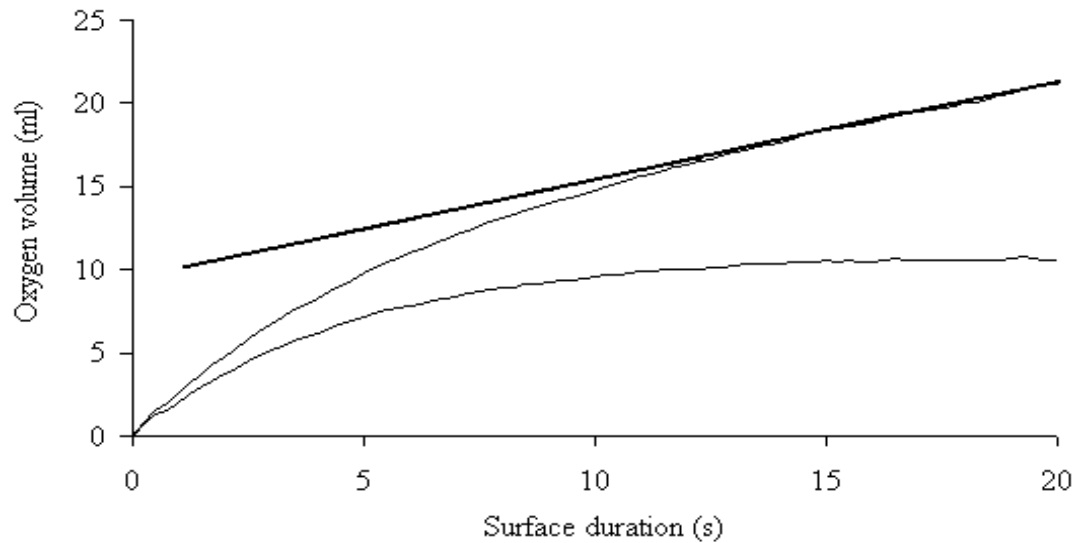
Figure II-3

Example of the generation of the oxygen reload curve from the oxygen uptake curve, where the oxygen uptake curve is associated with some dives in the substratum condition, in which stones were present on the food tray ($n = 1218$). The gradient of the thick black line (0.52 ml s^{-1}), estimates post-dive metabolic rate. This assumes that metabolic rate is constant during the surface interval although in reality it will gradually decrease over time to a point because of a reduction in activity by the bird such as decreases in ventilation frequency and heart rate. This value is removed from the oxygen uptake curve to produce the oxygen reload curve. The oxygen reload curve reaches an approximate plateau from around 13 s onwards.
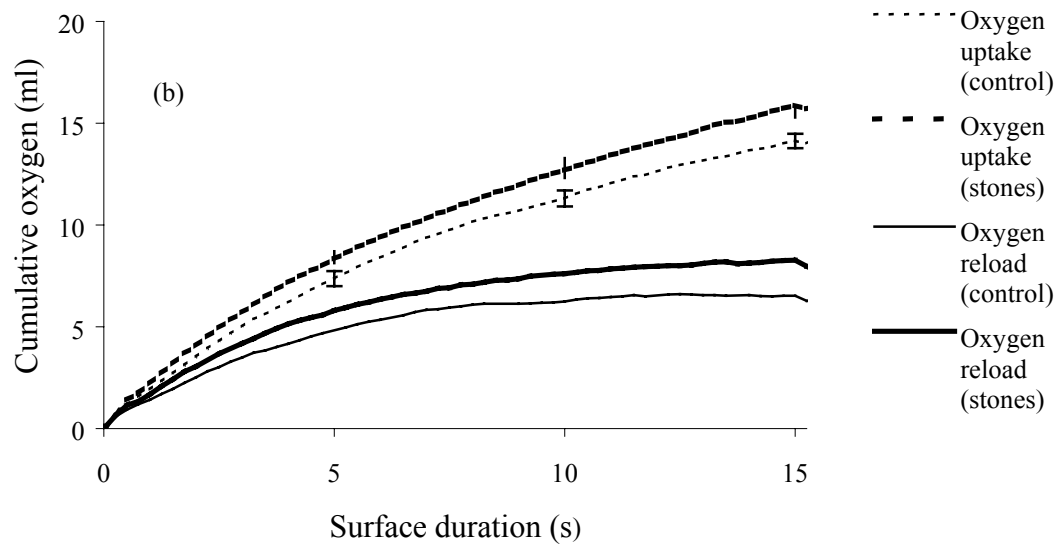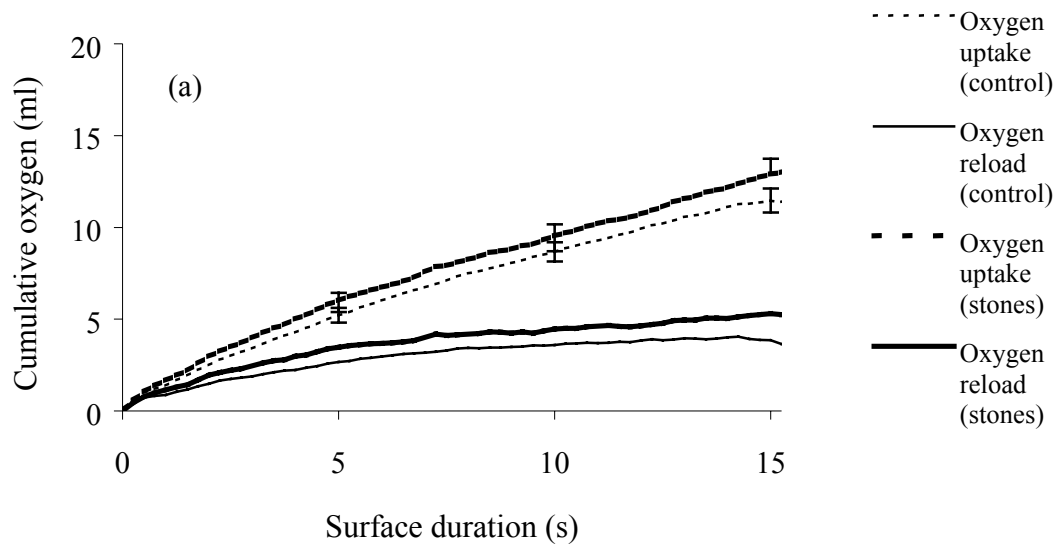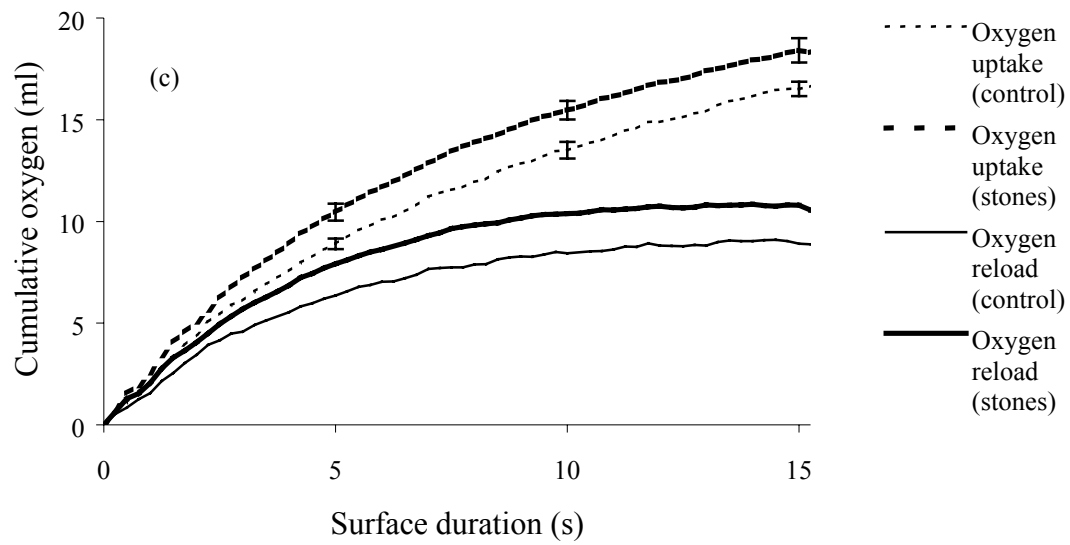
Figure II-4

Oxygen uptake and reload curves for the first 15 s post-dive of six tufted ducks in the two experimental conditions. Each graph represents a dive duration bin: (a) 5-9.75 s, (b) 10-14.75 s, (c) 15-19.75 s. Lighter lines: control condition (no stones present on the food tray; $n = 890$); darker lines: substratum condition (stones present; $n = 1218$); dashed lines: total amount of oxygen taken up by the birds; full lines: oxygen added to the stores in the respiratory system, blood and muscles. The vertical lines represent SE of the means at 5, 10 and 15 s.
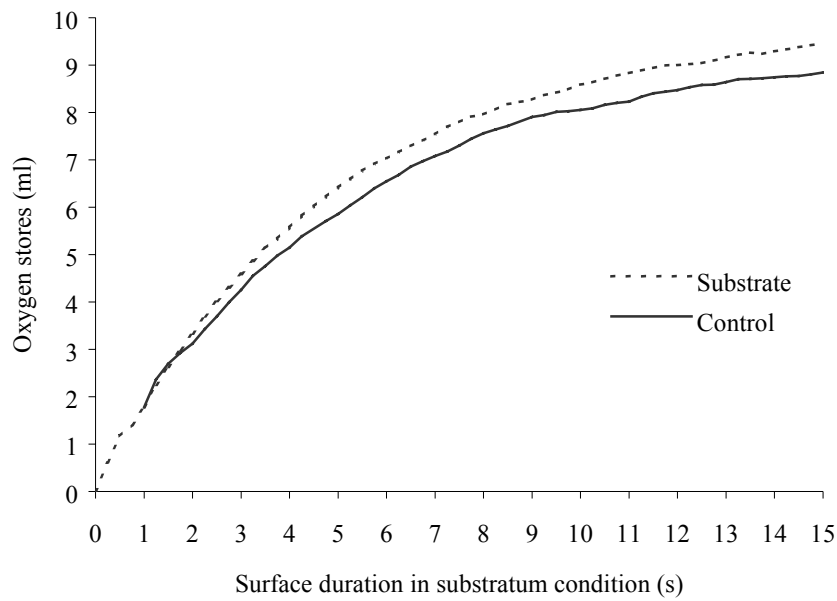
Figure II-5

Comparison of the shapes of the oxygen reload curve for six tufted ducks in the control condition (no stones present on the food tray; $n = 890$) and in the substratum condition (stones present on the food tray; $n = 1218$) from 1 s onwards.
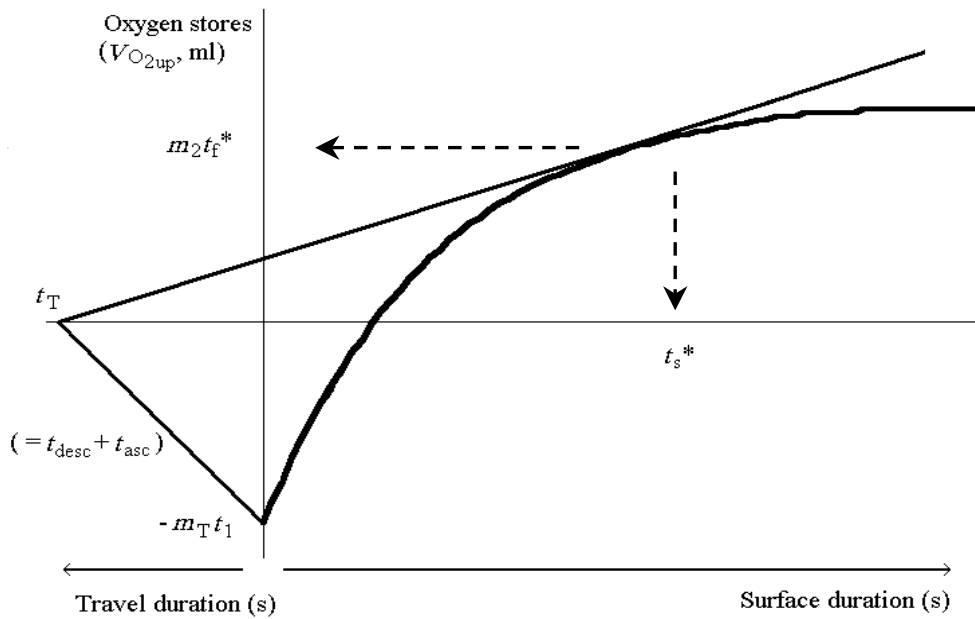
Figure II-6

Quantification of the optimal breathing model (Kramer 1988). Values for $-m_1 t_T$ and $m_2 t_f$ were generated from time budget data combined with estimates of power costs during different phases of the dive (Table II-4). To test the validity of the model, for each duck, we compared t_s^* to t_s and $m_2 t_f$ to the volume of oxygen consumed during the optimal surface period according to the model ($m_2 t_f^*$). t_s^* is represented by the value of X at the point of intersection of the tangent and $m_2 t_f^*$ is represented by the value of Y at that point. For definitions of abbreviations, see Fig. II-1.
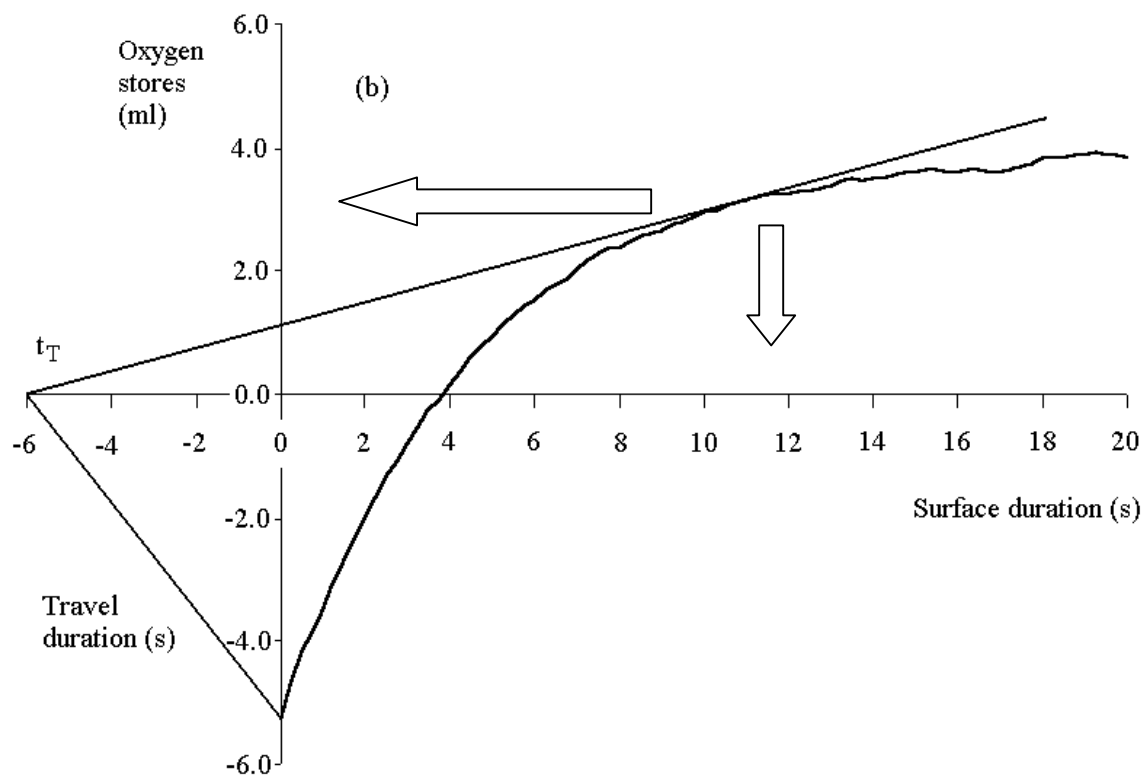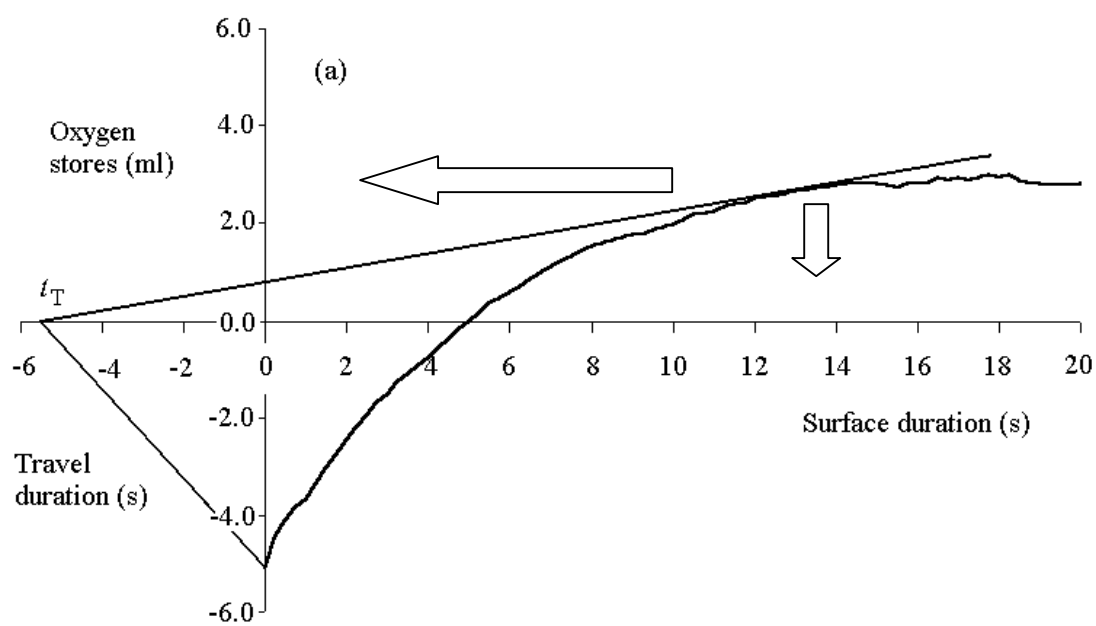
Figure II-7

Testing the optimal breathing model (Kramer 1988) using the mean values of six tufted ducks, under the two foraging conditions (a, control; b, substratum; for definitions see Methods). The tangent runs from the total travel duration (t_T) and touches the oxygen reload curve at a point determined by fitting the curve to a model and then calculating the intercept (for further details see Results). The arrow pointing to the ordinate indicates $m_2 t_f{}^*$ and the arrow pointing to the abscissa indicates $t_s{}^*$ calculated from the intercept of the tangent and the curve.
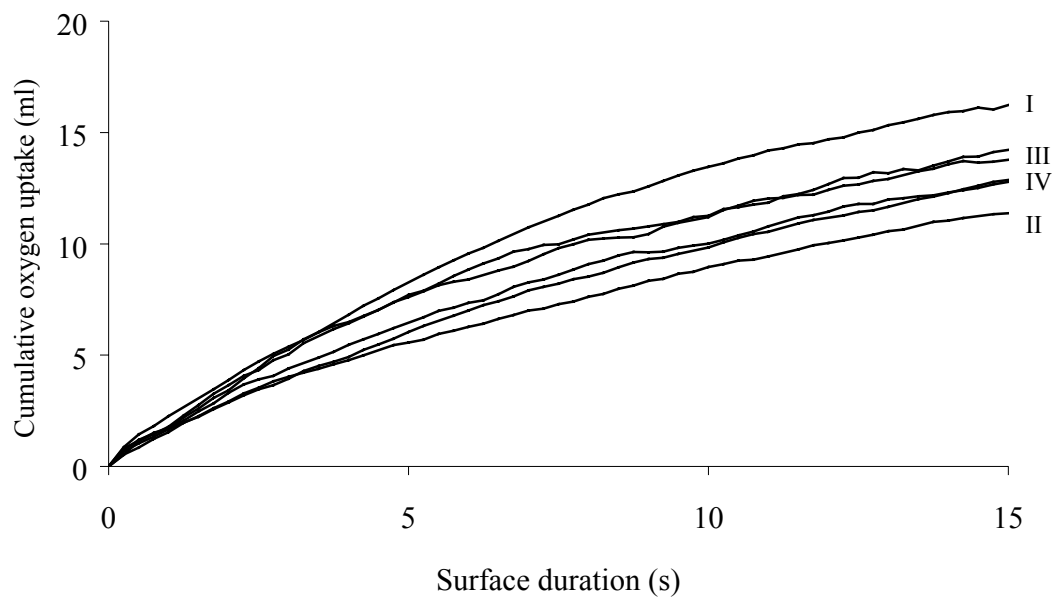
Figure II-8

Oxygen uptake curves for the six tufted ducks, categorised into four foraging strategies. Strategy I includes just one duck, lbdg; II includes just one duck, op; III includes two ducks, bdg and pr; IV includes two ducks, pinr and bblu.

III. The Influence of Oxygen and Carbon Dioxide on Diving Behaviour of Tufted Ducks, *Aythya fuligula*

Lewis G. Halsey, Jane Z. Reed, Anthony J. Woakes & Patrick J. Butler

While optimal diving models focus on the diver's oxygen stores as the predominant factor influencing diving behaviour, many vertebrate species surface from a dive before their oxygen stores are exhausted and may commence another dive well after their oxygen stores have been re-saturated. The present study investigates the influence of hypoxia and also hypercapnia on the dive cycle of tufted ducks, *Aythya fuligula,* in terms of surface duration and dive duration. The birds were trained to surface into a respirometer box after each dive to a feeding tray so that rates of oxygen uptake ($\dot{V}_{O_2}$) and carbon dioxide output ($\dot{V}_{CO_2}$) at the surface could be measured. Although $\dot{V}_{CO_2}$ initially lagged behind $\dot{V}_{O_2}$, both respiratory gas stores were close to full adjustment after the average surface duration indicating that they probably had a similar degree of influence on surface duration. Chemoreceptors, which are known to influence diving behaviour, detect changes in oxygen and carbon dioxide partial pressures in the arterial blood. Thus, the need to restore blood gas levels appears to be a strong stimulus to continue ventilation. Mean surface duration coincided with peak instantaneous respiratory exchange ratio due to pre-dive anticipatory hyperventilation causing hypocapnia. For comparison, the relationship between surface duration and oxygen uptake in re-analysed data for two grey seals indicated that one animal tended to dive well after fully reloading its oxygen stores while the other dived at the point of full reloading. More carbon dioxide is exchanged than oxygen in tufted ducks during the last few breaths before the first dive of a bout, serving to reduce carbon dioxide stores and suggesting that hypercapnia rather than hypoxia is more often the limiting factor on asphyxia tolerance during dives. Indeed,

according to calculations of oxygen stores and oxygen consumption rates over modal diving durations, a lack of oxygen does not seem to be associated with the termination of a dive in tufted ducks. However, factors other than carbon dioxide are also likely to be important, and perhaps more so, such as food density and rate of food ingestion. Because some predictive success has been demonstrated for optimal diving models, they should continue to incorporate oxygen stores as a variable but their validity is likely to be improved by also focusing on carbon dioxide stores.

Introduction

The majority of models on foraging behaviour by diving animals have examined strategies that maximise the proportion of time submerged while focusing on the management of oxygen stores (e.g. Kramer, 1988; Houston and Carbone, 1992; Walton et al., 1998). These models assume that usable oxygen stores are fully depleted at the end of each foraging dive because this maximises the rate of oxygen uptake ($\dot{V}_{O_2}$) at the surface, thus minimising surface time (Houston and Carbone, 1992). Thompson and Fedak (2001) point out that if these oxygen balance models realistically portray the optimal behaviour for air breathing divers then there is a paradox in that many marine vertebrates terminate their dives before their oxygen reserves are exhausted (e.g. Fedak et al., 1988; Thompson et al., 1991; Croxall et al., 1993; Webb et al., 1998; Le Boeuf et al., 2000). Tufted ducks (*Aythya fuligula*) have also been found to resurface after dives under natural conditions before their usable oxygen stores are depleted (Butler and Woakes, 1983; Stephenson et al., 1986). This suggests that the decision by diving vertebrates to terminate the dive and resurface may be influenced by factors other than the level of oxygen stores (Butler, 1982). Therefore, the decision to leave the surface and dive again may also be controlled by factors other than levels of oxygen stores. The predominant influence of oxygen on diving behaviour during the dive cycle is thus brought into question.

There is some evidence for the potency of hypercapnia over hypoxia in affecting the diving behaviour of mammals (Ridgway et al., 1969; Påsche, 1976; Gallivan, 1980; Ollenberger and West, 1998). Evidence that carbon dioxide levels in the body may control the duration of the diving portion of the dive cycle has come from research into human diving. Human divers are able to increase submergence time by hyperventilating prior to submergence which functions to reduce carbon dioxide levels in the body rather than increase oxygen levels because the arterial blood is already close to being saturated with oxygen during normal ventilation (Ferrigno and Lundgren, 1999). Voluntary breath-holds are terminated by inspiratory muscle

contractions intensifying until the airway is opened, and although the duration of these apnoeas varies, the partial pressure of alveolar carbon dioxide (P_ACO_2) at the end is always similar (e.g. Sterba and Lundgren, 1985). Butler and Woakes (1979) recorded hyperventilation and tachycardia in tufted ducks during the last few seconds prior to the start of some dives. These may be associated with a large loading of the oxygen stores or, more likely, with an unloading of the carbon dioxide stores as with humans. Jones et al. (1982) studied the role of arterial chemoreceptors in the diving responses of ducks during forced dives and found that they caused an increase in hind limb vascular resistance when they were stimulated by high partial pressures of carbon dioxide but not by changes in partial pressures of oxygen alone.

Concerning the influence of hypercapnia on surface behaviour during the dive cycle, breath-by-breath analysis of end-tidal oxygen and carbon dioxide in both harbour porpoises (*Phocoena phocoena*) and grey seals (*Halichoerus grypus*) showed that they were able to take up oxygen more quickly than they were able to remove carbon dioxide during the initial portion of a post-dive breathing bout (Boutilier et al., 2001). The oxygen stores of the harbour porpoises appeared to be saturated several breaths before the inter-dive surface period ended while excess carbon dioxide was still being removed, suggesting that carbon dioxide levels may be more influential than oxygen levels on terminating surface intervals in diving mammals. Supporting evidence is provided by Ridgway et al. (1969), who found that a bottlenose dolphin was unwilling to dive again until the partial pressure of carbon dioxide in its exhaled air (P_ECO_2) had returned to normal.

Butler and Stephenson (1988) recorded significant changes in both dive and surface durations in tufted ducks exposed to hypoxic (10 % oxygen), hyperoxic (40 % oxygen) and hypercapnic (5 % CO_2) gas compositions. Enstipp et al. (2001) reported significant changes in the surface duration of double-crested cormorants (*Phalacrocorax auritus*) exposed to a similar array of gas compositions and Furilla

and Jones (1986) noted significant changes in the dive duration of redhead ducks (*Aythya americana*) exposed to inspired oxygen concentrations between 10 % and 50 %. However, the relative control of the respiratory gases on the dive cycles of these birds is inconclusive. It is difficult to equate the effects of such inhaled gas compositions, which represent a variety of departures from ambient concentrations of oxygen and carbon dioxide, with the effects of changes in respiratory gas stores during the dive cycle. Little other direct evidence has been produced concerning the influences of oxygen and carbon dioxide on the diving behaviour of birds.

Avian divers have a fundamentally different respiratory anatomy from that of mammals with relatively small, rigid lungs and multiple air sacs to ventilate the lungs, which are not directly involved in gaseous exchange. Furthermore, the respiratory volume of birds is larger on a unit mass basis than that of mammals. Birds also have different diving behaviours from mammals, with generally shorter absolute diving and surface durations, but longer diving durations when accounting for body size (Schreer and Kovacs, 1997). These differences could affect the influence that the respiratory gases have during the dive cycle. The primary aim of the present study was to test the hypothesis that carbon dioxide removal is more important than oxygen replacement in determining surface duration in foraging tufted ducks. A secondary aim was to gain some understanding of the influences of these respiratory gases on the dive duration of this species. Finally, the influence of oxygen on diving behaviour in grey seals and tufted ducks was compared. Raw data on breath-by-breath measurements of end-tidal PO_2 in two grey seals were obtained from J. Z. Reed so that the relationships between reloading of the oxygen stores and termination of the surface period could be compared between these two species. The implications for optimal diving models are then discussed.

Methods

Six adult tufted ducks (mean mass 725 ± 27 g) were used in this experiment. They were raised from eggs collected from Kingsbury Water Park, Sutton Coldfield. When fully grown, they were housed in either indoor or outdoor holding facilities at The University of Birmingham. The experiments were performed on an indoor dive tank (1.0 x 1.6 x 1.7 m deep) incorporating an adjacent dry area (0.6 m x 0.8 m). The ducks were kept on the tank for several weeks before the experiments commenced so that they had time to become used to the noises and activities associated with the experiment, and to the concept of diving to a feeding tray (1.3 m depth) for their food. Food consisted of corn, pellets, and a variety of live foods including maggots and mealworms. This variety ensured that the masses of the ducks remained stable during the experimental period. On the day prior to the experiment, they were fed a reduced amount to encourage diving behaviour. Water temperature ranged between 15 ° C and 18.5 ° C while air temperature in the laboratory ranged between 18 ° C and 21 ° C.

The experimental procedure in the present study was similar to that described for the control condition in *Chapter II* but with some additions and adjustments (Fig. III-1). To obtain data on respiratory gaseous exchange after each dive to the feeding tray, the subject bird was confined to surfacing into a respirometer box (35 x 25 x 25 cm). Oxygen and carbon dioxide concentrations in the respirometer were recorded by passing separate gas samples through a fast response oxygen gas analyser (Ametek, model S3A-1/N.22) and a fast response carbon dioxide analyser (Morgan Capnograph, P.K. Morgan Ltd.), respectively. Due to some background noise in the voltage output signal from the carbon dioxide analyser, the signal was smoothed by the computer program (custom made in LabVIEW, National Instruments) with a running three-point average. Although the output of the oxygen analyser had very low background noise, because data from the two analysers were being compared, this signal was also smoothed.

Air was continuously pushed through the respirometer at 183 ml s^{-1} such that the concentration of carbon dioxide within the respirometer was always kept below 0.5 %. Humidity within the box ranged between 90 and 93 % across all experimental sessions and the range was generally less than this within each session. Injections of carbon dioxide into the respirometer at different points within the chamber showed that the levels of carbon dioxide produced by the bird were sufficiently small to ensure that absorption of carbon dioxide from within the respirometer box to the water was negligible. Leak tests (Fedak et al., 1981) were regularly conducted by bleeding a known amount of N_2 at different points into the respirometer to confirm that the calculated decrease in oxygen content, according to the rate of airflow through it, equalled the recorded decrease. A further 11.7 ml s^{-1} was drawn as the sample gas to the oxygen analyser and 2.5 ml s^{-1} to the carbon dioxide analyser. The response times of the analysers were less than 0.2 s and the lag time of the system due to the volume of the respirometer box and the tubing was 3.25 s. The residual time constant of the system after deconvolution (the conversion of oxygen concentration in the respirometer to rate of oxygen uptake, see later) was 0.4 s and was determined by nitrogen injections at various points within the respirometer box.

Each duck was present in the respirometer for up to 2 h. Recordings were collected when a dive bout commenced. At the end of longer periods of diving activity, the gas concentrations in the respirometer remained below 0.5 % for carbon dioxide and oxygen was reduced by a maximum of 0.5 % from its ambient level. To determine resting rates of oxygen consumption and carbon dioxide production ($\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ at rest, respectively), the ducks were placed in the respirometer box with the opening to the box covered with netting so that they could not dive. They remained in the box for 1-2 h and the respiratory exchange ratio (RER) was calculated for the 5 min period over which $\dot{V}_{CO_2}$ was lowest and stable (Withers, 2001) and the animal was assumed to be at complete rest. RER during rest is a measure of the exchange of $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ between the respiratory system and the air while in steady state, which is

therefore an estimate of $\dot{V}_{CO_2} / \dot{V}_{O_2}$ at the cellular level and is referred to as the respiratory quotient (RQ). This estimate does not account for carbon dioxide removal from the stores to form urea and uric acid. Accuracy of RER measurements in an open circuit respirometer system is dependent upon the fractional concentration of oxygen leaving the respirometer box and the accuracy of the oxygen and carbon dioxide gas analysers (Sotherland, 1985). The confidence interval for RER measurements in the present study was ± 0.01.

Analysis

Change in oxygen concentration and carbon dioxide concentration were converted to volumes of oxygen uptake (V_{O_2}) and carbon dioxide output (V_{CO_2}), respectively, at 0.25 s intervals, using a formula similar to that described in Woakes and Butler (1983). This allows measurement of fast changes in oxygen uptake and carbon dioxide output from an open circuit respirometer system:

$$V_{O_2} = (FO2_{(t_2)} - FO2_{(t_1)})V + \frac{(FO2_{(t_1)} + FO2_{(t_2)} - 2FO2_{(amb)})}{2}(t_2 - t_1)\dot{Q}$$

where,

V_{O_2} = Total oxygen consumption between times t_1 and t_2 (ml),

$FO2_{(t_1)}$, $FO2_{(t_2)}$ = fractional concentrations of oxygen at times t_1, t_2 leaving the chamber,

V = respirometer volume (ml),

$FO2_{(amb)}$ = fractional concentration of (ambient) oxygen entering the respirometer,

t_1, t_2 = start and finish of a period of time where variation of oxygen concentration in the respirometer is recorded and

$\dot{Q}$ = flow rate out of the respirometer (ml s^{-1})

To calculate V_{CO_2}, carbon dioxide values are interchanged for the equivalent oxygen values. V_{O_2} and V_{CO_2} were converted into $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ by dividing by (t_2-t_1). All values were corrected to STPD.

Analysis of a dive was carried out if the foraging tray was visited by the duck. All surface durations greater than 28 s were considered to be surface periods not associated with a diving bout and were eliminated from the analyses (Halsey et al., 2003). Normality tests on the data were performed using either the Kolmogorov-Smirnov test or the Ryan-Joiner test, depending on the number of data points constituting the data. Mean values were obtained for each bird and because these values were assumed to be a normally distributed, representative sample of the population, they were used to obtain the final mean (i.e. mean of means). Final mean values are given ± SE, usually for six ducks but sometimes for less than six. A significant difference between means was tested with paired *t* tests. Single sample *t* tests were also used where appropriate.

Results

A total of 1426 foraging dives (*n*) were recorded. Resting $\dot{V}_{O_2}$ was 0.25 ± 0.01 ml s^{-1}, resting $\dot{V}_{CO_2}$ was 0.20 ± 0.01 ml s^{-1} and RQ was 0.81 ± 0.01. The oxygen uptake and carbon dioxide output data for individual birds were normally distributed about the mean. The dive durations of each individual bird were normally distributed about the mean, while surface durations were not normally distributed. Since the surface durations still approximated to bell-shaped curves, the mean was used to describe the central tendency of the surface duration data for each individual bird.

Cumulative carbon dioxide output curves and oxygen uptake curves

Table III-1 shows statistical comparisons between the cumulative oxygen uptake curve and cumulative carbon dioxide output curve after 5 and 10 s at the surface for dive duration bins of 5-9.75 s, 10-14.75 s and 15-19.75 s. The two curves are statistically different from each other in all three dive duration bins. However, in all duration bins, the rate of growth of the carbon dioxide output curve is very similar to that of the oxygen uptake curve beyond the first few seconds. During the initial few seconds, $\dot{V}_{O_2}$ is higher than $\dot{V}_{CO_2}$.

Parkes et al. (2002) and Halsey et al. (2003) found that $\dot{V}_{O_2}$ was higher after dives of a longer duration. In the present study, dives were also split into post-dive surface duration bins to investigate whether post-dive surface duration correlated with a difference in $\dot{V}_{O_2}$, or $\dot{V}_{CO_2}$ (Fig. III-2; Table III-2). Although there does appear to be a higher V_{O_2} and V_{CO_2} during shorter surface periods, these are only significantly different between the < 10 s duration bin and the 20-29.75 s duration bin.

Fig. III-3 shows the oxygen uptake and carbon dioxide output curves for all dives combined. The rates of oxygen uptake and carbon dioxide output decrease with surface duration to an almost constant value somewhere between 10 s and 15 s (see also Parkes et al., 2002). These constant rates of gas exchange can be used as estimates of the rates of post-dive respiratory gas exchange that are ongoing during the surface period due to background metabolic processes (Halsey et al., 2003). These estimates assume that metabolic processes are constant during the surface interval although in reality they gradually decrease over time due to a reduction in activity by the bird such as decreases in ventilation frequency and heart rate. The gradient of the slopes between 20 s and 25 s were calculated and these values (0.46 ml O_2 s^{-1}, 0.44 ml CO_2 s^{-1}) were removed from the relevant cumulative curves to produce the oxygen reload curve (oxygen taken up to replenish body stores; Halsey et al., 2003) and the carbon dioxide washout curve (carbon dioxide removed to return body stores to

'normal'; Fig. III-3). The data points of the two slopes between 20 s and 25 s were linearly regressed to test that they were straight lines. The regressions returned an r^2 value of 0.997 for both slopes. To calculate the volume of oxygen taken up for the body oxygen stores to be fully replenished and the volume of carbon dioxide output for the body carbon dioxide stores to be at their 'normal', the slope gradients were extrapolated back to the abscissa (Fig. III-3). These values were then compared to the relevant curves to determine the duration of the surface period required for full adjustment to occur. In the present study, the oxygen reload curve for all dives reached an asymptote after 14.75 s at 8.20 ml, indicating that the oxygen stores were fully replenished at this point. The carbon dioxide washout curve for all dives reached an asymptote after 19.0 s at 6.25 ml.

Respiratory exchange ratio

The ratio of carbon dioxide output to oxygen uptake from the respiratory system (RER) was calculated for each second of surface time from 1 s to 11 s, and then over progressively longer intervals up to 40 s to account for the decrease in sample size of longer surface durations (instantaneous RER; Fig. III-4). RER was initially considerably below RQ and increased over time to a peak of 1.08 after 13 s, which coincided almost exactly with mean surface duration. Instantaneous RER remained above 1.0 beyond 20 s post-dive and decreased to 0.91 after 40 s. It only settled to the value of RQ during end of bout surface periods that lasted many minutes.

Pre-diving bout gaseous exchange

 Some limited data on the rates of gaseous exchange just prior to the start of the first dive of a bout were recorded in the present study (n = 18; Fig. III-5). Oxygen uptake and carbon dioxide output increased from between 3 s and 6 s before the first dive up to the start of the dive, with volumes of oxygen uptake and carbon dioxide output each ranging between 3 ml and 8 ml. The mean rates of oxygen uptake and carbon dioxide output were 0.93 ± 0.06 ml s^{-1} and 1.13 ± 0.09 ml s^{-1}, respectively, giving an

RER of 1.21. The increase in $\dot{V}_{CO_2}$ was greater than the increase in $\dot{V}_{O_2}$ between resting levels and the pre-dive anticipation ($P < 0.05$). Indeed, V_{CO_2} was significantly higher than V_{O_2} during this anticipatory period ($P < 0.05$).

Oxygen uptake curves of grey seals

The cumulative oxygen uptake curves for two grey seals (Fig. III-6) were generated from breath-by-breath measurements of end-tidal PO_2 (Reed et al., 1994). The mean surface period for seal *a* was 54.1 ± 2.5 s while the curve reached a fairly constant slope beyond 38 s, which suggests, as with tufted ducks (Parkes et al., 2002; Halsey et al., 2003), that the oxygen stores were saturated at this point. This was after a significantly shorter duration than the mean surface duration ($P < 0.001$). The mean surface period for seal *b* was 41.5 ± 3.6 s while the curve reached a fairly constant slope beyond 48 s, which was not significantly different from mean surface duration. Due to a limited sample size of long surface periods and a number of missing data points, there was considerable noise in the data beyond around 55 s for seal *a* and 44 s for seal *b*. This was improved by smoothing the noisy data with a running three-point average. For consistency, the rest of the curve in each case was also smoothed. To ensure that the smoothing did not affect the shape of the curves, the original and smoothed curves beyond 38 s for seal *a* and 48 s for seal *b* were regressed to calculate the gradient of the respective slopes. For both seals, the gradients of the slopes of the original and smoothed curves were identical (a, original, $y = 0.098x$, $r^2 = 0.997$; smoothed, $y = 0.098x$, $r^2 = 0.999$; b, original, $y = 0.035x$, $r^2 = 0.986$; smoothed, $y = 0.035x$, $r^2 = 0.989$).

Discussion

The values of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ for tufted ducks in the present study are in between the values recorded by Bevan and Butler (1992) for summer and winter conditions. The water temperature in the present study, which has a significant effect on resting

metabolic rate (Bevan and Butler, 1992), was also in between the values of the former experiment.

Mean dive duration (13.3 ± 0.8 s), mean surface duration (13.1 s) and mean RQ (0.81 ± 0.01) are comparable to the average values for tufted ducks diving in studies by Woakes and Butler (1983) and Halsey et al. (2003) using the same dive tank (Table III-3). The data from these studies is indicative of a trend predicted by Houston and Carbone (1992). Firstly, dive duration increases as travelling distance to the tray increases and secondly, surface duration increases in response to increased travelling distance.

Cumulative oxygen and carbon dioxide curves in tufted ducks

Parkes et al. (2002) found that $\dot{V}_{O_2}$ in tufted ducks during a surface period was not fixed, as was assumed by optimal foraging models, but was higher after dives of longer duration. Halsey et al. (2003) extended this by showing that $\dot{V}_{O_2}$ was higher after any dive that was energetically more expensive, whether due to dive duration or other factors such as cost of foraging. Furthermore, Halsey et al. (2003) offered evidence that the increase in $\dot{V}_{O_2}$, rather than being entirely due to higher partial pressure differentials of oxygen, was at least partly due to an increase in ventilatory effort by the ducks. In the present study, there is evidence to show that inter-dive surface durations also correlate with rates of respiratory gas exchange. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ are slower during longer surface durations after dives of the same duration, in which case the changes in $\dot{V}_{O_2}$ cannot be attributed in any part to changes in oxygen partial pressures since the energetic costs of the dives are the same. This offers further evidence that the ducks have some active control over the rate of respiratory gas exchange i.e. the ducks remained longer at the surface when they reduced the rate of adjustment of their stores.

The effects of respiratory gas exchange rates on surface durations

According to Boutilier et al. (2001), harbour porpoises remain at the surface for longer than the time taken for their oxygen stores to be saturated after prolonged dives. One of the two grey seals analysed in the present study also appeared to remain at the surface beyond full oxygen store reloading (see Fig. III-6a) since the mean surface duration of this individual is significantly longer than the point of inflection of the oxygen uptake curve. These findings are evidence that oxygen is not the dominant factor determining surface duration and that carbon dioxide maybe more influential in diving mammals. In contrast, however, seal *b* remained at the surface only as long as needed for full oxygen store reloading to occur and not significantly longer.

The oxygen stores of tufted ducks are close to being fully reloaded during the surface period (Fig. III-3), as would be predicted by optimal foraging models (e.g. Kramer, 1988; Houston and Carbone, 1992). Although the carbon dioxide output rate in tufted ducks is significantly lower than the oxygen uptake rate during the initial part of the surface period (Table III-3 and Fig. III-4), as was found in harbour porpoises (Boutilier et al., 2001), the carbon dioxide stores are also close to being fully adjusted at the end of the surface period, as can be seen from Fig. III-3, although it takes 19 s for total adjustment. Indeed, Fig. III-3 underlines the similarity in development of the respiratory gas exchange curves during the surface period in tufted ducks. Body gas concentrations are monitored by chemoreceptors detecting changes in partial pressure of oxygen in the arterial blood (P_aO_2), P_aCO_2, and pH levels (Butler and Stephenson, 1988; Enstipp et al., 2001). Thus, these data provide evidence that body gas concentrations are, perhaps not surprisingly, influential in the duration of ventilation in between dives in this species. They also offer evidence that neither respiratory gas is more important than the other.

Gas exchange before the start of a diving bout

In analysing gaseous exchange during surface periods, an assumption is often made that the surface period serves to enable the animal to recover from the previous dive, rather than to prepare for the next dive (e.g. Croxall et al., 1991; Wanless et al., 1993; Le Boeuf et al., 2000; Boutilier et al., 2001). However, tufted ducks are known to anticipate and prepare for the start of a voluntary diving bout, as shown by increases in heart rate and respiratory frequency (Butler and Woakes, 1979). Other bird species are also known to prepare for dives, often making physiological adjustments in anticipation of the required dive (e.g. Humboldt penguins, Butler and Woakes, 1984; South Geogian Shags, Bevan et al., 1997; eider ducks, Hawkins et al., 2000; Adélie penguins, Sato et al., 2002; Magellanic penguins, Wilson et al., 2002). Some tentative evidence suggests that diving mammals also anticipate and prepare for a subsequent dive (bottlenose dolphin, Elsner, 1969; Weddell seals, Kooyman and Campbell, 1972; harbour seals, Jones et al., 1973; Hill et al., 1987). Therefore, the assumption that all gaseous exchange during surface periods is a response only to the previous dive is probably not valid for most diving birds and mammals.

Preparatory periods before diving bouts by tufted ducks were recorded in the present study by increases in gaseous exchange (Fig. III-5). These periods were recorded before some of the first dives in a diving bout. Although these periods were not observed during surface durations within diving bouts, where recovery from the previous dive and preparation for the subsequent dive merged in to one another, anticipatory increases in heart rate and respiratory frequency do precede subsequent dives in a bout (Butler and Woakes, 1979). This has also been confirmed in some of the other species studied (Hawkins et al., 2000; Wilson, 2003; Wilson et al., 2003).

Analysis of the anticipatory period prior to the first dive can only represent preparation for the subsequent dive. The tufted ducks removed significantly more carbon dioxide than they took up oxygen during this period of hyperventilation,

taking advantage of the cross-current gas exchange system in their lung which enables P_aCO_2 to be lower than P_ECO_2 and thus increases the rate of carbon dioxide removal (Scheid and Piiper, 1972). Since both recovery and preparation occur during every surface period within a diving bout, peak RER, which occurs at the termination of an inter-bout surface period (Fig. III-4), may be primarily due to the pre-dive anticipatory hyperventilation causing hypocapnia.

According to the results of Boutilier et al. (2001), harbour porpoises and grey seals have also been found to commence the next dive at peak RER or close to it. Thus, the high RER in mammals may also be due to pre-dive preparatory hyperventilation rather than due to complete restocking of the oxygen stores while the carbon dioxide stores are still being adjusted, as Boutilier et al. (2001) suggest. Thus seal *b* in the present study, which was still reloading its oxygen stores at the time that it terminated its surface period, may still have a high RER towards the end of the surface period because of the high levels of carbon dioxide output associated with pre-dive preparation. Indeed, the end-tidal PO_2 data presented by Reed et al. (1994) for two grey seals during the entirety of their surface durations supports the theory that grey seals do not always dive after their oxygen stores are saturated. The data indicate that these seals were not remaining at the surface beyond the time that their oxygen stores were fully reloaded but rather were either diving before or at the point of complete oxygen re-saturation. Since the end-tidal values at the end of the surface period for both the grey seals in the study by Reed et al. (1994) are lower than that of a harbour porpoise reported in Boutilier et al. (2001), it is most likely that these seals were diving before complete oxygen store re-saturation.

For tufted ducks, the prominent function of the pre-dive hyperventilation to reduce body carbon dioxide levels in tufted ducks implicates carbon dioxide as important in determining dive durations but does not negate some influence of oxygen levels. The probable dual influence of the respiratory gases on dive duration is consistent with the

association between bilateral denervation of the carotid body chemoreceptors and increased dive duration (Butler and Woakes, 1982), since these receptors are sensitive to both hypoxia and hypercapnia in the blood (Butler and Stephenson, 1988).

The effects of respiratory gases on dive durations

Observations of tufted ducks diving in the wild suggest that oxygen is not a limiting factor on their dive duration (Butler, 1982; 1988). Under natural conditions, tufted ducks 'prefer' to dive to a depth of 1-3 m (Laughlin, 1972/73) and for a duration of around 18 s according to both Magnusdottir and Einarsson (1990) and L. Halsey (unpublished data). However, using measured values for the usable oxygen stores of this species (41.5 ml STPD kg^{-1}; Keijer and Butler, 1982), Woakes and Butler (1983) calculated that they could remain submerged for up to 51 s without needing to employ anaerobic metabolism if $\dot{V}_{O_2}$ was 0.57 ± 0.05 ml s^{-1} (although this may be an overestimate since the former study did not take into account that tufted ducks exhale upon submergence; Butler and Woakes, 1979). In the present study, the birds were estimated to take up about 8.0 ml into their oxygen stores in between dives (Fig. III-3). Assuming that $\dot{V}_{O_2}$ during a dive equals 0.57 ml s^{-1} (Woakes and Butler, 1983) and also assuming that on average across a diving bout these birds replace the oxygen used during each dive while at the surface, this volume of oxygen reload would predict an average dive duration of 14.1 s. This is similar to the observed mean dive duration of 13.3 s, confirming that the ducks in the present study were able to dive completely aerobically for considerably longer than they actually did. They are not consuming all of their oxygen supplies during each dive as is assumed by optimal foraging models, suggesting that body oxygen stores do not normally determine dive duration in this species.

Feeding duration, usually correlated with dive duration in tufted ducks, is probably influenced by factors other than respiratory gas levels such as food density (Halsey et al., 2003), particle selection time (Draulans, 1982), rate of food ingestion (Stephenson

et al., 1986) and predation risk (Heithaus and Frid, 2003). Furthermore, Fowler (1954) found that the end-point of asphyxia tolerance in human divers is partly attributed to increased tonus of the thoracic wall, although whether this factor plays an important role in adapted divers such as tufted ducks is unknown. Thus, the balance of various influences on ventilation during a dive is likely to be very varied (Butler, 1982). Tufted ducks simply may not dive for longer than they have to in order to forage sufficiently well, since diving for periods close to their aerobic limit may well, like any intense exercise, be physically uncomfortable.

In more unusual diving conditions, however, the relative influence of oxygen on dive duration may change. For example, Dewar (1924) recorded observations of wild tufted ducks living on a deep body of water diving for up to 40 s while Stephenson et al. (1986) trained tufted ducks to undertake voluntary feeding dives lasting over 40 s. Butler and Stephenson (1988) observed ducks diving from a hypoxic gas composition and in these cases, the ducks may have consumed nearly all of their usable oxygen stores making hypoxia the dominant stimulus for terminating the dive.

Implications for diving optimality models

Despite the proposition in the present study that oxygen often has relatively little influence on dive duration, popular models of optimal foraging in air breathing divers (e.g. Kramer, 1988; Houston and Carbone, 1992; Thompson et al., 1993; Mori, 1988), all of which assume that oxygen is the prominent factor determining the time budgeting of the dive cycle, have successfully predicted qualitative changes in diving behaviour. Furthermore, some predictive validity for the optimal breathing model (Kramer, 1988) has now been demonstrated quantitatively (Halsey et al., 2003). The inference here is that oxygen is an important influence during the dive cycle of diving birds. When considering the small proportion of a duck's oxygen stores that are used during most dives, indicating that it is not often very influential in determining dive duration, oxygen must be influential in determining surface duration to explain the

predictive validity often recorded for optimal diving models. This supposition is supported by the data on respiratory gaseous exchange in the present study. Carbon dioxide levels may well be involved in the termination of surface periods of birds as well, but if adjustments in body carbon dioxide levels often mirror adjustments in body oxygen levels, as illustrated in Fig. III-3, then it does not matter which of these respiratory gases diving optimality models focus on during inter-dive surface periods in birds. Such models should include consideration of the influences of both respiratory gases during the diving portion of the dive cycle and include the combined effect of these respiratory gases, RER, during the surface portion. Feeding parameters such as food density and particle selection time should also be considered to improve the predictive validity of these models.

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Table III-1. Statistical comparison between oxygen uptake and carbon dioxide output curves after dives of six tufted ducks ($n = 1426$) split into three dive duration bins after 5 and 10 s at the surface

Dive duration bins (s)	Volume of O_2 uptake after 5 s (ml)	Volume of CO_2 output after 5 s (ml)	Volume of O_2 uptake after 10 s (ml)	Volume of CO_2 output after 10 s (ml)
5-9.75	6.08 ± 0.40	4.59 ± 0.19**	9.23 ± 0.43	7.21 ± 0.25**
10-14.75	7.80 ± 0.29	5.64 ± 0.20***	11.98 ± 0.56	9.35 ± 0.37***
15-19.75	10.06 ± 0.38	7.29 ± 0.37***	14.71 ± 0.84	11.79 ± 0.56**

Values given are means $\pm$ SE.

Significant differences between volume of oxygen uptake and carbon dioxide output at 5 s and 10 s post-dive are represented by an asterisk: **$P < 0.01$; ***$P < 0.001$.

Table III-2. Statistical comparisons between oxygen uptake curves and between carbon dioxide output curves after dives of six tufted ducks ($n = 1426$) in three surface duration bins

Surface duration bins (s)	Volume of O_2 uptake after 5 s (ml)	Volume of CO_2 output after 5s (ml)	Volume of O_2 uptake after 10 s (ml)	Volume of CO_2 output after 10 s (ml)
< 10	9.12 ± 0.55	$6.78 \pm 0.32^{\dagger}$	$14.88 \pm 0.73^{\dagger}$	$12.41 \pm 0.80^{\dagger}$
10-19.75	8.27 ± 0.59	6.05 ± 0.32	12.98 ± 0.88	$10.41 \pm 0.51*$
20-29.75	$*7.77 \pm 0.61$	$*5.68 \pm 0.25$	$*12.04 \pm 0.81$	$*9.43 \pm 0.41$

Values given are means $\pm$ SE.

Significant differences are represented by an asterisk: $^{\dagger} P < 0.1$; $*P < 0.05$.

The asterisk to the right of the value represents a significant difference between the 10-19.75 s surface duration bin and the 20-29.75 s surface duration bin.

The asterisks to the left of values represent a significant difference between the 20-29.75 s surface duration bin and the < 10 s surface duration bin.

The cross represents $P < 0.1$ between the < 10 s surface duration bin and the 10-19.75 s surface duration bin.

Table III-3. Comparing time budget and gas exchange data to those obtained from previous studies on tufted ducks diving on the same tank

	Tray depth (m)	Dive duration (s)	Surface duration (s)	Respiratory quotient
Halsey et al. (2003)*	1.1	11.2 ± 0.7	12.3 ± 1.4	-
The Present Study	1.3	13.3 ± 0.8	13.1 ± 1.4	0.81 ± 0.01
Woakes and Butler (1983)	1.7	14.4 ± 1.9	16.1 ± 2.5	0.84 ± 0.03

Values given are means ± SE, where available.

* Means for the control condition.
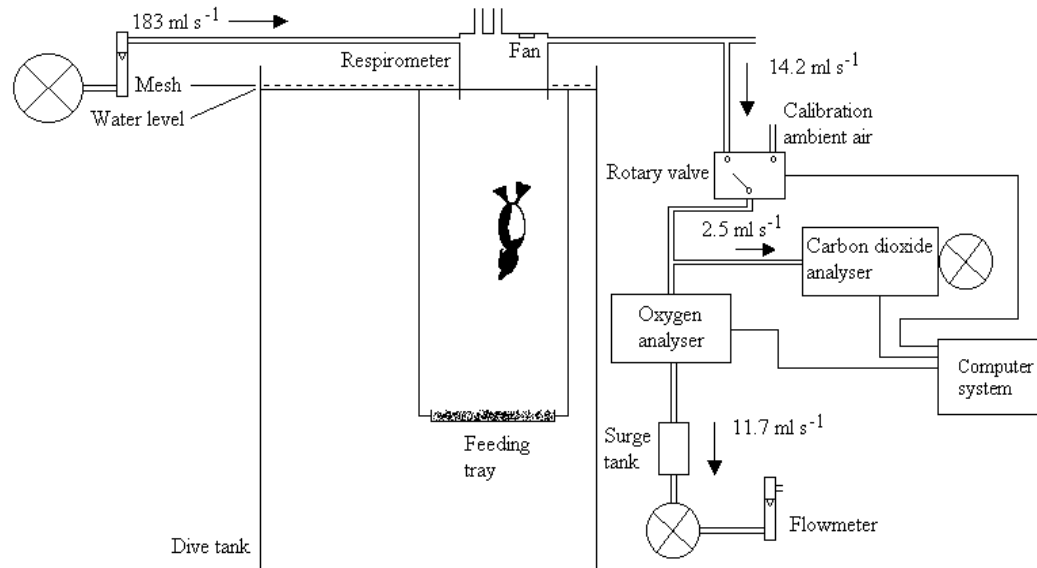
Figure III-1

Diagram of the experimental apparatus with a tufted duck diving from the respirometer. For further details see Materials and Methods.
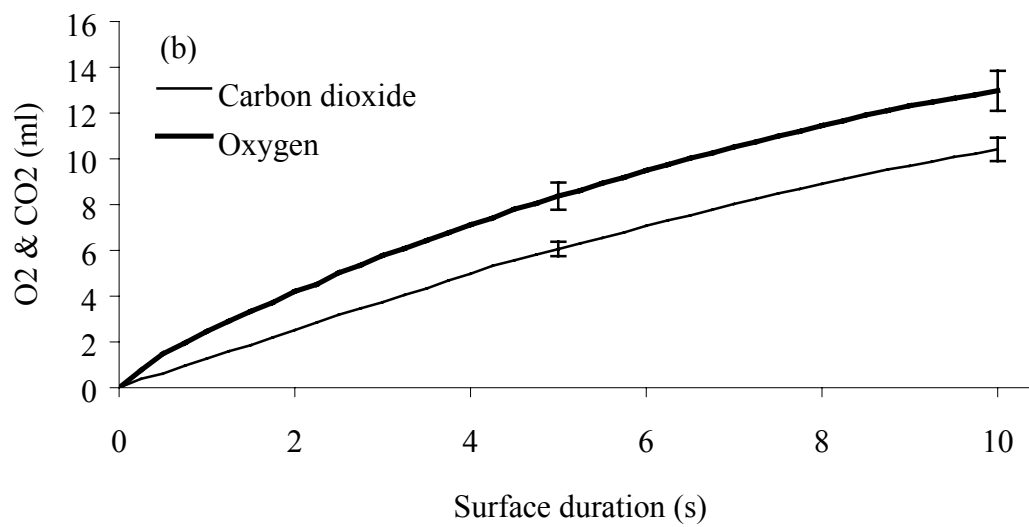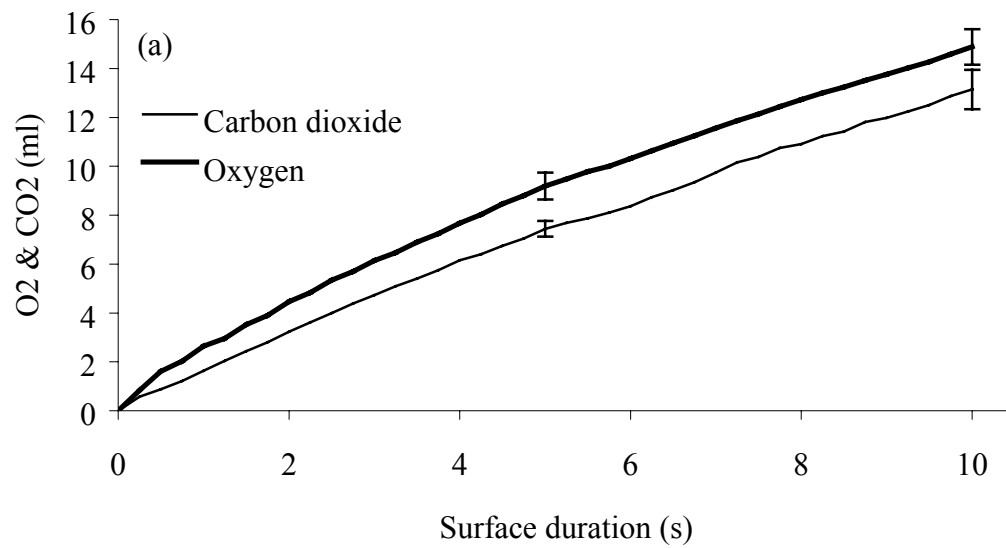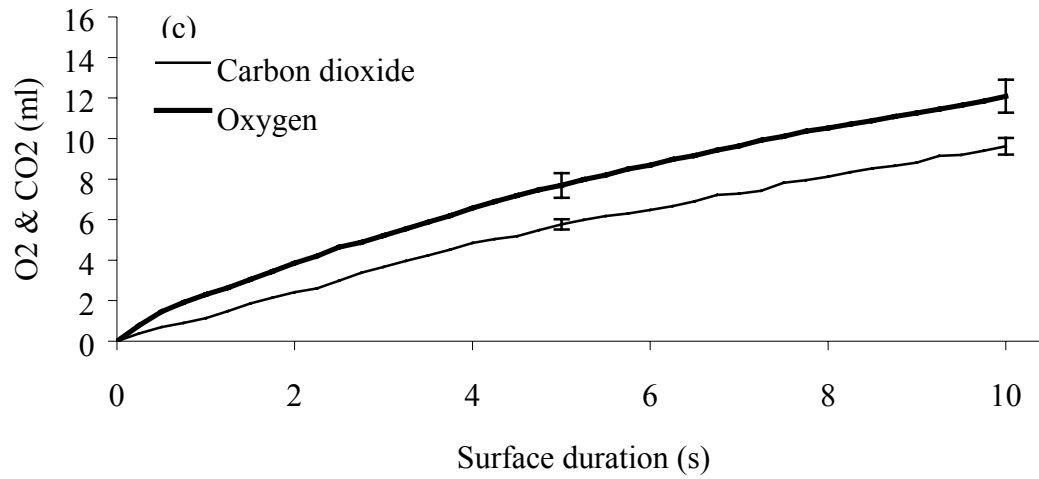
Figure III-2

Mean (± SE.) oxygen uptake and carbon dioxide output curves for the first 10 s surface duration of six tufted ducks. Each graph represents a surface duration bin: a) < 10 s, $n = 350$ b) 10-19.75 s, $n = 583$ c) 20-29.75 s, $n = 107$.
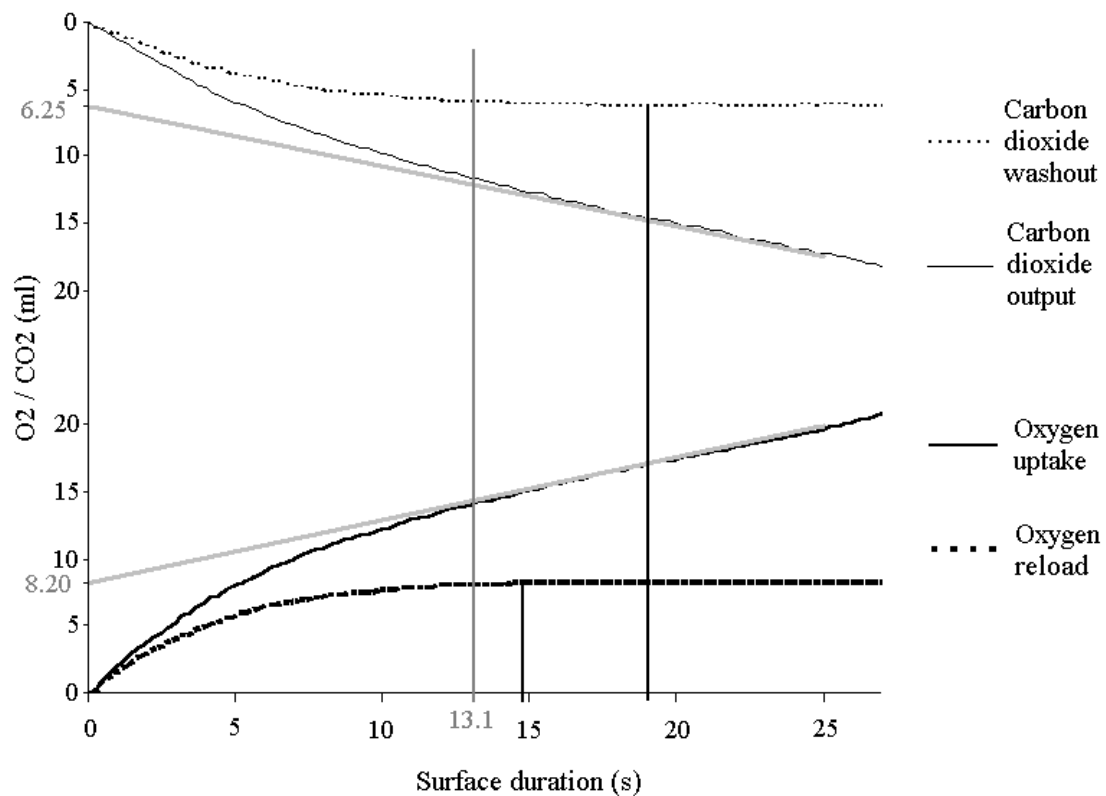
Figure III-3

Cumulative oxygen uptake and carbon dioxide output curves (the latter is inverted) for the first 27 s post-dive of six tufted ducks ($n = 1426$). The oxygen reload curve was calculated by removing an estimate of the rate of post-dive oxygen consumption from the oxygen uptake curve while the carbon dioxide washout curve was calculated by removing an estimate of the rate of post-dive carbon dioxide production from the carbon dioxide output curve (light grey tangent lines; see text). The vertical dark grey line indicates mean surface duration and the vertical black lines indicate the surface duration (to the nearest 0.25 s) for carbon dioxide washout (6.25 ml) and oxygen reload (8.20 ml) to occur (see text).
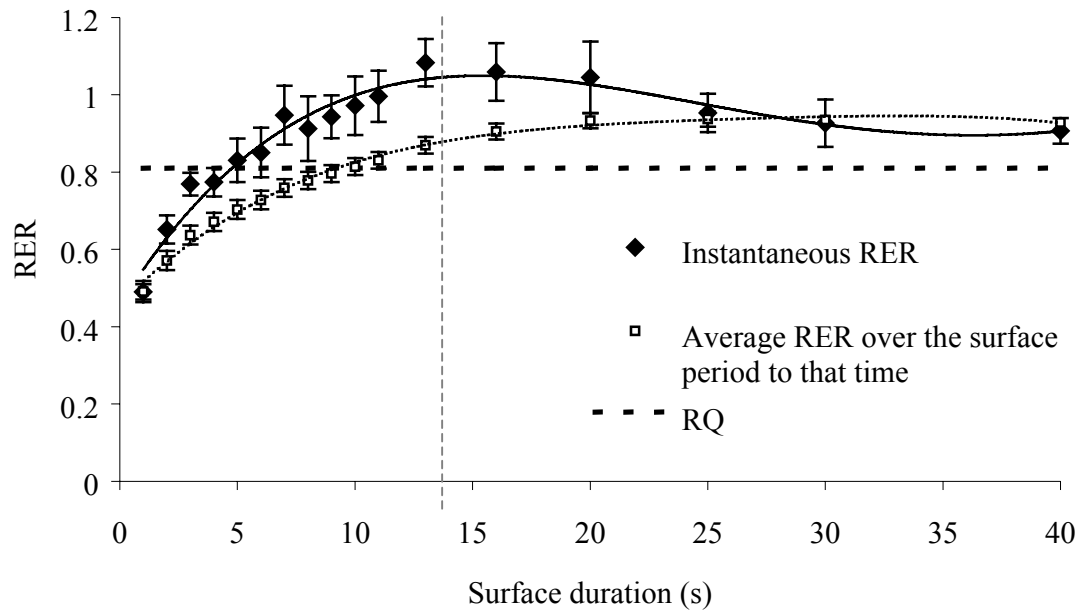
Figure III-4

Mean (± SE.) respiratory gas exchange ratio (RER) during the first 40 s post-dive by six tufted ducks ($n = 1426$). The vertical dashed grey line shows the mean surface duration (13.1 s). The solid diamonds show RER during the time at the surface since the last data point (instantaneous RER). RER increases to a maximum of 1.08 after 13 s and then gradually decreases over time until it equals RQ (indicated by the horizontal dashed line; 0.81 ± 0.01) after several minutes. The points are fitted with a 4th order polynomial curve to highlight the trend. The open squares show the average RER from the start of the surface period to the time of that data point, calculated from the instantaneous RER values. Again, a 4th order polynomial curve is fitted for clarity.
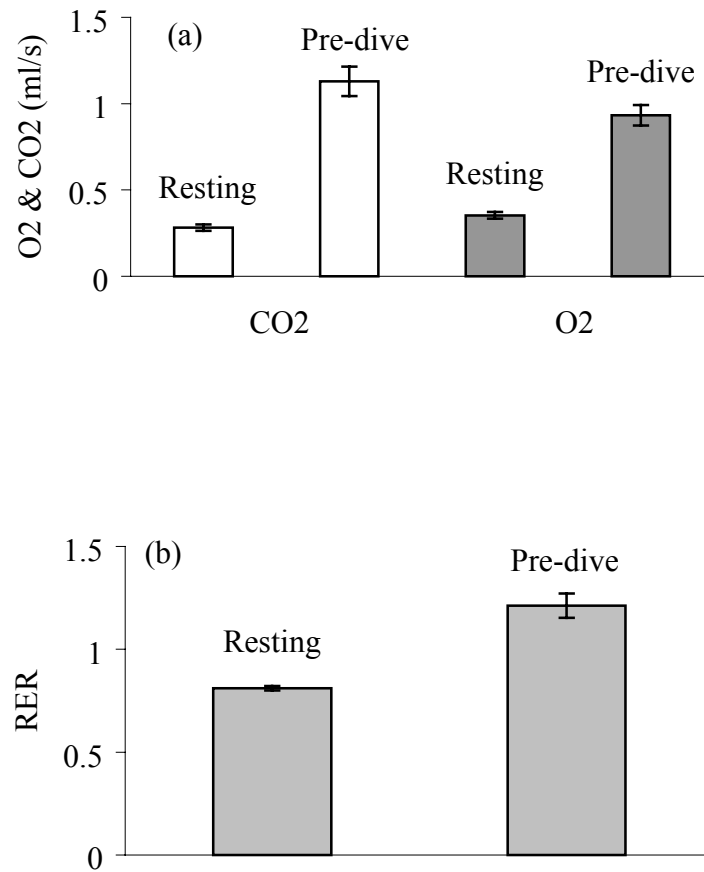
Figure III-5

(a) Mean values (± SE.) for $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ in six tufted ducks ($n = 18$) at rest on the

water and during the pre-dive hyperventilation prior to a diving bout.

(b) Mean values (± SE.) for RER at rest on the water and during the pre-dive

hyperventilation prior to a diving bout.
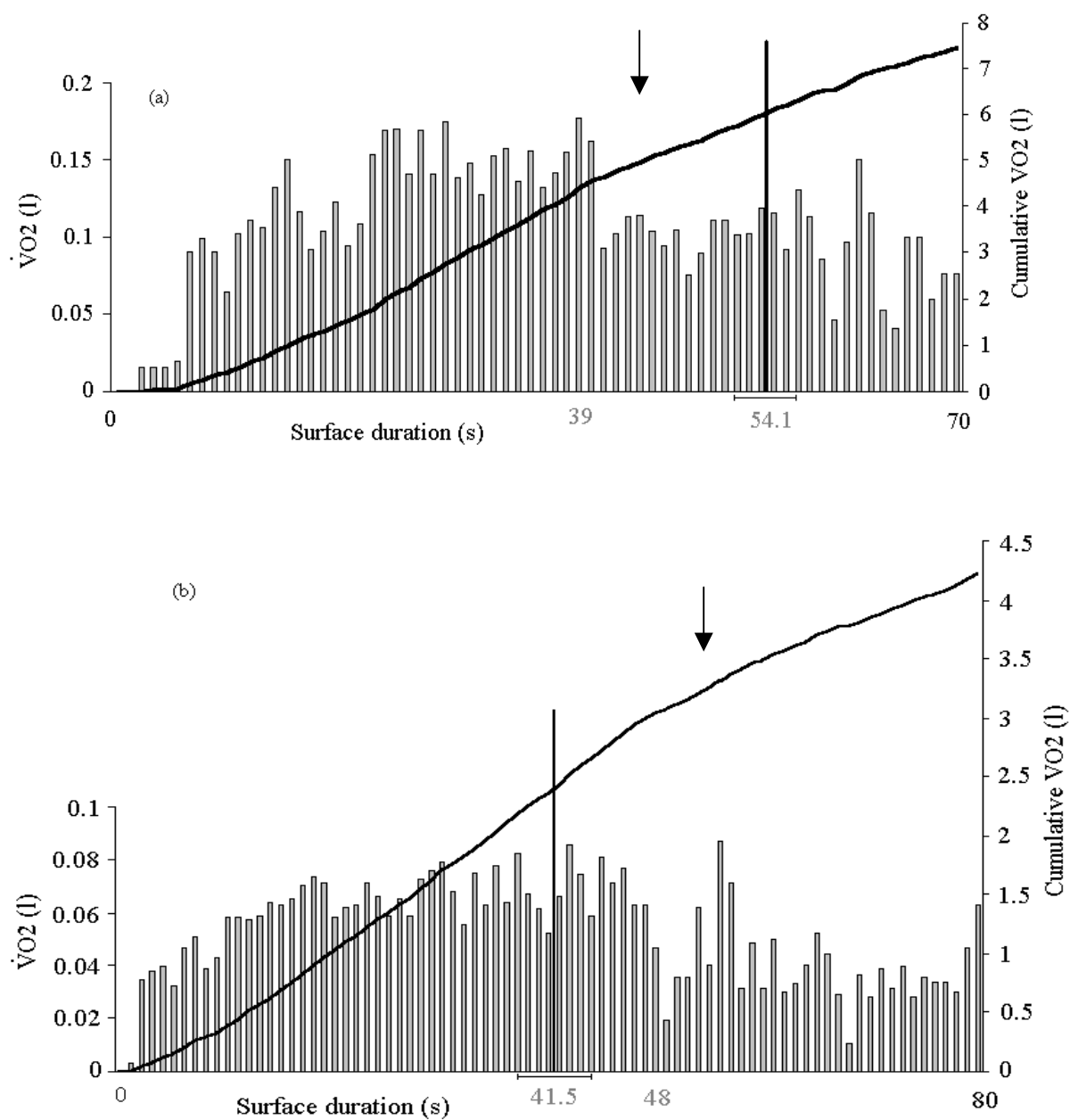
Figure III-6

$\dot{V}$ O_2 s^{-1} (bars) and cumulative oxygen uptake for the first 70 s post-dive of two grey seals (a, ♂, 250 kg, *n* = 34; b, ♀, 150 kg, *n* = 53). The arrows indicate the surface duration after which each curve becomes a constant gradient, representing saturation of the oxygen stores. The black vertical lines indicate mean surface times and below is the SE of the mean. The gradual reestablishment of peripheral circulation post-dive in grey seals (Reed et al., 1994), as well as possibly the completion of lung inflation during the initial ventilations, may cause re-saturation of the oxygen stores to accelerate over time. This would explain the exponential shape of the oxygen uptake curve up to 39 s for seal *a* and up to 48 s for seal *b*. In contrast, cumulative oxygen uptake over time in tufted ducks produces an exponential decay curve (Fig. III-2 and Fig. III-3), which is explained by decreases in respiratory frequency (Parkes et al., 2002) and the decline of the partial pressure differentials in the oxygen stores over time (Kramer, 1988). Respiratory frequency and tidal volume do not decrease over time in grey seals (Reed et al., 1994) and while the partial pressure differentials will decrease, this may not be sufficient to mask the effects of the reestablishment of circulation to the periphery of the seal and complete lung inflation.

IV. The Effects of Inspiring Hypoxic or Hypercapnic gas mixes on the Diving Behaviour and Respiration of Tufted Ducks

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Studies investigating the effects of hypoxia or hypercapnia on diving behaviour in ducks have assumed that changes in dive time budgeting are associated with changes in respiratory gas exchange between dives, which affect the readjustment of the respiratory gas stores. However, direct measurements have not been made and the effect of decreasing inspired oxygen or increasing inspired carbon dioxide on the oxygen and carbon dioxide stores in ducks is unclear. The present study quantifies the effects of exposure to hypoxia and hypercapnia on rates of respiratory gas exchange between dives in tufted ducks, *Aythya fuligula*, to investigate to what extent changes in diving behaviour can be explained by changes in rates of oxygen uptake and carbon dioxide output. The birds were trained to surface into a respirometer box containing either normal air, a hypoxic gas mix or a hypercapnic gas mix, after each dive to a feeding tray. This allowed rates of oxygen uptake and carbon dioxide output at the surface to be measured. As found in previous studies, the ducks adjusted their diving time budgets by decreasing dive duration (t_d) and increasing surface duration (t_s) when diving from a hypoxic or hypercapnic gas mix. Regression analyses of respiratory gas exchange against t_d produced estimates of the rate of oxygen consumed during the surface periods between dives and the rate of oxygen consumed during the foraging periods of dives ($\dot{V}_{O_2f}$). Oxygen uptake after a mean t_d was similar in the hypercapnic conditions and the hypercapnia control condition, indicating that the ability of the ducks to take up oxygen is not affected by hypercapnia. Thus, the changes in t_d and t_s in these conditions confirm an influence of carbon dioxide on diving behaviour. Increased hyperventilation in hypoxia (11 %

oxygen) helped reduce the decrease in mean oxygen uptake between dives compared to normoxia to only 2 ml of oxygen after a dive of mean duration. Furthermore, $\dot{V}_{O2f}$ decreased nearly three-fold in the hypoxia 11 % condition and therefore the decrease in t_d after inspiring a hypoxic gas mix is unlikely to be due to a decrease in the level that the oxygen stores reached during the subsequent dive. Estimates of respiratory exchange ratios during foraging periods were above 1 in the hypoxia 11 % condition, suggesting the use of anaerobic metabolic pathways during some dives from hypoxia. This may explain the adjustments in time budget variables in response to inspiring low oxygen concentrations.

The text and figures of this chapter are slightly altered from the published version. LGH developed the methodology, conducted the data collection, analysed the data and wrote the manuscript. PJB discussed the data analyses and conclusions and aided in the writing of the manuscript. AJW helped with methodological problems and discussed the data analyses and conclusions.

Introduction

The majority of aquatic animals are estimated to routinely consume only a proportion of the usable oxygen in their stores during dives (e.g. Fedak et al. 1988; Thompson et al. 1991; Croxall et al. 1993; Webb et al. 1998; Le Boeuf et al. 2000; Thompson and Fedak, 2001). While this suggests oxygen store levels might not be a very important influence on dive duration (t_d) in these species, Thompson and Fedak (2001) point out that most models of optimal foraging by diving animals have investigated behavioural strategies maximising the proportion of time submerged while focusing on the management of the oxygen stores (e.g. Kramer 1988; Houston and Carbone 1992; Walton et al. 1998).

For example, measurements of the capacity of tufted ducks (*Aythya fuligula*) to store oxygen (Keijer and Butler, 1982) and the mean rate that they consume oxygen during dives at a mean duration of 15 s ($\dot{V}_{O_2d}$; Woakes and Butler, 1983) suggest that this species could remain submerged and completely aerobic for around 50 s (calculated aerobic dive limit, cADL). However, they usually dive for durations considerably shorter than 50 s in natural conditions (e.g. Dewar, 1924; Magnusdottir and Einarsson, 1990; Halsey, unpublished data). Nevertheless, studies on diving birds where the partial pressure of oxygen in inspired air (P_IO_2) has been reduced, report reductions in t_d. Butler and Stephenson (1988) found that t_d in tufted ducks was reduced when inspiring hypoxic gas (9-11 % O_2) between dives. Furilla and Jones (1986) also noted significant decreases in t_d in redhead ducks (*Aythya americana*) exposed to increasingly hypoxic gases. This implicates oxygen as being influential in determining t_d in diving birds in hypoxic conditions.

Perhaps the most parsimonious hypothesis for these findings is that the birds were only able to take up a limited amount of oxygen in the hypoxic environment and thus, in contrast to diving in natural conditions, were fully depleting their usable oxygen stores during the dives, thus triggering their termination. However, if oxygen store levels do at least have some influence in determining t_d under natural conditions, since

the stores are only ever partially depleted in normoxia, this influence must be imparted through only a partial reduction in the oxygen stores. This presumably stimulates the body's oxygen sensors such as the carotid bodies (Butler and Woakes, 1982; Butler and Stephenson, 1988), triggering surfacing behaviour to enable ventilation of the lungs. Thus, a critical level of oxygen in the body stores could initiate the termination of a dive. If this second hypothesis is true, then under hypoxic conditions, the ducks may have taken up a reduced amount of oxygen between each dive so that the oxygen stores were depleted to the critical level more quickly than under normoxic conditions, rather than completely exhausted. This hypothesis would explain the large discrepancy between the cADL and the observed t_d of naturally diving tufted ducks and indeed of many other diving animals.

However, other factors seem just as likely as oxygen stores to be influencing the decision by diving birds and mammals to terminate a dive and surface (Butler, 1982). There are a growing number of studies indicating that carbon dioxide levels have a prominent effect on diving behaviour (Ridgway et al. 1969; Påsche 1976; Gallivan 1980; Butler, 1982; Jones et al. 1982; Butler and Stephenson, 1988; Ollenberger and West 1998; Ferrigno and Lundgren 1999; Boutilier et al. 2001; Halsey et al. 2003a). Evidence that pre-dive hyperventilation serves primarily to reduce the levels of carbon dioxide in the body of tufted ducks (Halsey et al., 2003a) and that t_d decreases in tufted ducks exposed to a hypercapnic gas mix (5-6 % CO_2; Butler and Stephenson, 1988) certainly suggest that carbon dioxide has an impact on t_d in this species. Carbon dioxide affects the respiratory drive in birds by the stimulation of several different receptors. The carotid body chemoreceptors are known to detect changes in carbon dioxide partial pressures in the arterial blood (P_aCO_2; Milsom et al., 1981), however they do not appear to instigate the end of a dive in response to an increase in P_aCO_2 (Butler and Stephenson, 1988). Other receptors that may have this role include the central chemoreceptors, and also the intrapulmonary chemoreceptors (Butler and Stephenson, 1988), which are stimulated by decreases in PCO_2 in the parabronchi (Powell, 2000).

The surface duration between dives in tufted ducks, according to Halsey et al. (2003a), is equally likely to be influenced by both oxygen and carbon dioxide levels in the body, since both stores are fully adjusted after the same surface duration (t_s). This conclusion is supported by the study of Butler and Stephenson (1988) who recorded a significant increase in the proportion of the dive cycle spent at the surface in both hypoxic and hypercapnic conditions and by Enstipp et al. (2001) who reported a significant increase in t_s in double-crested cormorants (*Phalacrocorax auritus*) exposed to hypoxic or hypercapnic gas compositions.

The results presented by Butler and Stephenson (1988) and Enstipp et al. (2001) suggest an importance for carbon dioxide levels throughout the dive cycle of aquatic birds. These studies, coupled with the study by Furilla and Jones (1986), also suggest an important role for oxygen levels in determining t_s, and an influence of oxygen levels on t_d, at least in hypoxic conditions. However, none of these three studies measured respiratory gas exchange at the surface in the subject birds, rather they assumed that hypoxic and hypercapnic conditions affect the ability of diving animals to take up oxygen and remove carbon dioxide, which therefore affects t_d and t_s. Previous studies by Parkes et al. (2002) and Halsey et al. (2003a, b) have quantified the cumulative oxygen uptake and reloading curves, and the cumulative carbon dioxide output and washout curves, during the surface period between dives in tufted ducks diving in normoxic environments. These studies have demonstrated associations between the shapes of the curves, i.e. the rates of respiratory gas exchange, and the diving behaviour of tufted ducks.

However, it is not clear how large the changes in ambient oxygen and carbon dioxide have to be to affect respiratory gas exchange in diving birds. For example, it has been shown that hyperventilation in a hypoxic environment, through increases in tidal volume and respiratory frequency (Jones and Purves, 1970), reduces the difference between P_IO_2 and P_aO_2 (Jones and Holeton, 1972), thus limiting the decrease in P_aO_2.

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Also, due to the increased effectiveness of oxygen uptake in the avian lung compared to that in the mammalian lung (Piiper and Scheid, 1975), which may be responsible for the increased hypoxia tolerance of birds (Dejours, 1982; but see Shams and Scheid, 1989), the parabronchial lung may have enabled birds to evolve a low blood oxygen affinity to maximise oxygen delivery within the tissues (Powell, 2000). Indeed, the avian respiratory system can considerably alter the amount of oxygen extracted from the air and used in the tissues under various environmental conditions (e.g. Tucker, 1968; Kiley et al., 1985). Furthermore, Rostorfer and Rigdon (1947) reported that duck blood is particularly effective in the transport of oxygen under conditions of hypoxia in comparison to that of many other bird species. This is due to a greater oxygen capacity of the blood and a higher percentage of saturation of the blood with oxygen at low oxygen tensions. Finally, intraspecific variability in diving ability between diving ducks has been reported (Stephenson et al., 1989) due to differences in blood volume and physiology affecting oxygen storage capacity. Consequently, some individual ducks are likely to be better predisposed to cope with diving from hypoxia than others.

There have been fewer studies on the effects of hypercapnia on carbon dioxide removal in birds. In response to increases in P_aCO_2, Jones and Purves (1970) reported an increase in respiratory frequency and tidal volume in ducks, however this did not result in a significant decrease in P_aCO_2. Although none of these studies measured P_aO_2 and P_aCO_2 in birds during diving bouts, they suggest that increases in P_ICO_2 are more likely to affect the ability of a diving bird to reduce the carbon dioxide stores than vice versa for P_IO_2.

The present study uses similar methodology to that used by Parkes et al. (2002) and Halsey et al. (2003a, b) to quantify the effects of exposure to hypoxia and hypercapnia on rates of respiratory gas exchange between dives in tufted ducks. The primary aim of the study is to investigate the extent to which the changes in diving behaviour of tufted ducks in hypoxic and hypercapnic environments can be explained by changes

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in rates and volumes of oxygen uptake and carbon dioxide output. This will help to elucidate the relative influences of oxygen and carbon dioxide on the budgeting of diving tufted ducks, thus extending the study by Halsey et al. (2003a). Associations between diving ability from hypoxia or hypercapnia and blood haematological indices were also investigated. The main questions this study addresses are: Does inspiration of hypoxic or hypercapnic gas mixes affect respiratory gas exchange between dives? Do the oxygen stores reach a critical level, or are they even exhausted, during dives after inspiration of a hypoxic gas mixture?

Methods

Six adult tufted ducks (0.57-0.75 kg) were used in this experiment. They were raised from eggs collected from Kingsbury Water Park, Sutton Coldfield, U.K.. When fully grown, they were housed in either indoor or outdoor holding facilities at The University of Birmingham. The experiments were performed on an indoor dive tank (1.0 x 1.6 x 1.7 m deep) incorporating an adjacent dry area (0.6 m x 0.8 m). Food consisted of corn, pellets and some live foods such as maggots and earthworms. This variety ensured that the masses of the ducks remained stable during the experimental period. On the day prior to the experiment they were fed a reduced amount to encourage diving behaviour. Water temperature ranged between 12 and 18 ° C for the hypoxic conditions and hypoxia control condition, and between 12 and 14.5 ° C for the hypercapnic conditions and hypercapnia control condition. Air temperature in the laboratory ranged between 15 and 21 ° C for all experiments. Humidity within the box was measured using a probe (Vaisala, Helsinki) and ranged between 90 and 93 % across all experimental sessions, the range being generally less than this within each session.

For the control conditions in the present study, the experimental procedure was very similar to that described in *Chapter III*. There were some additions and adjustments for the hypoxic conditions, and for the hypercapnic conditions (Fig. IV-1). To obtain

data on respiratory gas exchange after each dive to the feeding tray (1.7 m depth), the subject bird was confined to surfacing into a respirometer box (35 x 25 x 25 cm) after each dive. In the control and hypoxic conditions, differences between the concentration of oxygen and carbon dioxide in the gas entering and leaving the respirometer box were measured using fast response gas analysers such that oxygen uptake and carbon dioxide output of the duck could be calculated (Fedak et al., 1981). These gases were passed as separate samples through the oxygen gas analyser (Ametek, model S3A-1/N.22) and the carbon dioxide gas analyser (Morgan Capnograph, P.K. Morgan Ltd.). Due to some background noise in the voltage output signal from the carbon dioxide analyser, the signal was smoothed by the computer program (custom made in LabVIEW, National Instruments) with a running, weighted three-point average. In the hypercapnic conditions, changes in carbon dioxide concentrations could not be accurately recorded due to carbon dioxide diffusion into the water and so only oxygen concentrations were recorded with the fast response oxygen analyser. Carbon dioxide concentrations were recorded with a slower responding carbon dioxide analyser (ADC Ltd, model SS-100) to check that the correct gas mix was entering the respirometer box (e.g. 2.5 %). Because this analyser measured carbon dioxide concentration in the range of 0-1 %, the sample was drawn from the box by a pump attached to the carbon dioxide analyser and passed into a precision gas mixing pump (Wösthoff Pumps, Bochum) where it was mixed with nitrogen to dilute it 10-fold. The resulting gas mix was then passed through the carbon dioxide analyser.

11.7 ml s^{-1} were drawn as the sample gas to the oxygen analyser in all conditions and 2.5 ml s^{-1} were drawn to the fast response carbon dioxide analyser in the control and hypoxic conditions while 10 ml s^{-1} were drawn to the slow response carbon dioxide analyser in the hypercapnic conditions. The response times of the fast response oxygen and carbon dioxide analysers were less than 0.2 s and the lag time of the system due to the volume of the respirometer box and the tubing was 3.0 s. The residual time constant of the system after deconvolution (the conversion of oxygen

concentration in the respirometer box to rate of oxygen uptake, $\dot{V}_{O_2}$; see later) was 0.4 s and was determined by nitrogen injections at various points in the respirometer box.

In the control condition, air was pushed through the respirometer at 267 ml s^{-1} so that the concentration of carbon dioxide within the respirometer was always kept below 0.5 % during experimental sessions. For the hypoxic conditions, the oxygen concentration of the respirometer box was reduced by pushing a mixed flow of air and nitrogen through it. This was achieved by continuously flowing air and nitrogen into a mixing chamber (27 x 40 x 22 cm) placed upon oil to form an airtight seal. The gases were mixed using fans within the chamber to produce a gas mix of either approximately 15 % oxygen (hypoxia 15 % condition) or approximately 11 % oxygen (hypoxia 11 % condition) and overflowed to the respirometer box via impermeable tubing at 233 ml s^{-1}. Again, this maintained carbon dioxide concentration within the respirometer below 0.5 %. For the hypercapnic conditions (approximately 2.5 % CO_2 and 3-4.5 % CO_2; hereafter termed hypercapnia 2.5 % condition and hypercapnia max % condition, respectively; see later), air and carbon dioxide were flowed into the mixing chamber and exited to the respirometer box at 183 ml s^{-1}. The carbon dioxide concentration in the respirometer box was always below 5.1 %. Oxygen concentrations were approximately 20.0 % in the hypercapnia 2.5 % condition and 19.0 % in the hypercapnia max % condition. To maintain consistent concentrations of oxygen, carbon dioxide and nitrogen exiting the mixing chamber, the flows of air and nitrogen or carbon dioxide into the mixing chamber were passed through flowmeters incorporating units that maintained the gas flow at a constant rate (Flostat type MNBB21, Platon Flow Measurement).

The experimental sessions involving the hypercapnic conditions were performed after the hypoxic conditions. The ducks initially appeared more reluctant to dive during the hypercapnic conditions, including the hypercapnia control condition, often not diving at all. This could have been due to a number of possible factors including noise

disturbances near the dive tank around the time of these experiments. They were encouraged to dive by adding mealworms (*Tenebrio molitor*) to the feeding tray in place of maggots (*Calliphora vomitoria*), which were used in the hypoxic conditions, probably because the mealworms were novel. Since the food type available affects the foraging energetics of tufted ducks (Halsey et al., in prep.), data for a second control condition were obtained (hypercapnia control) with which to compare to the data from the hypercapnic conditions, so that food type was controlled for. Furthermore, because tufted ducks have been recorded adjusting their dive time budgeting and their buoyancy in response to foraging for different food types (Halsey et al., in prep.), nutritional tests were conducted on these two food types to ascertain differences in composition which might account for any differences in dive time budgeting or respiratory gas exchange. While all ducks repeatedly dived in the hypercapnia 2.5 % condition, the maximum carbon dioxide concentration of gas from which they would dive varied considerably. In order to gain data for another hypercapnic condition and maintain a high number of experimental birds, N, the carbon dioxide concentration of the gas mix used for the second hypercapnic condition was adjusted for individual birds and was designated the maximum carbon dioxide concentration that did not completely inhibit diving behaviour (hypercapnia max % condition).

Because two of the ducks had relatively high mean t_s in the control conditions, there was a concern that they were not behaving naturally in the control condition and therefore changes in t_s in response to exposure to hypoxic or hypercapnic gas mixes would not be apparent or would be a combined effect of the respirometer and the gas mix. Consequently, diving time budget data were recorded for all the ducks in a further condition where they were able to dive to the feeding tray and could resurface anywhere because the netting and respirometer had been removed from the surface (i.e. they were diving 'freely'). This allowed any effects on diving behaviour by the requirement to surface to and dive from the respirometer to be tested for through the comparison of time budget data.

Resting rates of $\dot{V}_{O_2}$ on water were determined for each condition using the same procedure described in Halsey et al. (2003a). Due to the importance of controlling for water temperature when measuring $\dot{V}_{O_2}$ (Bevan and Butler, 1992b), measurements of resting metabolic rate were recorded only when the water temperature was between 12 ° C and 13.5 ° C.

Time budget data for each foraging dive were also recorded. Along with dive t_d and t_s, foraging duration during a dive (t_f), descent duration to the feeding tray (t_{desc}) and ascent duration from the feeding tray (t_{asc}) were recorded. The proportion of a dive spent foraging ($t_{\%f}$) and the dive:pause ratio ($d{:}p$) were calculated from these measurements.

Respiratory data analysis

Oxygen concentration and carbon dioxide concentration were converted into volumes of oxygen uptake (V_{O_2}) and volume of carbon dioxide output (V_{CO_2}) respectively, at 0.5 s intervals, using a formula similar to that described in Woakes and Butler (1983). This allows measurement of fast changes in oxygen uptake and carbon dioxide output from an open circuit respirometer system:

$$V_{O_2} = (FO_{2(t_2)} - FO_{2(t_1)})V + \frac{(FO_{2(t_1)} + FO_{2(t_2)} - 2FO_{2(amb)})}{2}(t_2 - t_1)\dot{Q}$$

where,

V_{O_2} = Total oxygen consumption between times t_1 and t_2 (ml),

$FO_{2(t_1)}$, $FO_{2(t_2)}$ = fractional concentrations of oxygen at times t_1, t_2 leaving the chamber,

V = respirometer volume (ml),

$FO_{2(amb)}$ = fractional concentration of (ambient) oxygen entering the respirometer,

t_1, t_2 = start and finish of a period of time where variation of oxygen concentration in the respirometer is recorded

$\dot{Q}$ = flow rate out of the respirometer (ml s^{-1})

To calculate V_{CO_2}, carbon dioxide values are interchanged for the equivalent oxygen values. All values were corrected to STPD.

Analysis of a dive was performed if the foraging tray was visited by the duck. All t_s greater than 30 s were considered to be surface periods at the end of a diving bout and were eliminated from the analyses (Halsey et al., 2003a). Normality tests on the data were performed using either the Kolmogorov-Smirnov test or the Ryan-Joiner test, depending on the *n* value (total number of dives) of the data. Mean values were obtained for each bird and since these values were assumed to be a normally distributed, representative sample of the population, they were used to obtain a final mean. Final mean values are given ± SE, usually for six ducks but sometimes for less than six. A significant difference between final means was tested for with paired *t* tests. Where number of dives per diving bout were compared between conditions, consecutive dives of 3 or less dives were discarded as not sufficient to constitute true diving bouts. Only the data from three ducks were used to compare dives per diving bout between the hypoxic conditions and the hypercapnic conditions, because the data obtained from the other ducks regarding this particular measurement were sporadic, particularly in the more extreme conditions i.e. hypoxia 11 % and hypercapnia max % and were likely to incorporate strong confounds.

To compare the effects of the conditions on $\dot{V}_{O_2}$ and rate of carbon dioxide output ($\dot{V}_{CO_2}$), it was necessary to control for t_d. In each condition, for each duck, mean values of V_{O_2} and V_{CO_2} after 5, 10 and 20 s at the surface were calculated for a range of t_d at 1 s intervals. For example, for a 10 s dive, mean values of V_{O_2} after 5, 10 and 20 s t_s after the dive were calculated for each bird. This was repeated for each value of t_d, the range of which was somewhere between 5 s and 22 s, depending upon the duck and the condition. Final means for each t_s in each condition were calculated from the means of each duck. These final means were regressed against t_d. An example of the regressions is shown in Fig. IV-2. The equation of each regression was then used to

predict V_{O_2} and V_{CO_2} after a dive of mean duration in this study (12.4 ± 0.6 s) over the relevant t_s ($V_{O2\_12.4}$ and $V_{CO2\_12.4}$).

Food nutrition analyses

Samples of maggots and mealworms were dried at 80 º C for 36 hours to ensure that all water had been removed. Weights before and after drying (wet and dry weight, respectively) done in triplicate were used to calculate water content. All samples were ground up before nutritional analyses were carried out, in duplicate, and for all measurements, values are reported as both % of wet weight and % of dry weight. The gross energy of each sample was determined by bomb calorimetry (Parr 1356 Calorimeter, Parr Instrument Company). Mineral content was determined by heating a sample at 700 º C for 2.5 h, using a method similar to AOAC method 4.1.10 (official methods of analysis, 1995). Crude fat content was determined by Soxhlet extraction with petroleum spirit for 4 h, using a method similar to AOAC method 7.056 (official methods of analysis, 1980). After evaporation of the spirit, the residue was weighed and fat content was calculated. Crude protein content was determined using the Dumas dry combustion technique (NA 2000 Nitrogen and Protein Analyzer, Fisons Instruments). The methodology employed was similar to that described in AOAC method 4.2.04 (official methods of analysis, 1995) where nitrogen from the sample is measured in a nitrometer and converted to equivalent protein.

Blood measurements

The haematocrit levels and haemoglobin concentration of the blood of each duck were measured using blood samples removed from the brachial vein into heparinized capillary tubes and 2-3 ml into heparinized sample tubes, respectively. Haematocrit was measured by spinning whole blood in a microhaematocrit centrifuge (Hawksley and Sons Ltd) for 4 min. Mean values were obtained for each animal from duplicate samples. Haemoglobin concentrations were analysed at the Haematology Unit at the Queens Medical Centre, Birmingham, using an Abbott Automated Haematology Analyser (Abott Diagnostics Division, Berkshire). Each blood sample was split into

four aliquots. The first aliquot was lysed and the haemoglobin was converted chemically to cyanmethaemoglobin, which was then quantified spectrophotometrically. The other three aliquots were kept whole and the cell count measured using flow cytometry.

Results

A total of 5824 foraging dives were recorded. Some of the time budget data and some of the respiratory exchange data for each bird were normally distributed about the mean, while some of the data were not normally distributed. Since much of the data not normally distributed approximated to bell-shaped curves, the mean was used to describe the central tendency of all the data for each individual bird.

There was a significant difference in resting $\dot{V}_{O_2}$ in water between exposure to normoxic and normocapnic conditions and exposure to the hypoxia 15 % condition and the hypoxia 11 % condition (Table IV-1). There was also a significant difference in resting $\dot{V}_{O_2}$ in water between exposure to normoxic and normocapnic conditions and exposure to hypercapnia max % conditions.

The two food types produced no difference in diving behaviour between the two control conditions, nor any differences in the volume of oxygen taken up over mean t_s in each condition (V_{O_2mean}). However, in the study by Halsey et al. (in prep.), where changes in foraging energetics were associated with different food types, the ducks foraged for mealworms or zebra mussels. Presumably, the nutritional content of different food types influences the foraging energetics of diving ducks. Maggots and mealworms have similar nutritional and calorific values (Table IV-2), which explain the similarity in diving behaviour in the ducks when foraging for these food types.

Dive time budgets

Surface duration and t_d across all the ducks were not significantly different whether the birds were constrained to dive to and from the respirometer or were diving 'freely' (Table IV-3). Thus, the constraint of the respirometer had no adverse affect on the diving behaviour of the ducks i.e. the changes in diving behaviour exhibited in the hypoxic and hypercapnic conditions was affected only by the gas mixes and were not potentiated by the respirometer.

The time budget variables t_d, t_f, $t_{\%f}$ and $d{:}p$ were significantly lower in the hypoxia 15 % condition than in the control and were significantly lower in the hypoxia 11 % condition than in the hypoxia 15 % condition (Table IV-4). Surface duration only differed significantly between the control and the hypoxia 11 % condition. The variables t_{desc}, t_{asc} did not significantly differ between hypoxic conditions. In both the hypercapnia 2.5 % and hypercapnia max % conditions, t_s was significantly higher and $d{:}p$ was significantly lower compared to the control (Table IV-5). Dive duration and t_f were significantly lower in the hypercapnia max % condition compared to the control. The variables t_{desc} and t_{asc} did not significantly differ between hypercapnic conditions. Also, the variable $t_{\%f}$ did not significantly differ between the hypercapnic conditions.

The number of dives in a diving bout showed a decreasing trend as P_IO_2 decreased or P_ICO_2 increased (Table IV-1) in all the individual ducks compared. However, the differences were not significant because of large differences in how much the variable changed between conditions in individual ducks, coupled with low N values.

Cumulative oxygen uptake curves

Fig. IV-3a shows graphical comparisons between the hypoxic conditions of cumulative oxygen uptake over time at the surface, in a fashion similar to Parkes et al. (2002) and Halsey et al. (2003a, b). By subtracting an estimate of background metabolic rate while at the surface from the oxygen uptake curves, calculated from the linear part of the slope (Halsey et al., 2003 a, b), the volume of oxygen used to reload

the oxygen stores is gained (Fig. IV-3b). Table IV-1 shows V_{O2mean} for each condition. As P_IO_2 decreased, V_{O2mean} significantly decreased. There was a significant increase in V_{O2mean} between the hypercapnia control and the hypercapnia 2.5 % condition.

Regression of respiratory gas exchange against dive duration

Because t_d significantly differed between the conditions (Tables IV-4 and IV-5), V_{O2mean} does not provide an accurate comparison of changes in post-dive respiratory gas exchange purely due to changes in ambient gas compositions. Dive duration is related to $\dot{V}_{O2}$ during the subsequent surface period (Parkes et al., 2002; Halsey et al., 2003b), and so to compare the effects of the conditions in the present study on $\dot{V}_{O2}$ and $\dot{V}_{CO2}$, it was necessary to control for t_d. This was achieved by regressing V_{O2} and V_{CO2} against t_d at several values of t_s. Tables IV-6 to IV8 show the regression equations, $V_{O2\_12.4}$ and $V_{CO2\_12.4}$ after 5, 10 and 20 s at the surface.

Values of r^2 for all the regression equations for V_{O2} were high, with 12 of the 18 regressions having an r^2 above 0.90 and only two having an r^2 below 0.70. r^2 values for 7 of the 9 regression equations for V_{CO2} had with r^2 values above 0.78. The other two regressions had very low r^2 values of 0.24 and 0.30, mainly due to low n values, and were not used in the analyses.

The regression equations for V_{O2} at the two control conditions were similar at each t_s of measurement, despite the different food types available to the ducks. There were no significant differences between the slopes or between the intercepts at 5, 10 and 20 s V_{O2}, nor were there any significant differences between $V_{O2\_12.4}$.

The y intercept of each regression line represents an estimate of a fixed amount of oxygen consumed or carbon dioxide produced that is unrelated to the duration of the dive i.e. the oxygen consumed or carbon dioxide produced while on the surface of the water plus the oxygen consumed or carbon dioxide produced while travelling to and from the feeding tray. The y intercept increased as t_s increased in all V_{O2} and V_{CO2}

regressions, across all hypoxic conditions, which is expected since surfacing for longer inevitably demands greater metabolic requirements.

Estimates of oxygen consumption while travelling to the feeding tray in each condition were calculated from power cost estimates taken from the literature, in a fashion similar to that described by Halsey et al. (2003b). The cost of ascent from the feeding tray to the surface was assumed to be negligible (Lovvorn et al., 1991) and thus no oxygen consumption was attributed to it. These travel cost estimates were then subtracted from the relevant y intercepts to give estimates of oxygen consumption during the surface period (V_{O2s}). The y intercepts from the slopes of the regressions for 20 s t_s were used for this purpose because after this period of time the birds have fully replenished their oxygen stores (Parkes et al., 2002; Halsey et al., 2003a, b). The values in square brackets in Table IV-8 represent these estimates of V_{O2s} divided by 20 s to give the mean rate of oxygen uptake over the surface period ($\dot{V}_{O2s}$). Mean $\dot{V}_{O2s}$ over 20 s across all the conditions is 0.29 ± 0.01 ml s^{-1}, and is significantly lower than the values for this species given by Woakes and Butler (1983; 0.44 ml s^{-1}) and Bevan et al. (1992; 0.44 ml s^{-1}) which were estimated by multiple linear regressions. This suggests that the power cost estimates for descending may be too high. These estimates were derived from data on ducks diving to 1.2 m rather than 1.7 m in the present study, and diving deeper reduces the costs of working against positive buoyancy. However, since the error is constant, the estimates of $\dot{V}_{O2s}$ can still be compared. Estimates of $\dot{V}_{O2s}$ clearly increased as P_IO_2 decreased and were similar as P_ICO_2 increased.

Between the hypoxic conditions, after 5 s at the surface, the y intercepts of the V_{O2} regressions were similar while the regression slopes (representing oxygen consumed by the animal during the foraging part of the dive; V_{O2f}) significantly decreased as P_IO_2 decreased (Fig. IV-2). After 10 s and 20 s at the surface, the regression slopes still decreased as P_IO_2 decreased, however the y intercepts increased. The y intercepts of the V_{CO2} regressions at 5 s were similar. The slopes of the V_{CO2} regressions at 5 and

10 s both showed decreasing trends as P_IO_2 decreased, repeating the trends of the V_{O2} regressions, however the differences were not significant. Since V_{CO2} decreased more slowly than V_{O2} decreased, the respiratory exchange ratio (V_{CO2} / V_{O2}; RER) of the ducks while foraging increased as P_IO_2 decreased.

As P_IO_2 decreased, $V_{O2\_12.4}$ decreased at all surface durations, while $V_{CO2\_12.4}$ remained very similar at all surface durations. The confidence limits of an individual estimate from a regression are subject not only to a sampling error (from which the confidence zone is generated) but also to the error from the scatter about the regression line. Thus, the confidence limits of an estimate of V_{O2} or V_{CO2} are considerably larger than the breadth of the confidence zone of the regression line at that point (the 95 % confidence limits for individual estimates are calculated by adding 1 in the square root term in the equation for determining the confidence zone; Fowler et al., 1998). Subsequently, the differences between individual estimates need to be considerably larger than the breadth of the confidence zone to be statistically significant. V_{O2} was only significantly different between hypoxia control and hypoxia 11 % at 5 s, and hypoxia 15 % and hypoxia 11 % at 10 s. Between hypoxic conditions $V_{CO2\_12.4}$ was similar at both 5 s and 10 s.

Between the hypercapnic conditions, $V_{O2\_12.4}$ at 5, 10 and 20 s t_s was similar. While there were some significant differences between exponents of some of the regression equations, trends within the data are not clear and are therefore inconclusive. However, their possible implications are explored in the Discussion.

Blood values

Table IV-9 shows blood haematological indices of the six tufted ducks used in this experiment. Mean haematocrit was 41.3 ± 2.7 %, compared to 43.1 ± 1.4 % in tufted ducks measured by Stephenson et al. (1989). Mean haemoglobin concentration of the ducks in the present study was 11.6 ± 0.6 g dl^{-1} compared to 15.6 ± 0.4 g dl^{-1} (Stephenson et al., 1989). Haematocrit levels were statistically similar between the

present study and Stephenson et al. (1989) while haemoglobin concentration was significantly higher in the ducks in the study by Stephenson et al. (1989). There was no relationship between t_d or t_s and haematocrit or haemoglobin concentration in any of the hypoxic conditions.

Discussion

Diving behaviour in hypoxia and hypercapnia

From Butler and Stephenson (1988), Enstipp et al. (2002) and the present study, it is clear that dive time budgeting in aquatic birds is significantly affected by exposure to hypoxia. In all three studies, *d:p* decreased as P_IO_2 decreased. In the present study, and in Butler and Stephenson (1988), this was due to a decrease in t_d as well as an increase in t_s. Halsey et al. (2003b) showed that a decrease in t_f often accounted for the decrease in t_d and this is confirmed in the present study, where t_f decreased under hypoxic conditions while t_{desc} and t_{asc} remained constant. Therefore, aquatic birds not only forage for a shorter duration under hypoxic conditions, thereby reducing t_d and $t_{\%f}$, but also surface for longer between dives. Thus, the proportion of time spent foraging during each dive cycle is greatly reduced in hypoxic conditions. The basic model of Houston and Carbone (1992) predicts that t_d and t_f will decrease and t_s will increase as $\dot{V}_{O_2}$ between dives decreases. Decreases in P_IO_2 are associated with decreases in $\dot{V}_{O_2}$ in the present study (Fig. IV-3a), and thus the changes in dive time budget variables agree with the predictions of the model. Interestingly, the model also predicts that increases in t_s may not be very marked as $\dot{V}_{O_2}$ decreases, as found in the current results (Table IV-4).

Increases in P_ICO_2 had similar effects on dive time budgeting to decreases in P_IO_2. As P_ICO_2 increased, t_s increased while t_f and t_d decreased. Thus, *d:p* decreased, although $t_{\%f}$ did not decrease significantly. Again, these trends match the findings of Butler and Stephenson (1988). They also agree with the changes in t_s and *d:p* recorded in double-crested cormorants by Enstipp et al. (2001). In the hypercapnia 2.5 % condition, the

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ducks only adjusted the dive time budgeting by increasing t_s. As P_ICO_2 increased further, the response included a decrease in t_d as well as a further increase in t_s.

Regression analyses of respiratory gas exchange against dive duration

The V_{O_2} regressions showed consistently high correlations between the duration of the previous dive and $\dot{V}_{O_2}$ at the surface, and therefore a lack of confounding factors. Since V_{O_2d} is higher over longer dives (Woakes and Butler, 1983; Parkes et al., 2002), assuming that the oxygen stores are at the same level before each dive in a particular condition, the ducks are expected to take up oxygen more quickly during the subsequent surface period because of higher partial pressure differentials of oxygen between ambient gas and the blood of the bird (Kramer, 1988). Therefore, the high correlations imply that indeed the ducks did not vary the level of oxygen stores before the start of each dive, with only t_d affecting the oxygen store levels at the end of a dive. Fig. IV-3b confirms that the ducks are, at least on average, close to replenishing their oxygen stores fully after each dive and are therefore replenishing their stores to the same level, thus supporting the conclusions taken from the high r^2 values for the regression slopes. This assumption of consistent oxygen store levels before every dive has often been applied to diving animals (e.g. Woakes and Butler, 1983; Bevan et al., 1992; Houston and Carbone, 1992). Values of r^2 tended to decrease in regressions of V_{O_2} over greater t_s, however, indicating that as t_s increased, the ducks undertook more activities, which affected $\dot{V}_{O_2}$. For the first few seconds, the ducks were fairly sedentary while hyperventilating, however over 20 s at the surface, their behaviour sometimes included wing flapping and preening, which affected V_{O_2} and thus weakened the correlations.

Respiratory gas exchange during surface periods in hypoxia

Descent and ascent durations did not differ between any of the conditions, which may mean that oxygen consumed during travel to and from the feeding tray was a fixed value. Thus, trends in the y intercept values (representing a fixed volume of oxygen consumption that is unrelated to t_d) between the conditions and between the values of

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t_s within the conditions may indicate differences in V_{O2s}. The three regressions of V_{O2} after a t_s of 5 s in the hypoxic conditions have very similar intercepts indicating that the cardio-respiratory system of the ducks is equally active during the first few seconds after a dive, irrespective of P_IO_2. This would predict that V_{O2} after 5 s would decrease as P_IO_2 decreases due to a reduction in oxygen partial pressure differentials across the lung wall, and is confirmed by $V_{O2\_12.4}$ at 5 s in each condition. Indeed, there is a significant decrease in $V_{O2\_12.4}$ at 5 s in the hypoxia 11 % condition compared to the hypoxia control. The similarity in the y intercepts in the associated carbon dioxide regressions after 5 s at the surface confirms the trend for this exponent in the V_{O2} regressions. It also indicates a similar RER in each condition. These findings, indicating a similar respiratory effort during this period regardless of P_IO_2, may indicate that the ducks always hyperventilated as energetically as possible immediately after surfacing.

After 10 and 20 s at the surface, the y intercepts increase in the V_{O2} regressions (and V_{CO2} regressions after 10 s) as P_IO_2 decreases. The increase in $\dot{V}_{O2s}$ as P_IO_2 decreases is shown most clearly by the estimates of this value over 20 s, shown in square brackets in Table IV-8. Furthermore, there is less difference in $V_{O2\_12.4}$ between the conditions after these longer surface durations. This suggests that when the ducks surface after a dive into a low P_IO_2, the cardio-respiratory system is more energetic beyond the first 5 s than when they surface into a normoxic environment, as it compensates for the reduced V_{O2} during the first few seconds. Consequently, over the entire surface period, differences in oxygen uptake between hypoxic conditions were fairly small. Diving birds are known to hyperventilate immediately after, and directly before, a dive (Butler and Woakes, 1979; Parkes et al., 2002; Halsey et al., 2003a; Wilson, 2003; Wilson et al., 2003). Therefore, this increased respiratory effort in hypoxic conditions may represent a lengthening of the initial, post-dive hyperventilatory period, and / or of the pre-dive hyperventilatory period, and is probably a modification in respiratory behaviour to ensure sufficient adjustment in the body gas stores to enable another dive. Butler and Stephenson (1988) reported that the

duration of pre-dive hyperventilation was significantly longer in ducks exposed to hypoxia.

Butler (1970) found that mallard ducks at rest were able to maintain the same rate of oxygen consumption as P_IO_2 decreased to around 10 %, demonstrating that despite some reduction in P_aO_2, the same amount of oxygen was unloaded to the tissues as in normoxia. Furthermore, no apparent limitation to oxygen exchange in hypoxia during running exercise was found in Pekin ducks (Kiley et al., 1985) or emus (Schmitt et al. 2002). While bar-headed geese exhibited severe limitations to oxygen uptake during treadmill running under hypoxic conditions (Fedde et al., 1989), Butler and Bishop (2000) suggest that the subject animals may have been stressed by the surgical techniques employed in the study, evidenced by their high resting heart rates. Indeed, Faraci (1986) notes that bar-headed geese perform rigorous exercise at extreme altitude, flying at up to 11 000 m altitude (Swan, 1970; Fox, 2003).

The values of $V_{O2\_12.4}$ in the hypoxia condition regressions at 10 and 20 s did not decrease significantly as P_IO_2 decreased, and V_{O2mean} decreased significantly, but only by 17 %, in the hypoxia 11 % condition compared to hypoxia control, while there was an increase in t_s of only 1.1 s. Thus the inspiration of hypoxia down to 11 % oxygen does not affect respiratory gas exchange much between dives in ducks. The aforementioned experimental studies only measured $\dot{V}_{O2}$ during mild exercise in hypoxia. Since oxygen consumption during average t_d is known to be similar to that during swimming at maximum velocity (Woakes and Butler, 1983), the results of the present study extend these previous findings by demonstrating that at least some birds can also perform demanding exercise while exposed to hypoxic conditions.

Respiration during foraging periods in hypoxia

The slope of each regression line represents $\dot{V}_{O2f}$, assuming that the ducks readjust their oxygen stores to the same level between each dive. After 5 s at the surface, the bird is still replenishing its oxygen stores (Parkes et al., 2002; Halsey et al., 2003a, b)

while after 20 s, the animal has sometimes undertaken extraneous behaviours such as wing flapping and preening. Therefore, the regression slopes after 10 s at the surface probably represent the most accurate estimate of $\dot{V}_{O2f}$. The slope of the regression at 10 s in the hypoxia control (0.63 ± 0.06 ml s^{-1}) is similar to an estimate by Bevan et al. (1992) of the mean rate of oxygen consumption at the point of mean dive duration during a dive of 12.4 s in tufted ducks (0.60 ml s^{-1}).

In all hypoxic conditions, the regression slopes decrease as P_IO_2 decreases, suggesting that $\dot{V}_{O2f}$ decreases i.e. aerobic metabolism decreases during the foraging period. This decrease is considerable, but has been observed previously in tufted ducks. Bevan et al. (1992) recorded a decrease in mean $\dot{V}_{O2}$ at mean t_d in tufted ducks, coupled with a decrease in mean heart rate, as mean t_d increased. These birds had an estimated mean rate of oxygen consumption of 0.65 ml O$_2$ s^{-1} during dives of 10 s mean duration decreasing to 0.17 ml O$_2$ s^{-1} during dives of 35 s mean duration. In dives longer than 35 s, heart rate over the last few seconds was very close to resting level despite the ducks continuing to beat their legs. Since heart rate has been shown to decrease over time during long dives in tufted ducks (Stephenson et al., 1986), and in double-crested cormorants (Enstipp et al., 2001), these data presented by Bevan et al. (1992) may well be indicative of what happens to oxygen consumption during any one dive.

Thus, the ducks in the study by Bevan et al. (1992) and those in the present study may be increasing perfusion of the active muscles by producing a more severe vasoconstriction in non-active parts of the body during the dive (Butler et al., 1988; Bevan et al., 1992). Also, they may be replacing aerobic metabolism with anaerobic metabolism as the dive progresses. However, the physiological effects on diving birds of breathing hypoxic gas mixes are unclear, since while Enstipp et al. (2001) recorded a significant decrease in heart rate in cormorants diving after exposure to hypoxia, Butler and Stephenson (1988) recorded no change in heart rate in tufted ducks exposed to different P_IO_2 during diving bouts. Finally, Halsey et al. (in prep.) report adjustments in the buoyancy of tufted ducks depending upon dive depth and food

type, a very significant contributor to power costs during submergence (Lovvorn and Jones, 1991) and thus a reduction in pre-dive buoyancy would greatly reduce oxygen consumption during the dive. Thus the ducks in the present study may have been able to adjust one or more of these factors in response to a decrease in body oxygen stores, as well as tolerating a temporary build up of lactate and / or sacrificing some thermoregulatory control in order to dive (Bevan and Butler, 1992a).

The slopes of the carbon dioxide regressions in the hypoxic conditions provide evidence that anaerobic metabolism was occurring during some hypoxic dives. While the trends in the slopes of the carbon dioxide regressions agree with those of the oxygen regressions at both 5 and 10 s t_s, the decreases in carbon dioxide output were smaller than the decreases in oxygen uptake, indicating that, as the ducks reduced oxygen consumption in response to an increase in P_IO_2, RER increased (Tables IV-6 and IV-7). In the hypoxia 11 % condition, $\dot{V}_{CO2f}$ is higher than $\dot{V}_{O2f}$ both in the regressions at 5 and 10 s t_s, giving RER values greater than 1 during the foraging period of the dive. This indicates that not all of the carbon dioxide being produced during this time is due to the oxidisation of a substrate but rather that lactate has accumulated in the blood due to anaerobic respiration, forming additional carbon dioxide from bicarbonate (Maughan et al., 1997), which is blown off during subsequent hyperventilation at the surface. Thus, the exposure to hypoxia may have simulated the latter part of the longer dives in tufted ducks reported by Bevan et al. (1992), triggering a reduction in heart rate and oxygen consumption, and possibly increasing anaerobic metabolism. The decrease in t_d under such conditions might therefore be a behavioural response to limit or prevent a build up of lactate. While t_s did not markedly increase to allow lactate removal before the next dive, the number of dives in a diving bout decreased as P_IO_2 decreased (Table IV-2). This may indicate an accumulation of lactate during the bout, which does not occur, or occurs more slowly, during normoxic dives.

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Given these findings, it is worth noting here that, although the rates of oxygen consumption while travelling underwater may in fact not have been fixed, the alternative is that they decreased as P_IO_2 decreased due to an increase in vasoconstriction, an increase in anaerobic metabolism and / or a decrease in buoyancy. If so, the trend in $\dot{V}_{O2s}$ between the hypoxic conditions, indicated by the values in square brackets in Table IV-7, would be accentuated.

The effect of respiratory gas levels on dive duration in hypoxia

While V_{O2mean} did not decrease by a large amount in the hypoxia 11 % condition, the proportion of V_{O2mean} used to reload the oxygen stores between dives was less under more hypoxic conditions due to an increase in metabolic rate. Along with hyperventilation in hypoxic conditions, cardiac output also increases and redistributes blood flow so that oxygen is delivered to the heart and brain at normal volume (Faraci, 1986). These physiological adjustments increase metabolic rate, recorded in the present study by increased values of resting $\dot{V}_{O2}$ (Table IV-1) and by Butler and Stephenson (1988) as significant elevations in pre-dive and post-dive heart rates. Since t_s is longer under hypoxic conditions, the ducks are not only consuming oxygen at a higher rate but also for longer, further reducing the proportion of V_{O2mean} used to reload the body stores.

However, the increase in t_s in the hypoxia 11 % condition compared to the control was only 9 % (Table IV-3) and the estimate of $\dot{V}_{O2s}$ only increased by 84 % (Table IV-7), while Halsey et al. (2003a) point out that there is more than a three-fold difference between mean t_d and cADL in tufted ducks under natural conditions. Certainly, then, the ducks were diving after a surface period in 11 % oxygen with sufficient oxygen stores to be able to remain submerged for the same duration as when exposed to normoxia without exhausting their stores, even if oxygen consumption remained as high as during dives in normoxia. This does not support the hypothesis that t_d was shorter under hypoxic conditions because oxygen stores were exhausted. Since the increased hyperventilation during the surface period would have

produced a hypocapnia prior to diving, and since the t_d in the hypoxic conditions was shorter, allowing less time for carbon dioxide to build up, carbon dioxide levels are unlikely to be the main factor influencing t_d under these conditions. Furthermore, the volume of oxygen used to reload the stores is only about 3 ml less in 11 % oxygen compared to that in normoxia (Fig. IV-2), a reduction of about 35 %, while the oxygen used during foraging is several times less in 11 % oxygen (Table IV-4 and Table IV-7). This is evidence against the hypothesis that a critical level in the oxygen stores was reached more quickly during dives in the hypoxia 11 % condition, thus shortening the duration of the dives. Therefore, the results of this study suggest that oxygen levels have a limited influence on t_d in tufted ducks, thus supporting the conclusions of Halsey et al. (2003a).

The shorter t_d and decreasing number of dives in a diving bout in the hypoxic conditions, and in particular an estimated RER above 1 in the hypoxia 11 % condition, are evidence of an accumulation of lactate due to anaerobic respiration during dives after inspiring low levels of oxygen. It is difficult, however, to identify a reason for such a shift in the proportional use of anaerobic metabolic pathways given that the ducks were able to dive within their cADL in the hypoxia 11 % condition for the mean duration. However, the degree of vasoconstriction during normal dives in tufted ducks is also difficult to explain. Vasoconstriction in the body other than the leg muscles and the head occurs to a larger extent during dives than during surface swimming (Bevan and Butler 1992a). In contrast to the situation during surface swimming, virtually all of the blood flow during dives is directed through the ischiadic arteries and the brachiocephalic arteries i.e. oxygen is provided for the legs and head and very little is provided for other organs, which may metabolise anaerobically. This is surprising since tufted ducks have sufficient oxygen stores to maintain aerobic metabolism within all tissues during dives of normal duration, even when the rate of metabolism is elevated to levels during exercise (Woakes and Butler, 1983; Bevan and Butler, 1992a). Thus, ducks seem to employ physiological adjustments during dives under normal conditions in excess of that required to

perform a totally aerobic dive. They may, therefore, make further physiological adjustments, i.e. a switch towards anaerobic metabolism in parts of the body, when oxygen levels are lower than normal during the latter part of an extended dive or during shorter dives from hypoxia.

Oxygen consumption during surface and foraging periods in hypercapnia

In the hypercapnic conditions, while there are some significant differences between exponents of the regressions both between conditions and t_s, trends within these data are less clear. Larger variations in the pattern of response to carbon dioxide in comparison to oxygen have also been recorded in previous studies (e.g. Jones and Purves, 1970). Nevertheless, $V_{O2\_12.4}$ at each t_s are clearly very similar across conditions. This confirms that since an increase in P_ICO_2 only nominally reduces P_IO_2, the ability of the duck to take up oxygen in hypercapnia is not affected. Despite this, the ducks reduced t_d and increased t_s as P_ICO_2 increased, therefore suggesting that oxygen was not the main influence on t_s in the hypercapnic conditions.

The P_aCO_2 of ducks in normoxia in a study by Mangalam and Jones (1984) was about 4.3 kPa, while the P_aCO_2 of the same ducks in 4 % carbon dioxide (and 11.9 % O_2) was about 4.8 kPa, showing that increasing levels of P_ICO_2 above normal decreases the ability of ducks to prevent their carbon dioxide stores from rising. The diving behaviour of the ducks in the hypercapnic conditions, both in terms of t_s and t_d, may therefore be explained at least partly by a diminished ability to reduce carbon dioxide levels in the body during surface periods. This supports the conclusions of Butler and Stephenson (1988) and Halsey et al. (2003a) that carbon dioxide levels have an important influence on diving behaviour in tufted ducks.

The normal P_aCO_2 of chickens (*Gallus domesticus*) is approximately 4.7 kPa (Ray and Fedde, 1969), while Jones and Holeton (1972) measured the P_aCO_2 of muscovy ducks at rest in normoxia at 4.8 ± 0.2 kPa. This indicates that the increase in P_aCO_2 in the hypercapnia max % condition in the present study was small, despite the large

increase in P_ICO_2 relative to the control, confirming the high sensitivity of carbon dioxide receptors that affect respiratory drive, compared to the sensitivity of oxygen receptors (Butler and Taylor, 1974; Schmidt-Nielsen, 1975).

In contrast to the hypoxic conditions, $\dot{V}_{O2f}$ did not decrease as P_ICO_2 increased, a finding supported by measurements of heart rate in tufted ducks and diving cormorants, which did not change in hypercapnia compared to normoxia (Butler and Stephenson, 1986; Butler and Stephenson, 1988; Enstipp et al., 2001). Therefore, and perhaps not surprisingly, increases in carbon dioxide concentration do not produce the same physiological responses as decreases in oxygen concentration. Possibly then, the ducks could only respond to increases in P_ICO_2 behaviourally, by decreasing t_d to limit carbon dioxide build up and increasing t_s to maximise carbon dioxide removal.

Blood oxygen storing capacity and diving ability

Stephenson et al. (1989) found that tufted ducks trained to dive for long durations had reduced oxygen stores in the air sacs, off-set by a corresponding gain in blood oxygen stores due to hypervolaemia. Reduction in lung sac volume reduces buoyancy and thus the energetic cost of diving, representing a considerable energetic advantage to dive-trained ducks when diving for extended durations. Muskrats were also found to adapt when trained to dive through an increase in the capacity of the blood to store oxygen (MacArthur et al., 2003), though this time through increases in haematocrit and haemoglobin levels, and there was no decrease in lung capacity. Thus, the surprising observations of certain ducks in the present study diving many times in succession in the hypoxia 11 % condition, may be explained by an anatomy and / or physiology better predisposed to cope with the inhalation of hypoxic gases.

Although there was no relationship between haematocrit or haemoglobin levels, and diving ability measured in terms of t_d and t_s in any of the hypoxic conditions, these findings are in agreement with those of Stephenson et al. (1989) who reported that the increase in blood oxygen storage capacity in dive-trained ducks was due entirely to an

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increase in blood volume and not due to changes in blood haematological indices. Perhaps, then, the marked differences between the individual ducks diving in the hypoxic conditions can be explained by anatomical differences, for example a reduction in end-expiratory lung / air sac volume as measured by Stephenson et al. (1989) in dive-trained ducks.

Summary

The primary aim of this study was to quantify how much the inspiration of a hypoxic or hypercapnic gas mix affects respiratory gas exchange between dives in tufted ducks, and how much these differences can account for changes in diving behaviour. In a hypercapnic environment, while the ducks experienced no decrease in the ability to reload their oxygen stores between dives, they were presumably unable to reduce their carbon dioxide levels as much as in normocapnia. Thus, the increase in t_s and decrease in t_d cannot be due to oxygen driving diving behaviour in these conditions and confirm that carbon dioxide levels do play an important role in the diving behaviour of tufted ducks. In a hypoxic environment, even when P_IO_2 is halved, the ducks are able to substantially reload their oxygen stores after a dive. Thus, hypoxia does not significantly impair oxygen loading into the stores and delivery to the tissues. Although the proportion of oxygen taken up that is used to reload the respiratory system, blood and tissues is lower during hypoxia due to increased metabolic demands, the ducks still dived with sufficient oxygen to remain within their cADL. Despite this, they still reduced t_d and were therefore not exhausting their oxygen stores. Given that they also reduced $\dot{V}_{O2f}$ during hypoxia, neither were they reducing their oxygen stores to a critical level. The decrease in t_d, decrease in the number of dives in a diving bout and increase in estimated RER during dives in hypoxia indicates that they may have been responding to the decrease in oxygen in their stores by reducing oxygen consumption and metabolising anaerobically, accumulating lactic acid which could only be tolerated for a short time. Therefore, rather than a decrease in oxygen stores reducing t_d per se, as assumed by other studies,

lactate accumulation may have a strong influence on diving behaviour in hypoxic conditions.

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Table IV-1. Mean values of various measurements from six tufted ducks in the control conditions and the hypoxia and hypercapnia conditions

	Resting oxygen uptake while on water, resting $\dot{V}_{O_2}$ (ml O$_2$ s^{-1})	Oxygen uptake over mean surface duration, V_{O2mean} (ml)	Number of dives / diving bout
Hypoxia control condition ($n = 832$)	0.28 ± 0.03*	15.49 ± 1.29	20.52 ± 3.04
Hypoxia (15 % O$_2$) ($n = 1116$)	0.36 ± 0.06	15.03 ± 1.11**	14.96 ± 1.41
Hypoxia (11 % O$_2$) ($n = 767$)	*0.37 ± 0.05	***12.92 ± 1.06	*11.49 ± 1.52
Hypercapnia control condition ($n = 1129$)	0.27 ± 0.02*	15.43 ± 0.95*	20.25 ± 4.37
Hypercapnia (2.5 % CO$_2$) ($n = 1246$)	0.29 ± 0.02	17.21 ± 1.75	13.91 ± 4.50*
Hypercapnia (max % CO$_2$) ($n = 734$)	*0.41 ± 0.03	18.69 ± 1.70	10.01 ± 2.52

Values given are means ± SE.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Asterisks to the right of values represent a significant difference between that condition and the related condition involving either the next level of lower oxygen concentration or higher carbon dioxide concentrations (e.g. hypoxia control and the hypoxia 15 % condition).

Asterisks to the left of values represent a significant difference between that condition and the related control condition.

Table IV-2. Differences in water and nutritional content between maggots (*Calliphora vomitoria*) and mealworms (*Tenebrio molitor)*

	Maggots		Mealworms	
% Water	71		61	
Gross Energy (dry; MJ kg^{-1})	25		27	
	Dry	Wet	Dry	Wet
% Lipid	27	8	39	15
% Protein	55	16	50	20
% Mineral	4	1	4	1

Table IV-3. Time budget data from five tufted ducks in the hypercapnia control condition and in the condition where the ducks could dive 'freely'

	Dive duration, t_d (s)	Surface duration, t_s (s)
Hypercapnia control ($n =$ 1129)	12.82 ± 0.65	13.04 ± 1.76
'Freely' diving ($n = 710$)	11.22 ± 1.21	12.96 ± 1.77

Values given are means ± SE.

Table IV-4. Time budget data from six tufted ducks in the hypoxia control and the two hypoxia conditions

	Dive duration, t_d (s)	Surface duration, t_s (s)	Foraging duration, t_f (s)	Descent (s)	Ascent (s)	% Dive duration spent foraging, $t_{\%f}$	Dive:Pause ratio, $d{:}p$
Control ($n = 832$)	14.22 ± 0.46*	12.50 ± 1.67	7.65 ± 0.38**	3.80 ± 0.21	2.74 ± 0.18	51.98 ± 1.72*[†]	1.22 ± 0.13*
Hypoxia (15 % O$_2$) ($n = 1116$)	12.65 ± 0.56***	12.92 ± 1.69	5.90 ± 0.41**	3.99 ± 0.30	2.79 ± 0.17	45.36 ± 2.38**[†]	1.05 ± 0.11**
Hypoxia (11 % O$_2$) ($n = 767$)	***10.37 ± 0.54	*13.62 ± 1.43	***3.76 ± 0.22	3.83 ± 0.25	2.76 ± 0.20	***35.56 ± 1.40[†]	**0.79 ± 0.06

Values given are means ± SE.

[†]Values are arcsine transformed.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Asterisks to the right of values in the hypoxic conditions represent a significant difference between that condition and the condition with the next level of lower oxygen concentration (e.g. control and hypoxia 15 % condition).

Asterisks to the left of the value in the hypoxia 11 % condition represent a significant difference between that condition and the control condition.

Table IV-5. Time budget data from six tufted ducks in the hypercapnia control and the two hypercapnia conditions

	Dive duration, t_d (s)	Surface duration, t_s (s)	Foraging duration, t_f (s)	Descent (s)	Ascent (s)	% Dive duration spent foraging, $t_{\%f}$	Dive:Pause ratio, $d{:}p$
Control ($n = 1129$)	13.14 ± 0.62	12.84 ± 1.45**	5.88 ± 0.41	4.05 ± 0.46	3.12 ± 0.25	43.65 ± 3.11[†]	1.09 ± 0.12*
Hypercapnia (2.5 % CO_2) ($n = 1246$)	13.37 ± 1.04	15.63 ± 1.36	6.05 ± 0.61	4.02 ± 0.43	3.22 ± 0.23	44.01 ± 2.88[†]	0.87 ± 0.07*
Hypercapnia (max % CO_2) ($n = 734$)	*10.81 ± 1.57	*18.79 ± 1.57	*4.53 ± 0.76	3.67 ± 0.48	3.10 ± 0.23	39.64 ± 5.14[†]	**0.47 ± 0.03

Values given are means ± SE.

[†]Values are arcsine transformed.

*$P < 0.05$; **$P < 0.01$.

Asterisks to the right of values represent a significant difference between that condition and the condition with next level of higher carbon dioxide concentration (e.g. control and hypercapnia 2.5 % condition).

Asterisks to the left of the values in the hypercapnia max % condition represent a significant difference between that condition and the control condition.

Table IV-6. Regression of respiratory gas exchange against dive duration over 5 s at the surface in six tufted ducks, for each condition. The regression lines are described by the equation $y = bx + a$, where y is V_{O_2} and x is t_d

Condition	V_{O_2} Equation	V_{CO_2} Equation		V_{O_2} and V_{CO_2} after a 12.4 s dive, $V_{O_2\_12.4}$ and $V_{CO_2\_12.4}$ (ml)
Hypoxia control	$y = 0.41x + 3.99$ ($\pm$ 0.02*, 0.35) $r^2 = 0.96$	$y = 0.30x + 2.41$ ($\pm$ 0.02**, 0.31*) $r^2 = 0.95$	**0.73**	9.03* $\pm$ 0.48 6.17 $\pm$ 0.42
Hypoxia (15 % O_2)	$y = 0.31x* + 4.13$ ($\pm$ 0.04***, 0.51*) $r^2 = 0.84$	$y = 0.27x + 3.06$ ($\pm$ 0.03, 0.40***) $r^2 = 0.90$	**0.87**	8.02 $\pm$0.72 6.37 $\pm$ 0.57
Hypoxia (11 % O_2)	$y = ***0.20x + 3.95$ ($\pm$ 0.04, ***0.36) $r^2 = 0.83$	$y = 0.26x + 2.61$ ($\pm$ 0.04, ***0.37) $r^2 = 0.88$	**1.3**	6.44 $\pm$ 0.60 5.82 $\pm$ 0.62
Hypercapnia control	$y = 0.48x*** + 2.48***$ ($\pm$ 0.02***, 0.26***) $r^2 = 0.98$			8.46 $\pm$ 0.36
Hypercapnia (2.5 % CO_2)	$y = 0.40x + 4.36*$ ($\pm$ 0.03***, 0.36***) $r^2 = 0.94$			8.45 $\pm$ 0.46
Hypercapnia (max % CO_2)	$y = 0.47x + 3.00$ ($\pm$**0.03, ***0.49) $r^2 = 0.93$			8.01 $\pm$ 0.67

Table IV-7. Regression of respiratory gas exchange against dive duration over 10 s at the surface in six tufted ducks, for each condition. The regression lines are described by the equation $y = bx + a$, where y is V_{O_2} and x is t_d

Condition	V_{O_2} Equation	V_{CO_2} Equation		V_{O_2} and V_{CO_2} after a 12.4 s dive, $V_{O_2\_12.4}$ and $V_{CO_2\_12.4}$ (ml)
Hypoxia control	$y = 0.63x + 5.15$ (± 0.06, 0.84) $r^2 = 0.91$	$y = 0.44x + 4.25$ (± 0.05, 0.71) $r^2 = 0.88$	**0.70**	12.92 ± 1.15 9.75 ± 0.97
Hypoxia (15 % O_2)	$y = 0.53x*** + 6.16***$ (± 0.04, $0.58***$) $r^2 = 0.93$	$y = 0.37x + 6.30$ (± 0.04, $0.53***$) $r^2 = 0.83$	**0.70**	$12.69* \pm 0.82$ 10.83 ± 0.95
Hypoxia (11 % O_2)	$y = ***0.24x + *8.03$ (± 0.05, $**0.63$) $r^2 = 0.69$	$y = 0.32x + *6.27$ (± 0.05, 0.53) $r^2 = 0.85$	**1.3**	11.03 ± 0.96 10.28 ± 0.55
Hypercapnia control	$y = 0.69x*** + 4.47***$ (± 0.02, $0.26***$) $r^2 = 0.99$			12.96 ± 0.36
Hypercapnia (2.5 % CO_2)	$y = 0.47x* + 7.11**$ (± 0.02, $0.32***$) $r^2 = 0.97$			12.98 ± 0.44
Hypercapnia (max % CO_2)	$y = 0.58x + 5.51$ (± 0.03, $***0.45$) $r^2 = 0.96$			12.67 ± 0.67

Table IV-8. Regression of respiratory gas exchange against dive duration over 20 s at the surface in six tufted ducks, for each condition. The regression lines are described by the equation $y = bx + a$, where y is V_{O_2} and x is t_d

Condition	V_{O_2} Equation	V_{CO_2} Equation		V_{O_2} and V_{CO_2} after a 12.4 s dive, $V_{O_2\_12.4}$ and $V_{CO_2\_12.4}$ (ml)
Hypoxia control	$y = 0.76x + 9.35$ [0.19] (± 0.16, 2.34*) $r^2 = 0.76$	-		18.83 ± 3.24 -
Hypoxia (15 % O_2)	$y = 0.58x + 10.82$ [0.25] (± 0.05***, 0.71***) $r^2 = 0.92$	$y = 0.40x + 11.17$ (± 0.07*, 0.87***) $r^2 = 0.78$	**0.69**	18.08 ± 0.99 16.17 ± 0.99
Hypoxia (11 % O_2)	$y = 0.34x + 12.58$ [0.35] (± 0.12, ***1.16) $r^2 = 0.57$	-		16.75 ± 1.97 -
Hypercapnia control	$y = 0.59x + 11.33$ [0.27] (± 0.07, 0.84***) $r^2 = 0.89$			18.69 ± 1.23
Hypercapnia (2.5 % CO_2)	$y = 0.59x + 12.31$ [0.33] (± 0.06***, 0.80***) $r^2 = 0.90$			$19.66 \pm 1 .11$
Hypercapnia (max % CO_2)	$y = 0.59x + 11.96$ [0.34] (± 0.08, ***1.14) $r^2 = 0.79$			19.31 ± 1.62

Values in parentheses are ± SE of the regression exponents, *b* and regression coefficients, *a*, where *b* represents an estimate of total oxygen uptake (V_{O_2}) over the surface period and *a* represents an estimate of rate of oxygen consumed while foraging ($\dot{V}_{O_2f}$).

P < 0.05, **P* < 0.01, ***P* < 0.001.

Values in square brackets are estimates of $\dot{V}_{O_2s}$ (see Results for details).

Values in bold are estimates of RER during foraging, calculated from the *b* exponents of the V_{O_2} and V_{CO_2} regressions.

Asterisks to the right of the values for $V_{O_2\_12.4}$ represent a significant difference between that condition and the hypoxia 11 % condition.

Asterisks to the right of regression exponents represent a significant difference between that condition and the respective condition involving either the next level of lower oxygen concentrations or higher carbon dioxide concentrations (e.g. hypoxia control and the hypoxia 15 % condition).

Asterisks to the left of regression exponents represent a significant difference between that condition and the respective control condition.

Asterisks to the right of ± SEs of regression exponents in parentheses represent a significant difference between that condition and the condition involving either the next level of lower oxygen concentration or higher carbon dioxide concentration (e.g. hypercapnia control and hypercapnia 2.5 %).

Asterisks to the left of ± SEs of regression exponents in parentheses in the hypoxia 11 % condition and the hypercapnia max % condition represent a significant difference between that condition and the relevant control condition.

Table IV-9. Blood haematological indices in six tufted ducks

Duck	Venous haematocrit (%)	Haemoglobin concentration (g dl^{-1})
lbdg	34.5	10.4
op	46.5	11.1
bdg	46.0	10.4
pr	31.5	14.3
pinkr	42.5	11.0
bblu	47.0	12.4
Mean	41.3	11.6
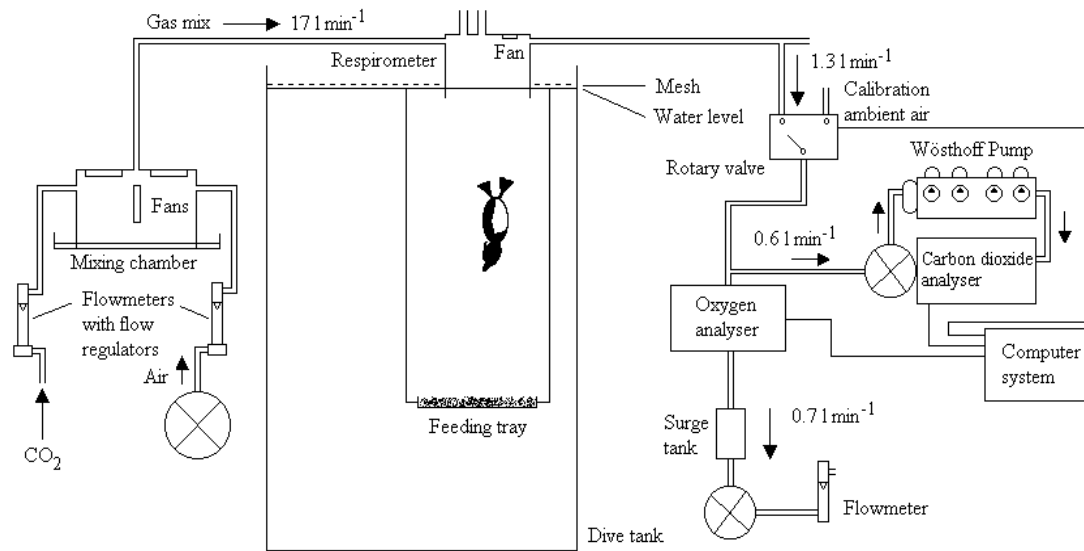
Figure IV-1

Diagram of experimental apparatus in the hypercapnic conditions, showing a tufted duck diving from the respirometer. For further details see Methods.
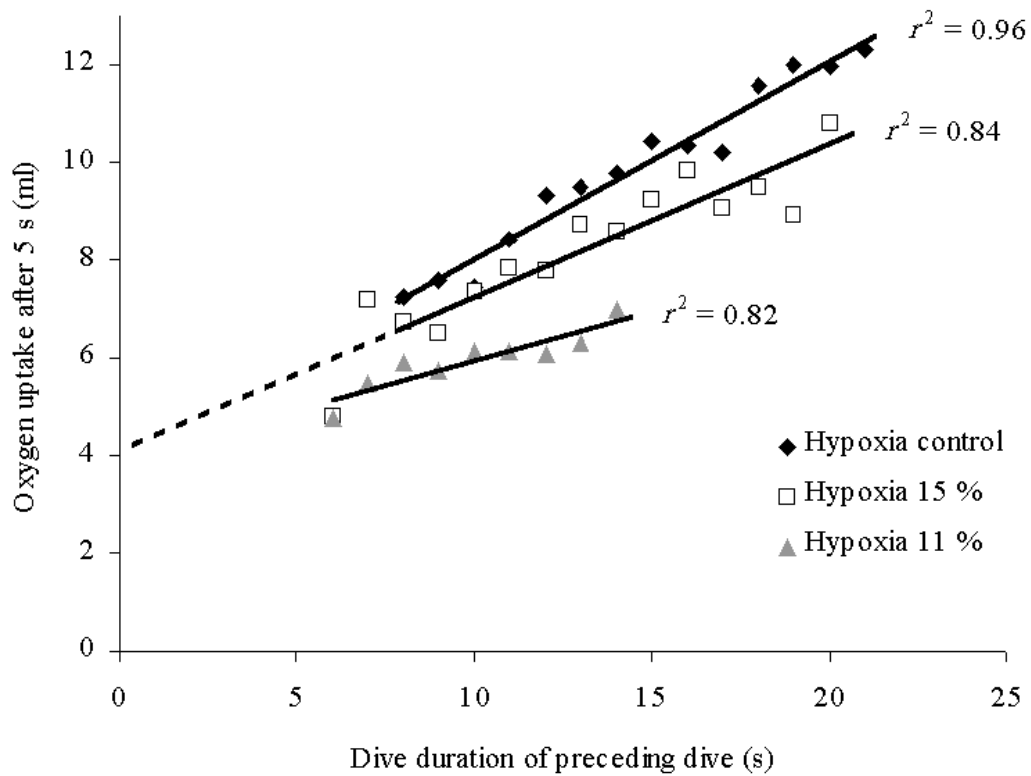
Figure IV-2

A graphical representation of the three hypoxia condition regressions in Table IV-6, of total oxygen uptake (V_{O2}) over 5 s at the surface against duration of the preceding dive (t_d; $N = 6$). The regression lines are described by the equation $y = bx + a$, where y is V_{O2} and x is t_d. The values of b, a and x for each condition are shown in Table IV-6. The dashed line represents an extrapolation of the regression line for the hypoxia 15 % condition data to the y intercept.
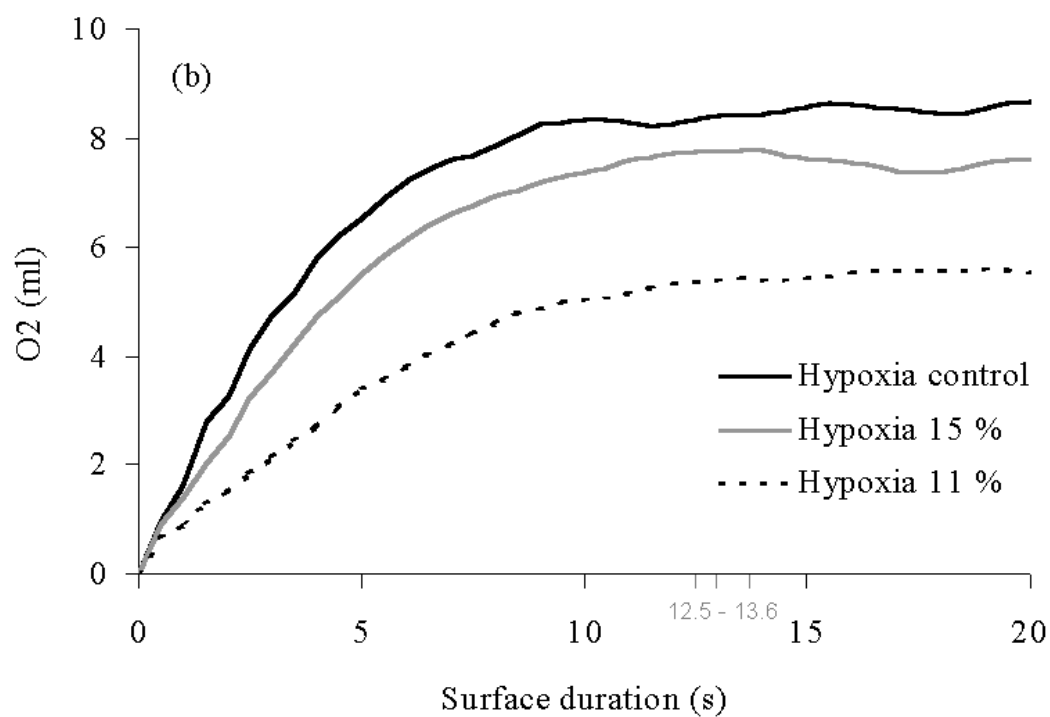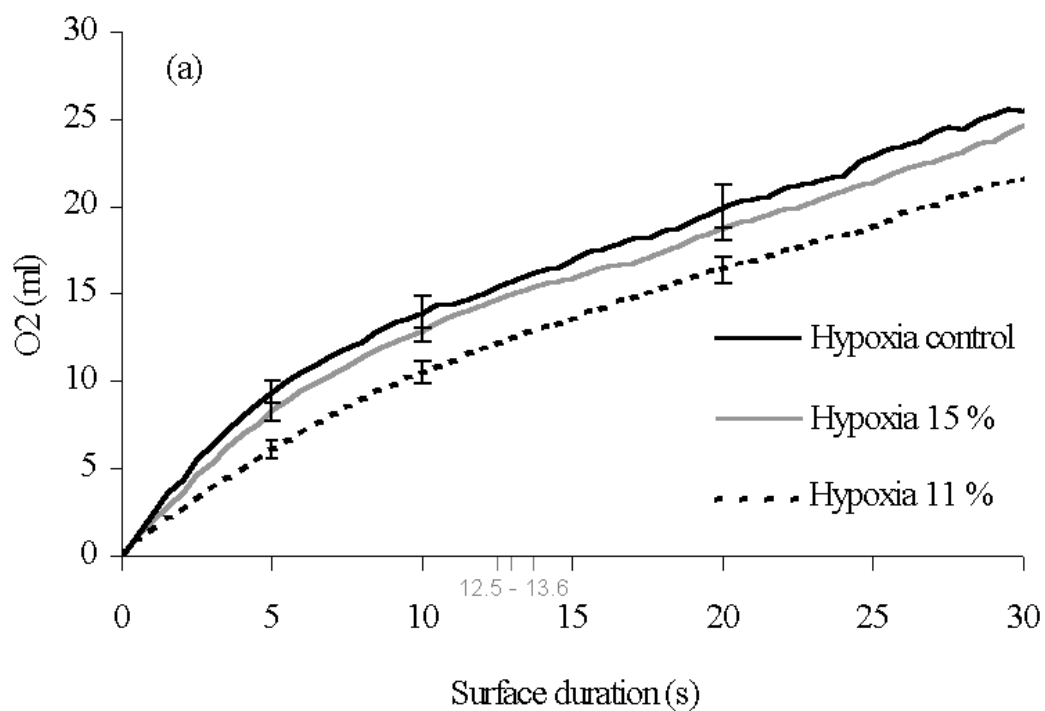
Figure IV-3

Mean (± SE at 5, 10 and 20 s) oxygen uptake curves and oxygen reloading curves for the first 30 s and 20 s surface duration (t_s), respectively, for six tufted ducks, $n = 2715$. The values in grey indicate mean t_s for each condition (t_s increases as P_IO_2 decreases). a) oxygen uptake in hypoxia conditions b) oxygen reloading in hypoxia conditions.

V. Contrasting Relationships of Diving Behaviour and Body Mass between Taxonomic Groups of Diving Vertebrates

Allometric relationships between diving parameters and body mass (*M*) in taxonomic groups of diving animals have been reported in a number of studies. However, none of these studies accounted for the phylogenetic inter-relatedness between species and therefore the claimed relationships may be spurious artefacts of the non-independence of species. In the present study, both across species regression analyses and regression analyses of independent contrasts (accounting for phylogeny) were performed on diving parameters and *M* for a large number of diving vertebrates. Information on the diving behaviour of each species was obtained through a literature search and correspondence with the authors, and also unpublished data. Mean dive duration, mean maximum dive depth and mean surface duration were regressed against *M* for alcids, cormorants, penguins and pinnipeds where enough data was available. Cormorants, penguins and pinnipeds mostly showed significant relationships between these diving parameters in the across species regressions, while the alcids did not. The lack of correlations in the alcids may be evidence of the use of anaerobic metabolism during dives across this taxonomic group, confounding the *M* specific metabolic advantage of larger species. In the regressions of independent contrasts, only the penguins still showed a significant relationship. This may indicate that many positive allometric relationships in earlier studies of diving animals have been misleading. These positive correlations in the penguin clade may tell us about how diving behaviour and *M* covary evolutionarily within this taxonomic group. Larger penguin species have a *M* specific metabolic advantage, enabling them to dive for longer and deeper. Of course, a number of other variables are also likely to affect diving behaviour, such as the diving environment, physiological and behavioural adaptations and the use of different metabolic pathways during dives. The effects of these factors are discussed using specific examples.

Introduction

As a method of comparative analysis between diving vertebrates, allometric relationships between body mass (M) and diving parameters have been tested for in many taxonomic groups of divers (e.g. Boyd and Croxall, 1996; Schreer and Kovacs, 1997; Watanuki and Burger, 1999). These analyses are tests of hypotheses derived from the theory that a larger M enables an animal to dive for longer due to M specific metabolic advantages (*Chapter VI*). For instance, longer dives correlate with deeper dives since the ability to remain submerged for longer periods allows extended transit durations. Also, many species increase bottom duration with increased travel duration to enhance foraging efficiency (Wilson, 1991), which also increases overall dive duration (t_d). In turn, increased dive depths (d_d) and t_d may require increased surface durations (t_s) because of the greater oxygen debts to be repaid (Kramer, 1988). The presence of a number of allometric relationships have been claimed within and across taxonomic groups of diving animals by such aforementioned studies, despite a number of confounding factors on diving behaviour, such as water depth and the employment of anaerobic metabolic pathways by certain species (e.g. Weddell seals, Castellini et al., 1988).

Earlier studies sometimes used more limited databases of species compared to the present study due to a lack of available data on diving animals (an exception being Schreer and Kovacs, 1997). Because of the limited species sample sizes (n), either n within taxonomic groups was small or distantly related clades were combined to increase group n. However, recent technological advancements have resulted in a rapid increase in the amount of data available on a wide number of air breathing divers, which have been incorporated into the database of the current study.

Furthermore, previous studies analysing the effect of M on diving parameters have failed to account for the non-independence of species despite employing statistical techniques that wrongly assume that species are independent points in a multivariate space (Maddison, pers. comm.). In other words, such 'traditional' analyses assume

that the species included have no hierachical relationships, i.e. a 'star-shaped' phylogeny, which is rarely the reality. Modern comparative biology explains species traits as changes along the branches of a phylogenetic tree. Closely related species tend to resemble each other since they share many characters through common descent rather than through convergent or parallel evolution (Harvey and Pagel, 1991). Thus, to understand causes, comparisons must be directed along the lines which evolution occurred (Garland, pers. comm.). Repeated convergence towards a particular association of traits, such as high M and deep diving, suggests that these characteristics may be fundamentally linked, but a single origin of a pattern provides no basis for the testing of statistical hypotheses (Purvis and Harvey, 1996). Therefore, strong correlations can simply be artefacts of the non-independence of species and show a statistical significance because standard errors are underestimated due to overestimating the degrees of freedom (Type 1 error). Consequently, phylogenetic relationships can show that there is little association between traits where traditional approaches have recorded a correlation. Thus, while analyses of variables using phylogenetically independent contrasts (PIC) do not always produce equations very different to those obtained using traditional analyses (e.g. Frappell et al., 2001), it is very important that phylogenetic relatedness is included in such analyses so that independent evolutionary events are considered. It should be noted here, however, that analyses of variables using PIC also has disadvantages (*Chapter VI*). Where phylogenetic information is limited, there can sometimes be a trade off between the N value (number of species) of the regression and the inclusion of phylogenetic information (*Chapters VI* and *VII*).

The present study includes 'traditional' across species regression analyses to test for correlations of dive parameters and M. The same data are re-analysed with regressions that take account of phylogeny within and between taxonomic groups (regression analyses of independent contrasts). This method is derived from a model designed to make independent contrasts between pairs of taxa at each node of a bifurcating phylogeny (Stone and Purvis, 1992). Two species below a node share a common

phylogenetic history to the point at which they split from a common ancestor. Therefore, any differences between them will be due solely to independent evolutionary change. Bias caused by the inclusion of particularly speciose taxa is also prevented. This method could affect the exponents of relationships generated by the traditional regression techniques or show that some relationships found to be present using the traditional approach are false, or even find that certain new relationships are present.

The primary objective of the present study, therefore, is to discover which taxonomic groups of aquatic vertebrates show valid correlations between dive parameters and M. In order to test for these correlations, relationships between M and multiple diving parameters are analysed within and across taxonomic assemblages, using linear regressions both including and excluding phylogenetic interrelatedness. These analyses allow the validity of previous studies to be tested, which have generally suggested a significant influence of M on diving behaviour within and between a wide range of taxonomies. The secondary objective of the study is to explore the main factors that can confound with these relationships between M and dive parameters. Three major confounding factors are flagged up during the Results and Comments and are considered in more detail in the Discussion. Anatomical and physiological confounds, and then ecological and environmental confounds, are considered using multiple example species from different taxonomic groups. Thirdly, the complications in assessing diving ability based on oxygen stores due to the use of anaerobic pathways by certain species are discussed, in particular with regards to the particularly strong relative diving ability of alcids in comparison to other avian divers. Given the limitations of the available diving data and data on phylogenetic relationships, these analyses should be considered as exploratory and the conclusions preliminary.

Methods

This study uses a database of diving parameters for a variety of air breathing vertebrate divers (*Appendix II*, Table AII-1). Data points were obtained from a literature search of published material, which was supported by correspondence with the authors, as well as from unpublished data. Data were recorded on *M* and a number of variables quantifying diving behaviour. Table AII-1 incorporates information on several 'main' taxonomic groups, defined as those taxa that included more than seven species. These groups were placed in traditional analyses and analyses of independent contrasts, both individually and in combinations, e.g. all 'bird groups'.

The four main species groups were the pinnipeds, cormorants, alcids and penguins. The cormorants are foot-propelled divers (Watanuki et al., 1996) that mostly feed on benthic prey such as bottom feeding fish and molluscs (www.antarctic.com.au.encyclopaedia / bio / Cormorants.html). The alcids are wing-propelled divers (Lovvorn and Jones, 1994), which often pursue their prey, usually small fish, through the water column (www.listgal.com/guillemot.html). Penguins are also wing-propelled divers that pursue prey during dives, however in contrast to the alcids, they cannot fly and might therefore be expected to be especially well adapted to aquatic locomotion. Their inability to fly might also affect how many times they can bring back food to their young, thus affecting how much food they attempt to catch during each foraging trip (Prince and Harris, 1988). Ducks and grebes form another clade, unfortunately incorporating too few species to be included separately in analyses. They are generally foot propelled, non-plunge divers that perform flat-bottom rather than bounce dives (Wilson, 1991). Table AII-1 also contains information on a number of secondary signal species that were contrasted to the main groups in the traditional regressions.

Developing the database of diving parameters

Masses of the species were often present in the literature and means were used when possible although sometimes midpoints of ranges were taken. If data on *M* were not present then they were either provided by authors or taken from alternative published sources (cited in Table AII-1). Mean dive durations were obtained from the literature, or calculated as accurately as possible from histograms or tabular data (e.g. Le Boeuf et al., 1988; Boyd and Croxall, 1996; Croxall et al., 1988; Kooyman et al., 1992). Other data were not always stated in the papers but could sometimes be calculated from tables or graphs, or were made available on request by the authors. Where short dives were known to be non-foraging dives they were discounted from these calculations. Mean surface durations were likewise obtained or calculated where possible, however such data were less common. Mean dive depth was also obtained or calculated when possible. Finally, the mean maximum dive duration (the mean of the single deepest dive of each individual; d_{dmax}) was taken from the data when available.

Some species have high sexual dimorphism. For such cases, where data were reported for only one sex (e.g. DeLong and Stewart, 1991), the sex was noted and in the traditional regression analyses, the data set was treated as independent from any data on the same species and other sex. For the analyses of independent contrasts, the data for a single sex was treated as the data representing that species, or where data on the two sexes were available, they were averaged to produce a single data point for that species.

The majority of data found in the literature were added to the database, however where values were deemed to be insufficiently valid or robust then the data for that species were not included. Data were not included from studies where water depths for the particular population observed were deemed to be unusually shallow for that species (e.g. Cooper, 1986) and where data collection techniques were considered

dubious. For example, Piatt and Nettleship (1985) recorded the depths at which individual alcids were found caught in fishing nets. Finally, no laboratory based data was used (e.g. Carbone et al., 1996) due to the prominent confounds that experimental work often introduces into the behaviour of species. In some cases, within-species seasonal or locational variations in diving behaviour were reported (e.g. Williams et al., 1992; Rodway, 1998). In such instances, decisions were made separately for each paper about how particular variables should be calculated. Finally, Table AII-1 holds data for several cormorant species taken from Cooper (1986). Where multiple field observations were recorded for the same species at different locations, an average was calculated from the most valid data points for each diving parameter. Valid data points were considered to be those where a sufficient sample size of dives was recorded (> 25 dives).

Phylogenetic relationships

For the regression analyses of independent contrasts, phylogenetic relationships for each main taxonomic group were produced using phylogenetic data from multiple papers (Fig. AII-1 to Fig. AII-4). Multiple phylogenetic trees on different subsets of a main taxonomic group were combined into one tree for the clade by connecting branches together e.g. true seals (Phocidae) and eared seals (Otariidae). Where the position of particular species differed between papers, the conclusions of the most recent paper were used. However the phylogenetic positions of a number of species in Table AII-1 could not be ascertained. These species were therefore not included in the regression analyses of independent contrasts.

Bivariate statistical analyses

Relationships between M and various dependent variables were fitted using linear regression (least squares). All relationships tested for were allometric so all variables were naperian log transformed prior to linear regression analysis. The distribution of

the data was not tested for normality, as has been the case with other allometric studies of diving behaviour (Boyd and Croxall, 1996; Schreer and Kovacs, 1997; Watanuki and Burger, 1999) along with many allometric studies on other physiological parameters (e.g. Tieleman and Williams, 2000). Therefore, any interpretation of the analyses must be done cautiously (Schreer, pers. comm.). The degree of error on linear regression slopes depends on the assumption of normality, which limits comparisons between slope gradients and intercepts (Boyd, pers. comm.). This is also true for the test of significance of a regression since the r^2 value assumes normality. Further, the statistical analyses were often performed on small n values

All traditional statistical analyses were conducted using SPSS (*v.* 10). This approach employed least squares regression techniques following naperian log transformation of the data to obtain a power equation ($Y = xM^b$), where Y is a diving parameter and M is the mass of the species in kg. The allometric M exponents of mean t_d, mean d_{dmax}, and mean t_s were calculated. Main groups were only placed in a particular traditional regression analysis where the number of species with a data point for both variables was greater than seven. Data points from species that were not placed into the main groups were not included in the analyses but were often used as secondary signals to provide contrasts in the traditional analyses, for example data on green turtles, *Chelonia mydas*, and the albatross family, *Diomedeidae*.

For regression analyses of independent contrasts, the naperian log transformed data were then converted to PIC and analysed using CAIC*v.*2 (Purvis and Rambaut, 1994). Regression analyses were performed using the 'crunch' algorithm since the characters being tested were continuous rather than dichotomous. All branch lengths were assumed to be equal. Regressions of contrasts in one variable against contrasts in another were forced to pass through the origin and the significance tests accounted for this loss of a degree of freedom.

Slopes of traditional regression equations within the present study, and between the present study and previous studies, were compared using *t* tests. Diving variables between clades accounting for *M* were also compared using *t* tests.

Results and Comments

Dive duration and body mass

Traditional, across-species regression of naperian log mean t_d and *M* (Fig. V-1) shows a significant relationship for cormorants, penguins, pinnipeds, the three main bird groups combined and all the main groups combined (Table V-1). Only the alcid clade did not show a significant correlation.

Alcids are often considered to be the North Atlantic equivalent of the penguins, since they are morphologically similar and exploit similar prey (Piatt and Nettleship, 1985). On a unit *M* basis, however, alcids have longer t_d than penguins, despite penguins being flightless and thus supposedly more able to adapt as specialised divers (*t* test, *P* < 0.001). This paradox is yet to be satisfactorily explained. However, one suggestion is that since many alcid species dive deeply, they have less adaptational conflicts between flying and diving than shallower divers because they have to work less hard against buoyancy due to reduced hydrostatic pressure (Lovvorn and Jones, 1994). Perhaps the parsimonious explanation is that alcids sometimes utilise anaerobic metabolic pathways, thus considerably increasing their dive durations. Indeed, Croll et al. (1992) recorded thick-billed murres diving deeply and spending enough time underwater that they were calculated to have to employ anaerobic metabolism during the period of submergence. See *Chapter VI* for further discussion on anaerobiosis. For discussions the physiological comparisons between alcids and other diving birds, see Watanuki and Burger (1999) and *Appendix III* '*Ultimate Divers*' and '*Take a deep breath…*'.

In the traditional analyses shown in Fig.V-1, the regression lines for penguins and cormorants lie very closely together and have very similar slopes ($t_{22} = 0.26$, $P = 0.81$) and the pinniped regression line also has a very similar slope (vs. penguins, $t_{31} = 0.196$, $P = 0.95$; vs. cormorants, $t_{30} = 0.33$, $P = 0.86$). This might indicate that similar underlying factors are influencing t_d in these taxonomic groups. This is in contrast to the alcids, which do not show a positive correlation in the traditional analyses, rather, the data points for the alcid clade are quite distinct from the pattern apparent for the other main taxonomic groups. The factors underlying t_d for the alcids may therefore be distinct from those of the other bird clades and the pinnipeds. This is discussed in more detail later.

A simple regression of mean t_d on M shows that t_d of ducks and grebes are similar to those predicted by extension to lower M of the relationship across penguins ($t_{15} = 2.13$, $P > 0.05$). Evidence from the literature indicates that the majority of penguin species rarely, if ever, exceed the limits of their calculated aerobic dive limits (cADL; e.g. Kooyman and Kooyman, 1995; Bethge et al., 1997; Kirkwood and Robertson, 1997). Since cormorants have similar t_d to M ratios as penguins, they may also tend not to exceed their cADLs, even when inhabiting deeper water. Unfortunately, very few data on cADLs are available on cormorants to confirm this. In laboratory experiments, duck species have not been recorded exceeding their cADLs, even in situations involving unusually long travel distances to obtain food (e.g. tufted ducks, *Aythya fuligula*; cADL of ~50 s v. maximum recorded t_d of 45 s; Butler, 1991b, Stephenson et al., 1986). The duck family *Anatidae* are often perceived as being rather poor divers (e.g. Butler, 1991a) due to their short mean t_d and poor t_d to M ratios. However, their t_d is very similar to those predicted for their M according to the allometric relationships for penguins and cormorants. Furthermore, their short t_d may be influenced by the shallow inland waters that they often occupy. They may well dive for longer if they inhabited deeper water, without necessarily using anaerobic pathways.

Fig. V-1 shows that no taxonomic groups, outside of the anomalous albatross family, have species with mean t_d below around 15 s. The little penguin *Eudyptula minor,* the common guillemot, *Uria aalge,* the long-tailed cormorant *Phalacrocorax africanus* and *A. fuligula* represent the shortest known diving species from the three avian taxonomic groups, the first two both diving for around 20 s on average and the latter two for around 17 s. These values may approximate to the minimum viable foraging duration for a bird preying in the benthic or pelagic zones.

This is as far as the data regressed in the traditional analyses can be analysed. The slopes of these analyses represent correlations generated by taxonomic artefacts and thus are invalid as tools for discussing causal or functional questions about the taxonomic groups, such as suggestions of similar physiologies. For instance, Boyd and Croxall (1996) suggested that because the mean t_d of female elephant seals lay very close to the regression line for the t_d of seabirds, this particular seal species may therefore use a physiological strategy more similar to seabirds than to other pinnipeds. However, the study used spurious results from invalid regression analyses and so their discussion about causal possibilities for a relationship between M and t_d was not valid.

Some of the secondary signal data on individual species provides interesting comparisons, however, to the regressed data of the main groups. The two largest whale species present in this study (the Northern bottlenose whale, *Hyperoodon ampullatus,* and the sperm whale, *Physeter macrocephalus*) have very large mean t_d. However, their data points are on or right of the regression line for all species (Fig. V-1) indicating that they tend to dive for only relatively average or even short durations when accounting for M compared to a multitude of other diving species. This is also seen in baleen whales, which also dive for relatively short durations, probably because they have high energy costs during foraging (Croll et al., 2001; Acedvedo-Gutierrez et al., 2002). The Pantropical spotted dolphin, *Stenella attenuate*, has a similar M to the harbour porpoise, *Phocoena phocoena*, but has considerably shorter dive durations. This may well be attributed to considerably lower myoglobin levels in skeletal

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muscles of *S. attenuata* and / or to differences in dive patterns based upon the duration of day (Baird et al., 2001).

C. mydas, as with other marine turtle species, often undertake resting dives; they sit at the sea bottom with a close to neutral buoyancy, which occurs at around 20 m (Hays et al., 2000). This behaviour in part explains the long t_d in comparison to d_d (see Fig. V-1). Their relatively smaller brains in comparison to mammalian divers may also afford them an evolutionary advantage for diving, since this organ thus requires less oxygen. However, the overriding reason for their long t_d is the fact that, unlike all other species discussed in this study, *Chelonia mydas* is an ectotherm, and therefore has a metabolic rate about 10 % of that of similar sized mammals.

The sea otter (*Aonyx capensis*) has a very short t_d compared to *M*. Whether this represents a physiological limit or simply a limit imposed by the environment is unclear. *A. capensis* forages for food on the seabed but only in areas where the waters are very shallow i.e. several metres at most. Short dive depths may only require a short t_d, particularly since *A. capensis* surfaces after each catch to process its prey. If this species were in fact able to stay comfortably submerged for much longer than its normal t_d, this data would indicate a particularly acute environmental effect on the diving behaviour of the species.

The albatross species *Diomedea cauta* and *D. chrysostoma* both have particularly low t_d in comparison to any other taxonomic group in this study. This is intriguing since they are known to pursue prey underwater during 'swimming dives' (Hedd et al., 1997) with similar characteristics to some alcid species, which are much smaller birds but dive for much longer durations. Again, then, the question arises as to whether such short a t_d is due to environmental or physiological factors. Water depth is certainly not a limiting factor in this case, however if the target prey of *D. cauta* and *D. chrysostoma* inhabit the upper part of the water column then shallow dives would be optimal and likely to be associated with a short t_d. Alternatively, are albatrosses

limited physiologically or anatomically to a short t_d? Albatrosses primarily use their wings (half folded) to row through the water (Wilson, pers. comm.) One possibility is that their wings are poorly adapted for this task since they are designed for soaring flight. In comparison, alcids flap their wings during flight and may therefore have been able to evolve wings at an adaptive 'mid-point' between aerial and sub-aquatic locomotion that is more successful for the latter than have albatrosses. Albatrosses may subsequently expend very high levels of energy while pursuing prey, metabolising their oxygen stores very rapidly and thus needing to surface quickly, which would be predicted by the oxygen store / usage hypothesis (see *Chapter VI*).

When the similarities between species due to shared ancestry are controlled for using independent contrasts, the relationships between naperian log M and naperian log mean t_d in the three main birds groups combined and all the main groups combined (Fig V-5) are still significant (Table V-2). However, we should not interpret the across-taxa slope as necessarily saying anything about the way that mean t_d and M have shown correlated evolution within taxa. The analyses of independent contrasts indicate fewer significant relationships overall than the traditional analyses since the majority of individual groups did not show significant regressions. The results of the traditional analyses conducted in earlier studies of dive parameters across taxonomic groups (Burger, 1991; Boyd and Croxall, 1996; Schreer and Kovacs, 1997; Watanuki and Burger, 1999) can therefore be considered to be often misleading.

The penguin clade is the exception since the regression analysis remains significant when regressing contrasts (Fig. V-2), demonstrating that the correlation is not a taxonomic artefact. The nature of the statistics may offer an explanation for the presence of a significant correlation within the penguin clade but a lack of significance for other clades. The taxonomic groups not showing a significant relationship may have had too small n values or a lack of range of M in comparison to the degree of scatter around the regression line. However, while the alcids and cormorants offered a truncated range of M in comparison to the penguins, along with

smaller *n* values, this was not the case with the pinnipeds, which still did not show a significant correlation.

The positive correlation between *M* and mean t_d in penguins may tell us about how these two characters covary evolutionarily within this taxonomic group. It offers evidence that larger penguin species have a *M* specific metabolic advantage. That is, larger penguins take advantage of their high blood to body size ratio and low metabolic rate to *M* ratio to store more oxygen and consume it more slowly and so are able to dive for longer. A similarity in physiologies between penguin species with little variation in life history traits, other than size, would increase the likelihood that the correlation would be apparent from the regression. If this is the case, large species, such as the emperor penguin, are similar to overgrown versions of smaller species such as the little penguin.

In contrast, alcids, cormorants and pinnipeds may vary physiologically far more than the penguins such that other life history traits mask the advantage that *M* affords. Alternatively, they may be affected more than the penguins by the environments in which they forage, which could influence t_d more than *M* and again mask this effect. Such environmental factors include different water depths and prey availability.

Maximum dive depth and body mass

Due to the bimodality of diving exhibited by a large number of vertebrate divers, measures of central tendency of t_d could be somewhat misleading. A value representing the mean d_{dmax} of a species may reflect physiological dive limits, which could reduce the error around a regression statistic and would be informative with regards to understanding divers and their ecologies (see *Chapter VI*). Hence mean d_{dmax} was regressed against *M*.

In the traditional regression analyses (Fig. V-3), mean d_{dmax} was related to M in penguins and pinnipeds but not in alcids. There were also significant correlations between these variables in the three main bird groups combined and all main groups combined (Table V-3).

Alcids dive more deeply than penguins accounting for M ($t_{19} = 3.76$, $P < 0.01$) and more deeply than pinnipeds ($t_{29} = 4.73$, $P < 0.001$). Penguins also dive more deeply than pinnipeds ($t_{30} = 8.93$, $P < 0.001$). If mean d_{dmax} indicates diving capacity then alcids should be considered particularly good divers, supporting the measures of mean t_{d}. Again, the parsimonious explanation is that alcids utilise anaerobic pathways whereas other taxonomic groups do not, or at least less extensively. Certainly the data points for the alcids are again quite different to the patterns for the other main groups. Boyd and Croxall (1996) suggest that some seabirds exceed their cADLs much more frequently than pinnipeds and that alcids may regulate oxygen consumption differently depending on t_{d}. There seem at present to be no physiological explanations for why penguins can dive for longer than pinnipeds on a M specific basis. Boyd and Croxall (1996) argue that penguin species do not use anaerobic metabolic pathways during extended dives and multiple studies on penguin species have concluded that they rarely, if ever, exceed their cADLs (see earlier).

The values for ducks and grebes lie very closely to an extrapolation of the regression line for pinnipeds towards the y intercept. This indicates that duck and grebe species are capable of making dives as long as pinniped species for their M, again offering evidence that ducks are good divers for their size.

While *S. attenuata* had lower than expected mean t_{d} according to M, a comparison of mean d_{dmax} to other species indicates that this species closely matches predicted diving capacity based on M (Schreer and Kovacs, 1997). Since the physiological limits of this dolphin species are as expected, the low mean t_{d} value is probably due to

the environmental factor of dive patterns at certain times of the day rather than the physiological factor of myoglobin stores.

The analyses of independent contrasts show significant relationships for all the main bird clades together and all the main groups together. Again, the penguins are the only single taxonomic group showing a significant correlation (Fig. V-2), indicating that the correlation is not a taxonomic artefact for this clade, but is likely to be for the other clades. However, the r^2 values of individual clades in the traditional analyses are high enough to suggest that noise within the data is minimal. Therefore, some of the individual groups may not be showing significant relationships simply because n values are too small, rather than because of a lack of correlation between the contrasts of mean d_{dmax} and M.

Surface duration and body mass

A relationship between t_s and t_d is predicted by an optimal foraging depth model (Mori, 1998), which anticipates that larger divers should make deeper and longer dives and stay longer on the surface than smaller divers. Furthermore, the 'surface law hypothesis' dictates that the ratio between lung surface area and M is smaller in larger animals and so the oxygen loading rate is lower, causing larger animals to surface for longer (Mori, 1998). This relationship is shown in a number of diving species. For example, Watanuki et al. (1996) found that post-dive t_s was significantly correlated with t_d in the Japanese cormornant, *Phalacrocorax capillatus*. Hays et al. (2000) recorded a direct proportionality between t_s and t_d in *C. mydas*, despite a very high dive:pause ratio (*d:p*) of approximately 25:1. Thompson and Fedak (1993) reported an isometric linear function between t_d and t_s in grey seals, *Halichoerus grypus*, with a *d:p* of approximately 5:1, however only up to a certain t_d. Brünnichs guillemots, *Uria lomvia* also demonstrates isometric linear proportionality (Croll et al., 1992) but again only for short t_d. In the present study, a relationship was tested for between mean t_s

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and M, which would be expected in taxonomic groups where t_d and M are related and t_d and t_s are related.

In a model explaining variation in naperian log mean t_s in terms of naperian log M (Fig. V-4), correlations were significant in the cormorants and the pinnipeds along with all the main group combinations (Table V-5). The clear contrast in the slopes for pinnipeds and cormorants is discussed in *Chapter VII*.

Boyd and Croxall (1996) did not find this relationship in pinnipeds, probably because they used multiple measures of central tendency and also because they had a smaller data set. However, the correlation for pinnipeds in the present study disappeared when phylogenetic relatedness was accounted for. The n value and range of M were both large enough to indicate that the effects of M may have been masked by other life history traits. Indeed, a significant correlation was only obtained when all the main groups were combined in the regressions of independent contrasts (Table V-6, Fig. V-5).

Despite the minimal data available on t_s in penguins (therefore not included in Fig. V-4), according to traditional regression analysis, it appears that alcid species generally have considerably higher t_s when accounting for M than do penguins ($t_{11} = 2.76$, $P < 0.05$; Fig. V-4). This is further evidence that alcids dive anaerobically more often than penguins (which may not do so at all) since lactate generated from anaerobic metabolism is slow to clear and thus anaerobic dives require longer subsequent t_s than completely aerobic dives. Furthermore, the lack of a relationship between t_s and M in alcids may be because t_s required by such species is the result of a more complex set of factors than for purely aerobic divers, who are highly dependent upon their oxygen stores and thus might be more clearly affected by oxygen replenishment during the surface portion of the dive cycle.

Discussion

Comparing regression techniques

For each set of regressions between a diving parameter and *M* in the present study, controlling for phylogenetic effects using independent contrasts produced different results to those obtained in the traditional, across species analyses. In all cases, there were fewer significant correlations when phylogenetic effects were accounted for. However, often the slopes were similar after phylogenetic effects had been controlled for. In many instances, the apparent correlations across species are probably phylogenetic artefacts, although small *n* values and truncated ranges of *M* may have concealed correlations in some cases. In all but one instance of significant correlations in both regression techniques, the percentage of variance accounted for by *M* was higher in the traditional analyses.

Confounding variables

Traditional regression analyses and regression analyses of independent contrasts of diving parameters against *M* have shown up a number of significant correlations. These correlations have been analysed and commented upon within the Results and Comments section. However, a number of relationships tested for in this study were not significant, while many significant relationships had considerable scatter around the regression line. Thus, variables other than *M* are imparting considerable influence upon the diving behaviour of many species. From the literature search conducted in conjunction with this study, three key factors appear to be particularly influential on diving behaviour other than *M*, namely environmental and ecological factors; physiological, anatomical and behavioural adaptations; and the use of different metabolic pathways. All have been discussed briefly within the Results and Comments section and are considered in more detail here.

Physiological, anatomical and behavioural confounds

Some species may have the potential to dive deeper and for longer durations because of M specific metabolic advantages, but their diving capacity is limited in other ways. Two sibling murre species provide an example of anatomical and behavioural effects on diving performance. *U. lomvia* is known to feed on the sea bottom, in comparison to *U. aalge* that eats pelagic fish. This difference is consistent with theories based on anatomical comparisons (e.g. Spring, 1971) showing that *U. lomvia* is adapted to deep diving while *U. aalge* is better adapted to pursuit swimming (Bradstreet and Brown, 1985). Theoretically, for a given oxygen store volume, and assuming similar metabolic rates, a pursuit diver is unlikely to be able to dive for as long as a benthic feeder since the former tends to perform more energetic locomotion while submerged and benefits less from a decrease in buoyancy due to depth. Indeed, these behavioural and anatomical differences result in very different modal t_d between the two species, with *U. lomvia* generally diving many times longer than *U. aalge*, despite little difference in M.

A second example is given by comparing Weddel seals, *Leptonychotes weddelli*, and female Southern elephant seals, *Mirounga leonine*, that have similar M, however the latter consistently dive for deeper and longer (Castellini et al., 1991). Elephant seals have had to adapt to not having the option to haul out of the water at will, particularly during migrations to feeding grounds, probably by increasing their ability to remain submerged for longer durations.

Peters et al. (1998) suggest that substantial differences in diving energetics between closely related species may indicate that behavioural traits are more influential than morphological characteristics. Puffins, *Fratercula arctica* undertake shallow dives of short t_d, even when taking M into account, but swim very rapidly while submerged in pursuit of their prey for up to 50 m (Bradstreet and Brown, 1985). In contrast, black guillemots, *Cepphus grylle*, are less than 0.5 kg and have been reported staying

underwater for well over a minute at a time, which probably reflects an adaptation, perhaps to a metabolic reduction or a tolerance to lactate, to cope with foraging in pack ice where there may be few openings to surface (*Appendix III 'Ultimate Divers'*). Acevedo-Gutierrez et al. (2002) propose that the high energetic costs of lunge-feeding undertaken by blue whales (*Balaenoptera musculus*) and fin whales (*B. physalus*) confine them to very short t_d relative to other large whales with more energy efficient foraging behaviours.

Environmental and ecological confounds

Intraspecific differences in diving behaviour have been regularly recorded, often due to environmental differences between populations (e.g. Bradstreet and Brown, 1985; Cooper, 1986; Rodway, 1998; Williams et al., 1992; Wanless et al., 1993; Hyvarinen et al., 1995; Schreer and Kovacs, 1997). Such factors can produce considerable variation in the behaviours of different species populations, or in the same individuals in different seasons.

Animals foraging underwater would be expected to optimise their foraging efficiency by not only maximising the proportion of time spent underwater (Houston and Carbone, 1992) but also by taking into account the water depth of highest prey density (Mori, 1998). Thus a species population inhabiting a niche environment where viable prey exists at a different depth to their normal d_d will adjust their diving behaviour (Dewar, 1924). *Alle alle* will normally dive to around 15 m during the night when the zooplankton upon which they feed is most dense around that depth. During the day when the zooplankton is at the water surface, d_d dramatically decrease (Bradstreet and Brown, 1985). At Guba Arkangel's skaya, males tended to forage during the 'day' and females at 'night' (Golovkin et al., 1972) and thus, despite minimal sexual dimorphism, females perform much deeper diving bouts than males. King et al. (1998) observed nocturnal foraging in double-crested cormorants (*Phalacrocorax auritus*), a very rare behaviour in the cormorant family and probably induced by easy

exploitation of a food source existing at a shallow depth at night. Seasonal variations can also be significant, probably due to factors such as water temperature, prey distribution, breeding times and day lengths. For example, patterns of diving depth were found to vary significantly at different times of the year in *L. weddelli* (Castellini et al., 1991). Longer t_s in the freshwater turtle, *Rheodytes leukops*, during summer is explained by the decrease in oxygen concentration in the water and increase in metabolic cost due to the higher water temperature, causing a switch from facultative to obligate air breathing (Gordos et al., 2003).

The walrus (*Odobensus rosmarus rosmarus*) is a very large pinniped but undertakes only short and shallow dives. While walruses are likely to be able to dive for longer than they have been recorded, they have no need to because they prey on abundant benthic prey in shallow waters (Wiig et al., 1993; Schreer et al., 2001). Unless walruses have survived in an environment, which evolutionarily, has only recently changed in terms of the water depth that prey are available, their diving behaviour indicates that their large size is not an adaptation to prolonged t_d. Their presumed ability to dive for long periods is thus a 'spandrel' (Gould and Lewontin, 1979) of some other adaptive advantage of size. Is this also the case for large bodied deep divers? Did the large *M* of Emperor penguins, *Aptenodytes forsteri*, and Northern elephant seals, *Mirounga angustirostris*, evolve due to a selection pressure to dive deeply and for long durations or are they simply filling an available niche due to the adaptive spandrel of large *M*? They are almost certainly quite capable of surviving in an environment where water levels are much shallower so long as food is available, as has been demonstrated by ringed seals, *Phoca hispida*. This species inhabits a wide range of water depth as shown by the shallow dives of saimaa ringed seals (*P. h. saimensis* Nordq) compared to sea-dwelling ringed seals (*P. hispida*). In Fig. V-1, *P. h. saimensis* Nordq are on the right side of the regression line for pinnipeds, showing that pinniped species can still be successful when not diving to the depths and durations they are capable of as predicted by *M*. Therefore, increased *M* may not

always be an adaptation for diving behaviour but as a spandrel might be useful for some species by providing an option for deeper diving.

Consideration of the examples discussed generates the question: Would the diving behaviour of a taxonomic group of species be homogenous if they were studied in the same body of water with prey inhabiting the same depth in the water column, or would some species be too sub-optimal for that environment? In other words, do physiological or ecological factors have a bigger influence on diving behaviour? Mori (2002) produced diving models predicting that the depth of prey in a habitat determines predator body size even though environmental factors are influential. Peters et al. (1998) suggest that interspecific differences in the penguin genus *Spheniscus* may be nothing more than a manifestation of a flexible foraging strategy adopted by all the species. Investigations of this question require research comparing the actual depths obtained by species in their natural environments against the (unforced) physiological limits of submergence.

Theoretical aerobic dive limits

While larger divers might be expected to dive for longer than smaller divers due to lower metabolic rates relative to *M*, those species which can tolerate lactate accumulation can utilise anaerobic metabolic pathways while diving and are thus not constrained by their oxygen stores. Since very few studies have directly measured plasma lactate levels in conjunction with diving bouts (except in Weddell seals; Kooyman et al., 1980 and Castellini et al., 1988), most work on anaerobic diving is theoretical and highly speculative. Often, cADLs may be very different to true ADLs, partly due to a lack of understanding of the mechanisms used by diving vertebrates to reduce oxygen utilisation. Kooyman and Kooyman (1995), for example, recorded patterns of diving behaviour in *A. forsteri* indicating that their ADL was around twice that previously calculated.

Often, cADLs seem to be underestimates of true ADLs, partly due to underestimates of volumes of usable oxygen stores, for instance blood oxygen levels not being accounted for, or the oxygen volume in the trachea of bird species. Underestimates of ADLs can also be due to overestimation of metabolic rates, partly because of the variation in energy expenditure of diving at different depths due to changes in buoyancy (Wanless et al., 1993). Futhermore, there is probably a lack of appreciation of the mechanisms in diving vertebrates to reduce metabolic rates. Hypometabolism by heterothermy may serve to reduce metabolic rate through the consumption of cold foods and water (Butler, 1993; Boyd, 1997). Wilson and Culik (1991) and Woakes et al. (1995) found that abdominal temperatures were reduced in some seabird species during diving bouts, while Caputa et al. (1998) recorded selective brain cooling in Pekin ducks (*Anas platyrhynchos*). In contrast, however, stomach temperatures of some Phalacrocorax species remained constant during cold water dives (Gremillet et al., 1998) and the metabolic rates of common and thick-billed murres increased as water temperature decreased (Croll and McLaren, 1993), both probably representing thermoregulatory reactions. Circulatory adjustments during diving such as reduction of skeletal blood flow (Butler and Jones, 1982) may extend to areas of the body not yet fully appreciated. Diving vertebrates are able to tolerate more severe levels of hypoxia than purely terrestrial animals (Bryan and Jones, 1980), quite possibly to a greater degree than is presently realised. Some species may have further physiological adaptations and they may make use of different strategies to regulate their rate of oxygen consumption depending upon the t_d (Boyd and Croxall, 1996). Furthermore, aerobic and anaerobic metabolic pathways may be utilised simultaneously during some dives (Carbone and Houston, 1996). Thus it is very difficult to ascertain which species allow blood lactate accumulation, and how frequently, by comparing dive durations against a cADL.

Surface durations have also been used as evidence of the presence or absence of anaerobic dives during diving bouts, however their validity is presently unclear. Surface durations after anaerobic dives are often considered to increase with a power

function against t_d (e.g. Kooyman, 1989; Croll et al., 1992; Burger et al., 1993) because lactate accumulation is presumed to require prolonged t_s in order metabolise it. However, Wanless et al. (1993) found that dives by shags, *Phalacrocorax aristotelis*, became shorter as their dive bout progressed. Thompson and Fedak (1993) interpreted the lack of increase in surface duration in *H. grypus* after dives of about 7 min as representing a tolerance to lactate during subsequent dives. Butler (1991b) states that the sub-resting heart rate of tufted ducks during long horizontal dives suggests that blood lactate accumulates but it does not have a large inhibitory effect on exercise in tufted ducks because they can still undertake a number of dives in succession. However, a cumulative effect might exist since the number of bouts in a dive is lower than normal (Stephenson et al., 1986).

The proportion of dives in which a species chooses to utilise anaerobic metabolic pathways is probably very variable, from being the modal choice in *U. lomvia* (Croll and McLaren, 1993) to only occurring during very long, horizontal dives or forced submergence in *A. fuligula* (Butler and Woakes, 1982). Duck and grebe species may not normally have to prolong their dives due to the shallow waters that they tend to inhabit. An interesting question then is whether anaerobic dives offer the optimal diving pattern, in terms of the proportion of time spent underwater, for any species, or whether these dives are undertaken by certain species due to the necessity of staying underwater long enough to forage. Ydenberg and Clark (1989) argue that anaerobic diving strategies may be adaptive under certain circumstances, for instance western grebes, *Aechmorphus occidentalis*, will remain submerged beyond its ADL if by doing so it is likely to capture the prey it is pursuing. Furthermore, there may be a general trend of an increased use of anaerobic metabolism in smaller diving species as a way to push the physiological limits to compensate for size disadvantages (Mori, 2002). However, many researchers believe that diving seabirds seem to avoid anaerobic dives where possible (Burger, 1991) and some species may spend different proportions of their dives using anaerobic pathways depending upon the available prey and the water depth they inhabit. Cooper (1986) studied two populations of great

cormorants, *Phalacrocorax carbo*, and recorded a Canadian population averaging 51 s underwater while a New Zealand population averaged only 21 s underwater. The number of anaerobic dives are very likely to be much lower, and quite possibly negligible, in the New Zealand based population and would seem to be due to their inhabitation of waters less than 2 m deep.

In the present study, evidence from data on t_d, d_d and t_s have been used to argue that many alcid species are likely to utilise anaerobic pathways extensively during dives. In comparing their considerable diving ability against the larger penguins, which are considered to rarely, if at all, dive anaerobically, this is the most parsimonious explanation since other physiological differences between these two taxonomic groups do not predict the differences recorded (Watanuki and Burger, 1999). However, until more data are obtained from directly recording the level of plasma lactate in an individual during stages of a dive cycle and a dive bout, conclusions about anaerobiosis can only be based on indirect evidence. Consequently, the extent of anaerobic metabolism as a confound to correlations between diving parameters and M will remain unclear.

In summary, despite a plethora of variables influencing the diving behaviour of diving vertebrates, M often seems to correlate significantly with a number of diving parameters such as t_d, d_d and t_s. Therefore, M often accounts for a considerable amount of the variation in diving parameters between species. However, if the phylogenetic relatedness between species is accounted for, ensuring the independence of each data point placed in the regression, some of the relationships are no longer significant. In some cases, this may be due to low n values within taxa or truncated ranges of M, however it may be due to large physiological and behavioural variations between species in a clade, masking the effect of M. Nevertheless, the penguin clade still showed significant relationships between both t_d and d_{dmax}, and M, offering

evidence that penguin species have similar physiologies and thus M has a strong influence on diving behaviour.

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Table V-1. Allometric relationships between mean dive duration (t_d) and body mass (M) for various taxonomic groups

Taxonomic Group	N	Regression equation	SE_{slope}	r^2	t	P
Alcids	9	$t_d = 3.9M^{0.17}$	0.25	0.06	0.66	0.533
Cormorants	12	$t_d = 3.2M^{0.68}$	0.28	0.38	2.45	< 0.05
Penguins	12	$t_d = 3.5M^{0.63}$	0.08	0.86	7.67	< 0.001
Pinnipeds	20	$t_d = 2.5M^{0.65}$	0.18	0.41	3.52	< 0.01
Bird groups	33	$t_d = 3.7M^{0.40}$	0.08	0.48	5.32	< 0.001
All groups	53	$t_d = 3.7M^{0.42}$	0.04	0.74	12.07	< 0.001

Table V-2. Allometric relationships between the contrasts of mean dive duration and the contrasts of body mass for various taxonomic groups

Taxonomic Group	N	Regression equation	r^2	P
Alcids	9	$y = x^{0.30}$	0.17	0.305
Cormorants	6	$y = x^{0.61}$	0.29	0.268
Penguins	11	$y = x^{0.66}$	0.88	< 0.001
Pinnipeds	13	$y = x^{0.38}$	0.40	0.051
Bird groups	26	$y = x^{0.48}$	0.47	< 0.001
All groups	39	$y = x^{0.37}$	0.51	< 0.001

Table V-3. Allometric relationships between mean maximum dive depth (d_{dmax}) and body mass (M) for various taxonomic groups

Taxonomic Group	N	Regression equation	SE_{slope}	r^2	t	P
Alcids	7	$d_{dmax} = 4.1M^{0.36}$	0.18	0.44	2.00	0.102
Penguins	13	$d_{dmax} = 3.3M^{0.82}$	0.15	0.73	5.40	< 0.001
Pinnipeds[1]	14	$d_{dmax} = 1.9M^{0.72}$	0.24	0.44	3.07	< 0.05
Bird groups[†]	24	$d_{dmax} = 3.8M^{0.46}$	0.09	0.55	5.17	< 0.001
All groups[†1]	39	$d_{dmax} = 3.9M^{0.34}$	0.05	0.59	7.27	< 0.001

[†] These groups included data from the cormorant group.

[1] Data on the walrus (Wiig et al., 1993) was removed from this regression due to the particularly shallow depth of the water they inhabited.

Table V-4. Allometric relationships between the contrasts of mean maximum dive depth and the contrasts of body mass for various taxonomic groups

Taxonomic Group	N	Regression equation	r^2	P
Alcids	7	$y = x^{0.30}$	0.37	0.149
Penguins	12	$y = x^{0.67}$	0.55	< 0.05
Pinnipeds[1]	8	$y = x^{0.21}$	0.25	0.307
Bird groups[†]	21	$y = x^{0.41}$	0.38	< 0.01
All groups[†1]	29	$y = x^{0.40}$	0.44	< 0.001

[†] These groups included data from the cormorant group.

[1] Data on the walrus (Wiig et al., 1993) was removed from this regression due to the particularly shallow depth of the water they inhabited.

Table V-5. Allometric relationships between mean surface duration (t_s) and body mass (M) for various taxonomic groups

Taxonomic Group	N	Regression equation	SE_{slope}	r^2	t	P
Alcids	7	$t_s = 3.1M^{-0.14}$	0.652	0.01	-0.22	0.837
Cormorants	11	$t_s = 2.3M^{1.18}$	0.408	0.48	2.90	< 0.05
Pinnipeds[1]	17	$t_s = 2.8M^{0.37}$	0.152	0.28	2.44	< 0.05
Bird groups[†]	22	$t_s = 3.1M^{0.31}$	0.21	0.10	1.48	0.213
All groups[†1]	31	$t_s = 3.1M^{0.30}$	0.04	0.57	7.04	< 0.001

[†] These groups included data from the penguin group.

[1]Data on the walrus (Wiig et al., 1993) was removed from this regression due to the particularly shallow depth of the water they inhabited.

Table V-6. Allometric relationships between the contrasts of mean surface duration and the contrasts of body mass for various taxonomic groups

Taxonomic Group	N	Regression equation	r^2	P
Alcids	7	$y = x^{0.61}$	0.17	0.416
Cormorants	6	$y = x^{1.99}$	0.29	0.270
Pinnipeds[1]	11	$y = x^{0.46}$	0.26	0.164
Bird groups[†]	17	$y = x^{0.70}$	0.19	0.100
All groups[†1]	28	$y = x^{0.41}$	0.25	< 0.05

[†] These groups included data from the penguin group.

[1]Data on the walrus (Wiig et al., 1993) was removed from this regression due to the particularly shallow depth of the water they inhabited.
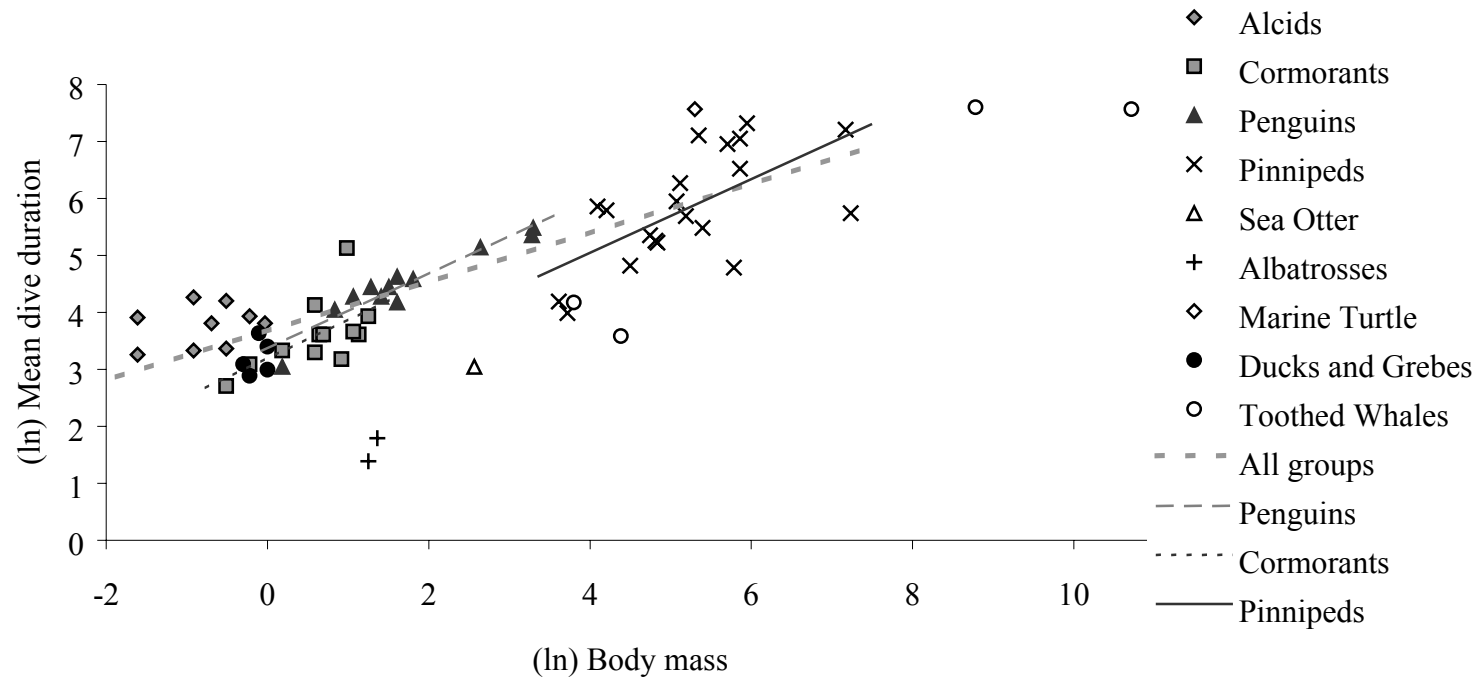
Figure V-1

Allometric relationships on naperian log scales between mean dive duration and body mass of the main taxonomic groups. Best fit lines are included for groups that showed a significant relationship and labels are included in the legend for clarity. Secondary signal species are also included. The trend line for all main bird groups combined has not been included for clarity.
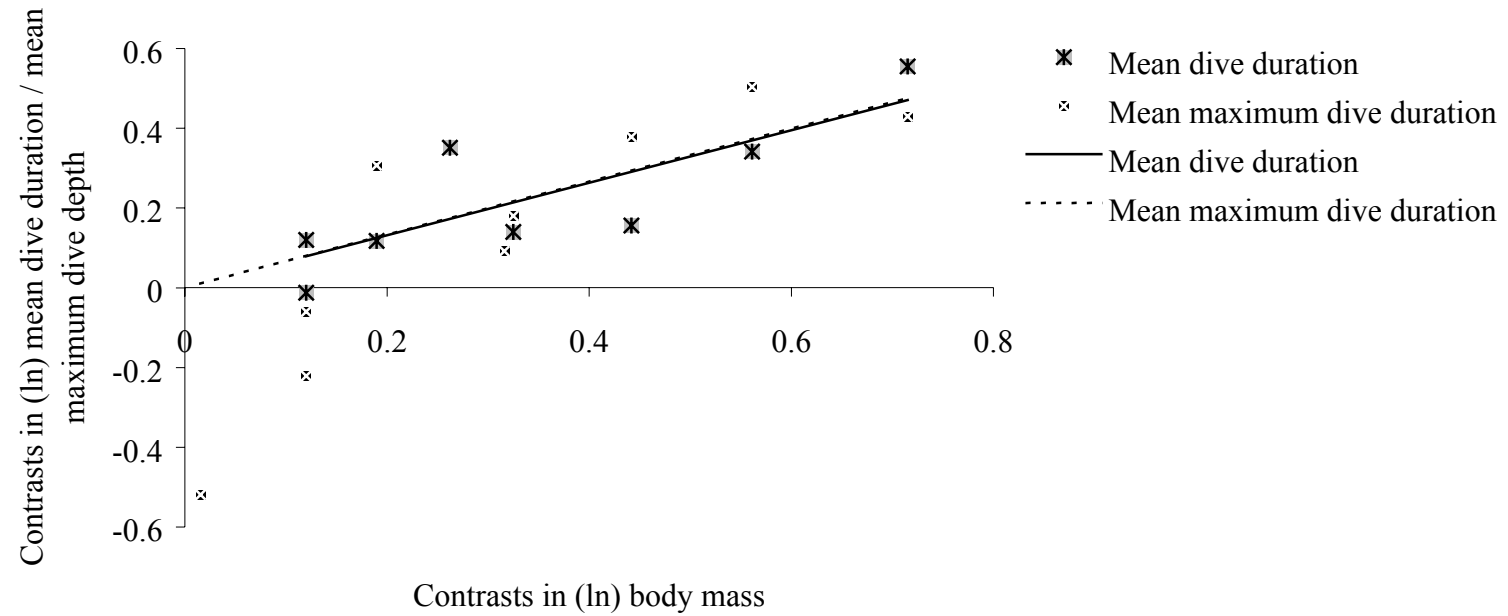
Figure V-2

Relationships on naperian log scales between contrasts in mean dive duration and mean maximum dive depth against body mass (*M*) of the penguins. Best-fit lines are included for the two dependent variables regressed against *M*.
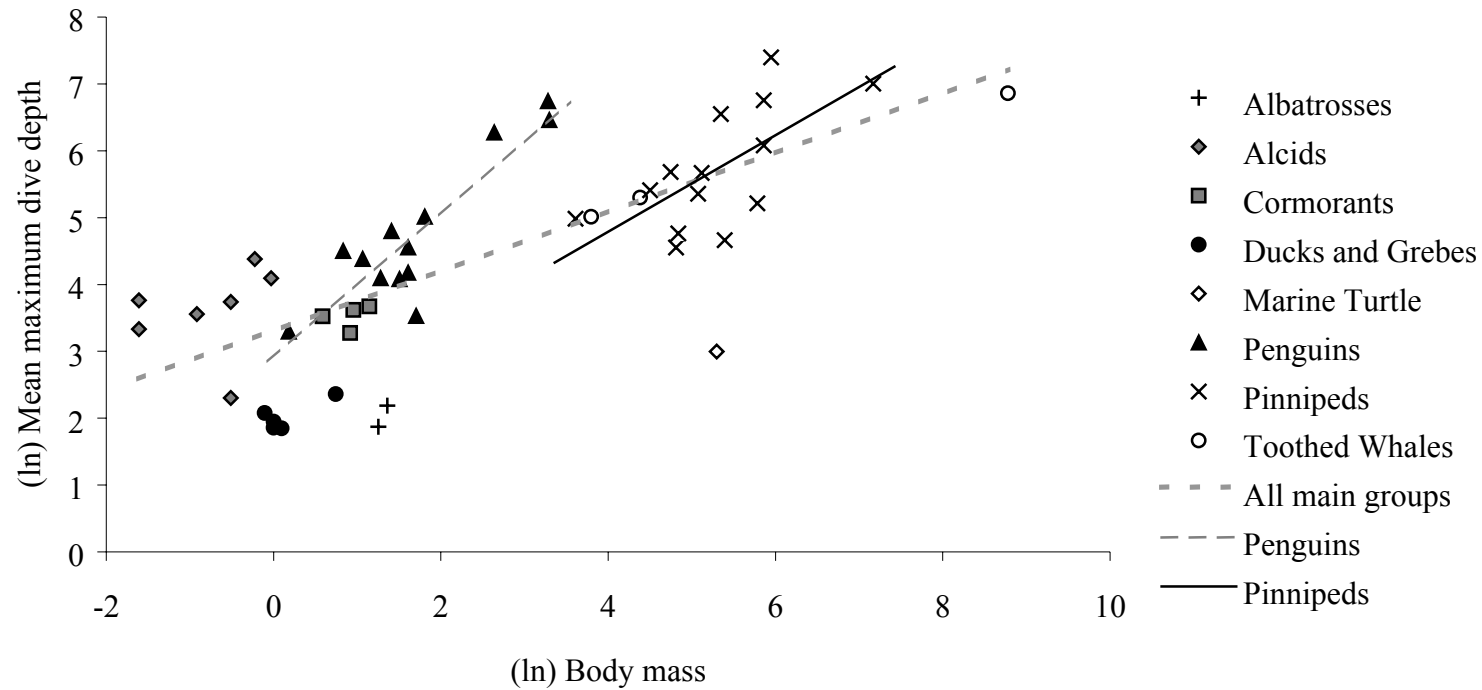
Figure V-3

Allometric relationships on naperian log scales between mean maximum dive depth and body mass of three of the main taxonomic groups. Best-fit lines are included for groups that showed a significant relationship. Secondary signal species are also included. The trend line for all main bird groups combined has not been included for clarity.
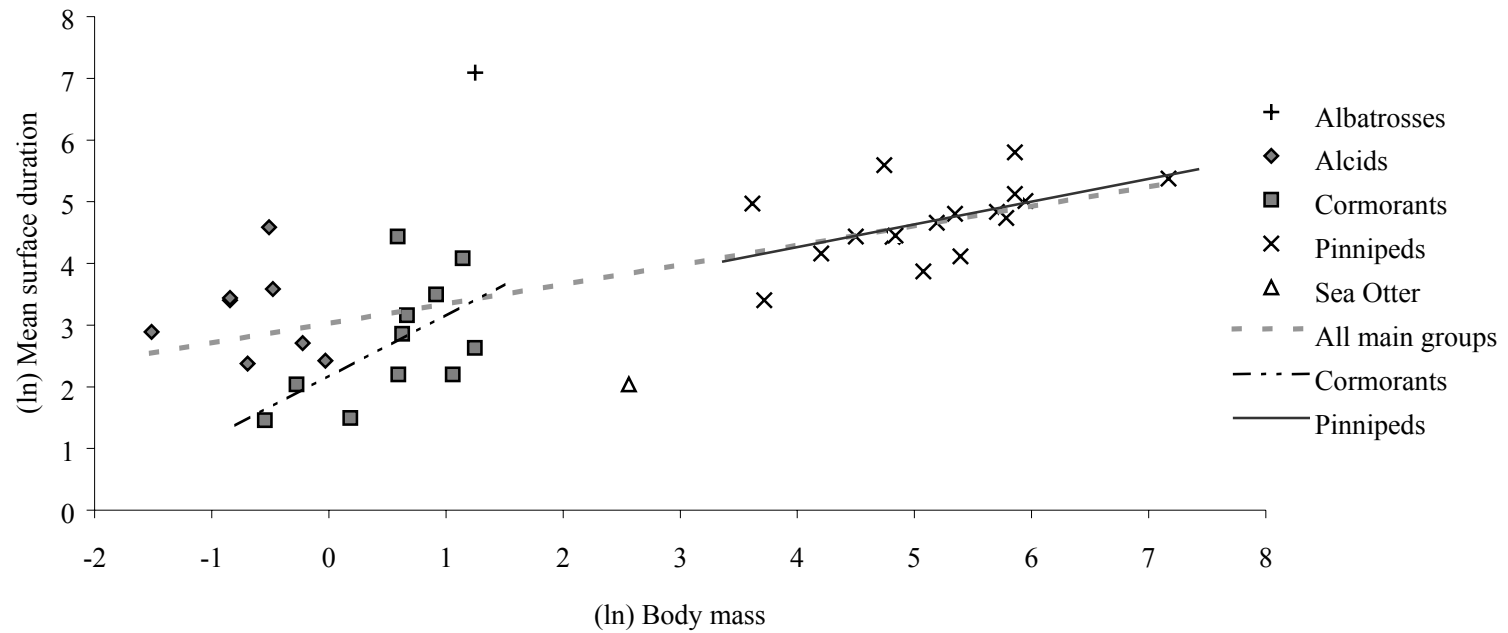
Figure V-4

Allometric relationships on naperian log scales between mean surface duration and body mass of three of the main taxonomic groups. Best-fit lines are included for groups that showed a significant relationship. Secondary signal species are also included. The trend line for all main bird groups combined has not been included for clarity.
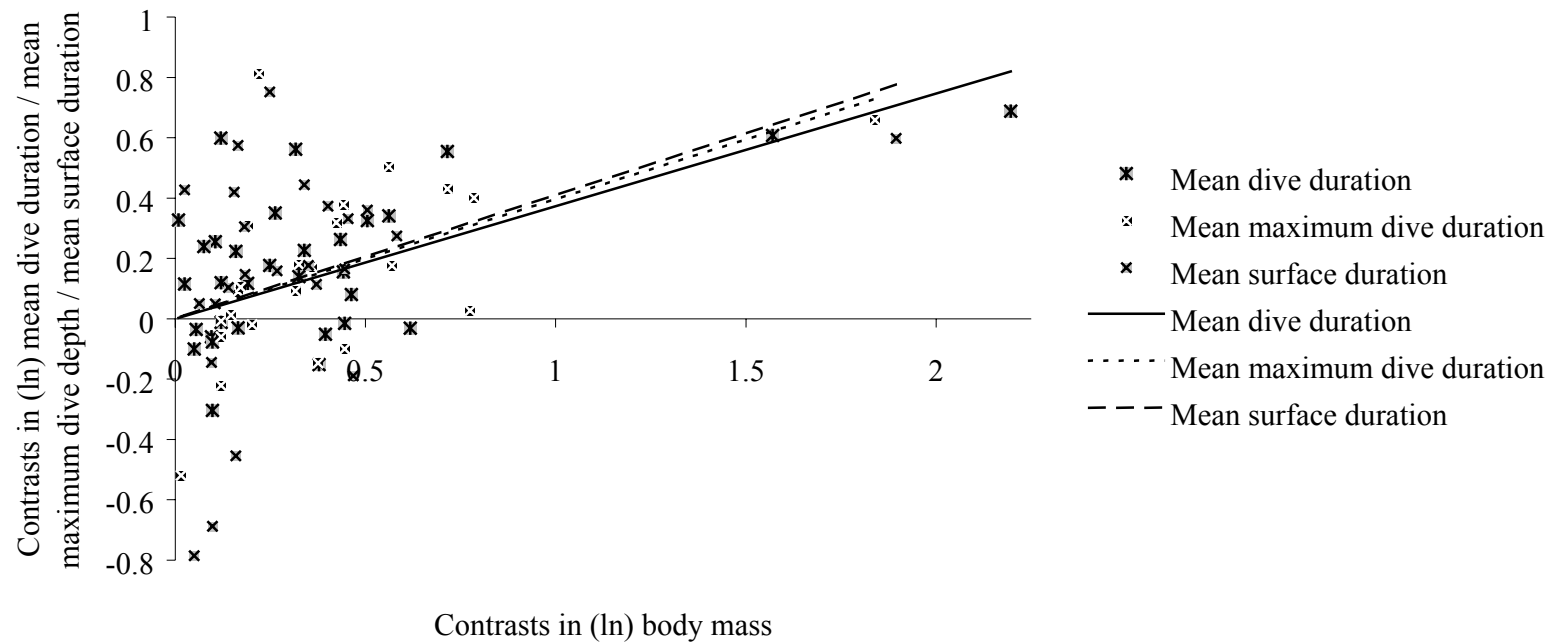
Figure V-5

Relationships on naperian log scales between contrasts in mean dive duration, mean maximum dive depth and mean surface duration against body mass (*M)* of all the main taxonomic groups combined. Best fit lines are included for each dependent variables regressed against *M* and labels are included in the legend for clarity.

VI. Predicting the Diving Limitations of Aquatic Vertebrates

Comparing model predictions of the diving limits of diving animals to measurements of observed maximal behaviour in natural conditions will help elucidate current levels of understanding about vertebrate diving physiology. The oxygen store / usage (S / U) hypothesis is a simple model that estimates the maximum duration of an aerobic dive based on body mass (M), with an expected exponent of 0.25. The most applicable exponents of oxygen store and oxygen usage against M for pinnipeds and penguins found in the literature were substituted in to the hypothesis, in an attempt to predict maximum dive duration (t_{dmax}) in these two taxa. The proportionality of the equations for t_{dmax} against M were 0.69 for pinnipeds and 0.68 for penguins, which may indicate a dominant influence of metabolism on dive duration. These exponents are both significantly higher than the predictions of the S / U hypothesis, showing that our present knowledge of diving physiology, based mostly on captive birds in laboratory studies, cannot explain the scaling of diving capacity with M in pinnipeds and penguins.

Introduction

To better understand the diving behaviour of diving animals, knowledge of their physiologies must be progressed. Since a large number of diving vertebrates are estimated not to use anaerobic metabolism significantly during dives (Thompson and Fedak, 2001), an important facet to understand about their physiologies is the duration limits of aerobic dives. The oxygen store / usage hypothesis (S / U hypothesis) is a simplistic, logical model for determining the maximum aerobic dive durations (aerobic t_d) of air breathing vertebrates. The hypothesis states that maximum aerobic t_d is equal to the volume of usable oxygen within the body during submergence divided by the rate at which that oxygen is metabolised. Body mass (M) is thought to be approximately proportional to oxygen storage capacity (e.g. Lasiewski and Calder, 1971; Hudson and Jones, 1986) and proportional to the rate of oxygen metabolism at rest when raised to the power of approximately 0.75 (Table VI-1). Body mass should therefore also be related allometrically to maximum aerobic t_d. These relationships are defined by Eqs. i and ii:

Maximum aerobic t_d = maximum O_2 storage / rate of O_2 metabolism   (i)

Maximum aerobic $t_d \propto M / M^{0.75}$
$$\propto M^{0.25} \qquad (ii)$$

Therefore, a 10 fold increase in M should be associated with a 1.8 times increase in t_d (Butler and Jones, 1982). This predicts that larger divers should dive for longer than smaller divers because they have lower M specific metabolic rates (Schreer and Kovacs, 1997).

In attempting to refine Eq. ii for specific taxonomic groups, it must first be noted that the S / U hypothesis assumes that oxygen metabolism occurs at resting rate (Table VI-1), despite the formula modelling a form of ecological energetics (i.e. t_d). Table VI-2 shows examples from the literature of allometric relationships between metabolic

rates during various activities for various taxonomic groups. Bennett and Harvey (1987) showed that the common practice of assuming a constant ratio between resting metabolic rate and metabolic rate during periods of activity is invalid. This can be seen by comparing Tables VI-1 and 2. The most relevant values in Table VI-2 for the present study are those recorded by de Leeuw (1996) who investigated the metabolic rates of various diving birds and mammals. The value for mammals was found to be 0.73 while the value for bird species was 0.70. Lasiewski and Calder (1971) recorded comparisons of allometric estimates relating respiratory variables to M in birds and mammals. They calculated a slope value of 0.91 for the logarithmic relationship between M and the volume of the respiratory system in birds, and a value of 1.06 for mammalian species.

The following study tests the proportionality of the S / U hypothesis with two taxonomic groups that are considered to rarely, if at all, exceed their aerobic dive limits - the pinnipeds and the penguins (Boyd and Croxall, 1996). Replacing the original values in the S / U hypothesis (Eq. ii) with the above values most relevant for pinnipeds and penguins produces the following predicted relationships:

Pinnipeds:

Maximum aerobic $t_d \propto M^{1.06} / M^{0.73}$

$$\propto M^{0.33} \tag{iii}$$

Penguins:

Maximum aerobic $t_d \propto M^{0.91} / M^{0.70}$

$$\propto M^{0.21} \tag{iv}$$

Assuming that maximum dive duration (t_{dmax}) represents the aerobic diving limit, the proportionality of Eqs. iii and iv can be tested by calculating the exponents of interspecific allometric equations for t_{dmax} against M in pinnipeds and penguins. Despite the advantages of controlling for phylogenetic relatedness when testing for allometric relationships (*Chapter V*), this was not accounted for in the analyses of the

present study. Firstly, because otherwise the number of species for each taxon would be reduced due to limited information on phylogenies for penguins and pinnipeds. Secondly, because the equations that the regression slopes of the present study (Eqs. iii and iv) were being compared against were derived from exponents taken from previous studies that also used traditional regression analyses not including phylogenetic information. Finally, because the confidence limits around regression slopes including phylogenetic independent contrasts are larger than around standard regressions and so the chances of making a Type 2 error when comparing equations would be high.

Methods

The data used in this study were taken from the same database utilised in *Chapter V* (*Appendix II*, Table AII-1). The taxonomic groups used in this study were chosen from Table AII-1 according to the criteria of sample size and calculated minimal anaerobic metabolism during dives. The majority of the literature on pinnipeds argues that few, if any, of their dives are in excess of the calculated aerobic dive limit (cADL; e.g. Thompson and Fedak, 1993; possibly excluding the exception of Weddell seals, *Leptonychotes weddellii*, Kooyman et al., 1983), which are likely to be underestimates anyway (Kooyman, 1989). Relevant literature also indicates that the majority of penguin species rarely, if ever, exceed their cADL (e.g. Kooyman and Kooyman, 1995; Bethge et al., 1997; Kirkwood and Robertson, 1997). Of particular importance to the current study is that data on species were not included in Table AII-1 where water depths were deemed to be a heavily confounding factor on diving depths and durations i.e. the water depth reported in the study was considerably lower than the maximum diving depth recorded for that species elsewhere.

Mean maximum dive durations (the mean of the single longest dive of each individual) were frequently reported in studies of pinnipeds, however, only rarely was this parameter reported in studies of penguins, although mean maximum dive depths

(d_{dmax}) were often present. The former variable was, therefore, estimated from the latter for penguins by extrapolating from the relationship between mean dive depth (d_d) and mean t_d within the penguin group. Further explanation of this statistical analysis is presented in the Results section. The slopes of the regression equations of t_{dmax} against M for pinniped and penguins were compared using student *t* tests. The t_{dmax} of pinnipeds and penguins were also compared using a student *t* test. Exponents of the aforementioned regression equations were compared to expected exponent values using single sample *t* tests. All statistical analyses were conducted using SPSS (*v.* 10).

Results

To test the accuracy of Eq. iii, mean values of t_{dmax} and mean *M* for pinnipeds were taken from Table AII-1 and naperian log transformed before linear regression analysis was conducted.

Pinnipeds: ($SE_{slope} = 0.122$, $r^2 = 0.73$, $t_{14} = 5.682$,   (1)

$t_{dmax} = 4.4M^{0.69}$ $P < 0.001$; Fig. VI-1)

To test the accuracy of Eq. iv, firstly the relationship between mean t_d and mean d_d for penguins was calculated.

Penguins: ($SE_{slope} = 0.132$, $r^2 = 0.94$, $t_{12} = 12.180$,   (2)

$t_d = 1.61 d_d + 40$ $P < 0.001$; Fig. VI-2)

Assuming that penguins are totally aerobic divers and therefore their maximum dive depth is influenced by their maximum aerobic dive time, by substituting mean d_{dmax} in place of mean t_d into Eq. 2, an estimate of mean t_{dmax} of each penguin species was calculated. The allometric *M* exponent of estimated mean t_{dmax} was then obtained:

Penguins: ($SE_{slope} = 0.085$, $r^2 = 0.87$, $t_{12} = 8.023$,   (3)

$t_{dmax} = 3.1M^{0.68}$ $P < 0.001$; Fig. VI-1)

Only four studies found on penguins have provided data on t_{dmax} (Kooyman et al., 1992; Williams et al., 1992; Kooyman and Kooyman, 1995; Bethge et al., 1997). There was no significant difference between these data points and the calculated values in the present study ($t_3 = -0.188$, $P = 0.86$).

The regression line exponent of Eq. 1, representing the relationship between maximum aerobic t_d and M in pinnipeds, was significantly different to the value predicted by Eq. iii ($t_{13} = 2.95$, $P < 0.05$). The regression line exponent of Eq. 3, representing the relationship between maximum aerobic t_d and M in penguins, was significantly different to the value predicted by Eq. iv ($t_{11} = 5.53$, $P < 0.001$). The slopes of the regressions of Eqs. 1 and 3 were not significantly different from each other ($t_{24} = 0.56$; Fig. V-1). Penguins had significantly longer mean t_{dmax} than pinnipeds when controlling for M ($t_{24} = 6.72$, $P < 0.001$; Fig. VI-1).

Discussion

The accuracy of the estimations of mean t_{dmax} in penguin species from regressions of mean t_d against mean d_d suggests that penguins maintain the same behaviour patterns during dives of increasing depth right up to t_{dmax}. For example, in penguins, it seems likely that their longest dives serve the same function as their normal dives, most obviously foraging, while species that settle on the sea bottom for extended periods are likely to have longer mean t_{dmax} than predicted from their mean d_{dmax} (e.g. green turtles, *Chelonia mydas*, Hays et al., 2000; *Appendix III 'Ultimate Divers'*). Even some pinniped species may sleep while submerged (Kooyman, 1989) and certainly explore uncharted waters during some extended bouts (Fedak and Thompson, 1993). The accuracy of these estimations also serves to support the assumption made in this study that t_{dmax} represents the aerobic t_d limit, which is arguably difficult to justify otherwise. This is because maximum events are often freak events that may explain little about normal behaviour, in particular when number of dives in a study are large,

since this increases the chances of recording an unusual occurrence. The results and conclusions in the present study should be considered in light of this assumption.

Another interesting aspect of this study is the similar slopes of the regression lines for pinnipeds and penguins for the log plotted relationships between M and mean t_{dmax} (Fig. VI-1). This suggests the same underlying relationship between either M or a correlate, and mean t_{dmax}. However, penguins have longer mean t_{dmax} than pinnipeds for their M suggesting either a greater diving ability or the need to sometimes dive relatively more deeply. The slopes of Eqs. 1 and 3 are fairly similar to the M exponent of metabolic rate (0.75), which may indicate a dominant influence of metabolism on t_d (Watanuki and Burger, 1991). Furthermore, the r^2 values for Eqs. 1 and 3 are very high, showing that M accounts for a large proportion of the variation in mean t_{dmax} i.e. that mean t_{dmax} of a pinniped or penguin can be predicted by their M. However, causal or evolutionary associations between mean t_{dmax} and M cannot be concluded since the regression analyses do not account for phylogenetic inter-relatedness (*Chapter V*).

The exponent relating mean t_{dmax} to M was significantly higher than that predicted by the S / U hypothesis for both penguins and pinnipeds. Discrepancies from the model's estimate have also been reported in other studies. The M exponent for maximum forced dive endurance in captive Pekin ducks (*Anas platyrhynchos*) measured by Hudson and Jones (1986) was 0.64, while the same exponent for 15 cormorant species was 0.66 ± 0.11 (Watanuki et al., 1996), which are both also considerably higher than predicted by the hypothesis. A more extreme example is offered by Watanuki et al. (1996) who reported that mean t_{dmax} among Japanese cormorants (*Phalacrocorax capillatus*) was a function of M raised to 1.87. So, why is the prediction of the S / U hypothesis often so inaccurate? These findings imply that diving species vary physiologically far more than is presently understood, for example Watanuki and Burger (1999) claim that cADLs, which are mostly based on metabolic studies of the tissue and lung oxygen stores of captive birds, may not accurately reflect the physiological limits of diving for many species. There are only two variables included

in the S / U hypothesis; capacity of oxygen stores and rate of oxygen consumption. The accuracy of current estimates of these two factors in diving animals are considered next in terms of our present understanding of diving physiology.

Body oxygen stores

Boyd and Croxall (1996) suggest that there is often inaccuracy in the assumed volumes of body oxygen stores of diving animals. For example, Ponganis et al. (1993) determined that previous estimates of the oxygen stores of *L. weddellii* were considerable underestimates of their volume. Hudson and Jones (1986) found the *M* exponent in Pekin ducks to be 1.19 for total oxygen stores, which is considerably greater than the original value in the model of 1.00 or indeed the values for pinnipeds and penguins used in this study. Hudson and Jones (1986) attribute this large value partly to the fact that body *M* and organ *M* do not always scale very closely (see also Hayes, 2001). Given that organ size is the main influence on oxygen consumption, *M* does not therefore reflect oxygen consumption very well. Furthermore, Hudson and Jones (1986) point out that the S / U hypothesis might fail to predict the situation during prolonged dives since the *M* relationship of the organs expected to receive most of the stored oxygen (i.e. the brain and heart) is not considered.

The *M* exponent value of oxygen stores can also depend partly on whether only oxygen stores in the lungs are measured (see Butler and Jones, 1982), or whether oxygen carried in the blood and muscle tissues is taken into account as well. For example *L. weddellii* have relatively more oxygen stores in their bodies than many other diving species (Castellini et al., 1991), because they extensively utilise muscle myoglobin stores. Estimated oxygen storage capacity can also vary depending upon whether the species in question is perceived to be able to utilise oxygen stored elsewhere in the respiratory system (e.g. the trachea; Boggs et al., 1998).

Oxygen consumption during dives

The value of the regression slope for rate of oxygen metabolism against M, (0.75 in the original hypothesis), is not a fixed value for all groups of homeotherm species (Table VI-1 and Table VI-2), but varies at the taxonomic level (e.g. Hayssen and Lacy, 1985; Bennett and Harvey, 1987) and is often notoriously difficult to define (Thompson and Fedak, 1993). For example, when taxonomic assignment of the species is disregarded then the slopes are closer to 0.67 than 0.75 in mammals (Hayssen and Lacy, 1985) as well as in birds (Daan et al., 1989). This may be because they have, as yet poorly understood, physiological mechanisms for reducing oxygen uptake. For example, Watanuki et al. (2003) reported adjustments by birds in forward thrust frequency with changes in buoyancy and depth to increase locomotor efficiency. Woakes et al. (1995) measured reductions in body temperature in penguins that may afford them a diving advantage over pinnipeds since their low thermal inertia and high surface area to volume ratio increase the rate of body cooling during dives (Boyd, 1997), in turn reducing metabolic rate (Butler, 1993). Abdominal temperatures in penguins can decline by 5-10 º C (Woakes et al., 1995; but see Green at al., 2003 and Ponganis et al., 2003), which at a Q_{10} of 2, could result in a 30-50 % reduction in metabolic rate (Boyd and Croxall, 1996). Animals are also likely to consume oxygen at different rates depending upon swimming speed (Hindell et al., 2000), the depth to which they travel (since buoyancy costs might be affected; Lovvorn and Jones, 1991), and the energetic costs of their feeding behaviour (e.g. Acevedo-Gutierrez et al., 2002).

The slope is also affected by the type of active behaviour during which metabolic rate is recorded. For example, de Leeuw (1996) recorded different values for diving birds and mammals depending on whether they were undertaking a foraging trip or undertaking a diving bout in which case the type of dive also affected metabolic rate. Therefore, no single allometric scaling rule for homeotherm metabolic rates is available (Daan et al., 1990) but rather the differences between closely related species may be significant. Thus, despite the attempt in the present study to refine the S / U

hypothesis by tailoring it to specific taxonomic groups i.e. pinnipeds and penguins, there is still an incorrect underlying assumption in the model that metabolic rate follows a common allometric pattern across fairly diverse groups of species.

Anaerobic metabolism

Pinnipeds and penguins have often been assumed not to metabolise anaerobically although some species may sometimes utilise anaerobic pathways, which, of course, the S / U hypothesis does not account for (e.g. Weddell seals; Castellini et al., 1988; Emperor penguins; Kooyman et al., 1980). Even occasional use of anaerobic pathways will bias the model since they will probably have been utilised during the longest dives. If, in fact, the majority of species to some extent metabolise anaerobically during dives, then the S / U hypothesis is invalid for exploring diving capability. One taxonomic group that seems very unlikely to use anaerobic pathways in the wild is the duck family Anatidae, since they typically inhabit shallow water. Unfortunately, this ecological factor also confounds the data since the mean t_{dmax} of these species are unlikely to represent physiological extremes, exemplified by the tufted duck (*Aythya fuligula*), which consistently dives well within its aerobic dive limits (*Chapter III* and *Chapter IV*). Furthermore, few data points were available on maximum dive parameters for these species. However, experimental work on tufted ducks has shown that mean oxygen consumption at mean t_d is lower in those ducks that perform longer dives (Bevan et al., 1992), which could indicate that aerobic metabolism declines as a dive proceeds with, possibly, increasing anaerobiosis (see also *Chapter IV*).

Concluding remarks

Values of t_d for species included in the present study as well as from several other studies are considerably different to that predicted by the S / U hypothesis. Underestimations of oxygen stores due to a lack of appreciation of the volume of usable oxygen within the body, and overestimations of oxygen metabolism at least in

part due to yet unappreciated energy-saving mechanisms may account for this. For example, Watanuki and Burger (1999) were not able to explain the longer t_d of alcids compared to penguins when accounting for M with this hypothesis, despite concluding that oxygen stores were similar in both taxonomic groups, as were their M specific metabolic rates during diving. Therefore, enhanced knowledge of the physiological adaptations of these species to diving is required to explain the relationships between M and diving capacity.

The utilisation of anaerobic pathways is a possible explanation for a large proportion of the differences between observed and expected figures of mean t_{dmax}. Oxygen storage volumes may only be miscalculated by relatively small amounts in many diving animals, leaving oxygen metabolism as the factor with the larger error value, and since anaerobic metabolism could allow considerable increases in t_d, its utilisation would dramatically effect the prediction of the S / U hypothesis (Kooyman, 1989; Croll et al., 1992). Anaerobic metabolism may be a more common occurrence in diving vertebrates than is presently thought (e.g. evidence of anaerobic metabolism during extreme dives by tufted ducks; Bevan and Butler, 1992; *Chapter IV*) and would also explain the longer t_d in alcids compared to penguins (Watanuki and Burger, 1999; however, see *Appendix III 'Ultimate Divers'*). Thus, hard evidence of the degree of utilisation of anaerobic metabolic pathways by diving animals would undoubtedly represent an important increment in our understanding of the behaviour of diving animals.

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Table VI-1. Slopes of allometric relationships between resting metabolic rate and body mass

Author	Species / Taxonomic group	Slope
Lasiewski and Dawson (1967)	Non-passeriformes	0.72
	Mammals	0.76
	Seabirds	0.70
Ellis (1984)	Seabirds	0.72
Hayssen and Lacy (1985)	Eutherians	0.70
Bennett and Harvey (1987)	Birds	0.68
Daan et al. (1989)	Birds	0.68
	Raptors	0.68
Daan et al. (1990)	Birds	0.68
	Eutherians	0.70

Table VI-2. Slopes of allometric relationships between metabolic rates during various high-energy activities

Author	Metabolic type	Species / Taxonomic group	Slope
Walsberg (1983)	Daily energy budget	Birds	0.61
Bennett and Harvey (1987)	Active metabolic rate	Birds	0.61
Nagy (1987)	Field metabolic rate	Eutherians	0.81
		Marsupials	0.58
		Passerines	0.75
		Non-passerines	0.75
Daan et al. (1990)	Daily energy expenditure during parental care	Birds	0.66
De Leeuw (1996)	Diving	Birds	0.70
	Diving	Mammals	0.73
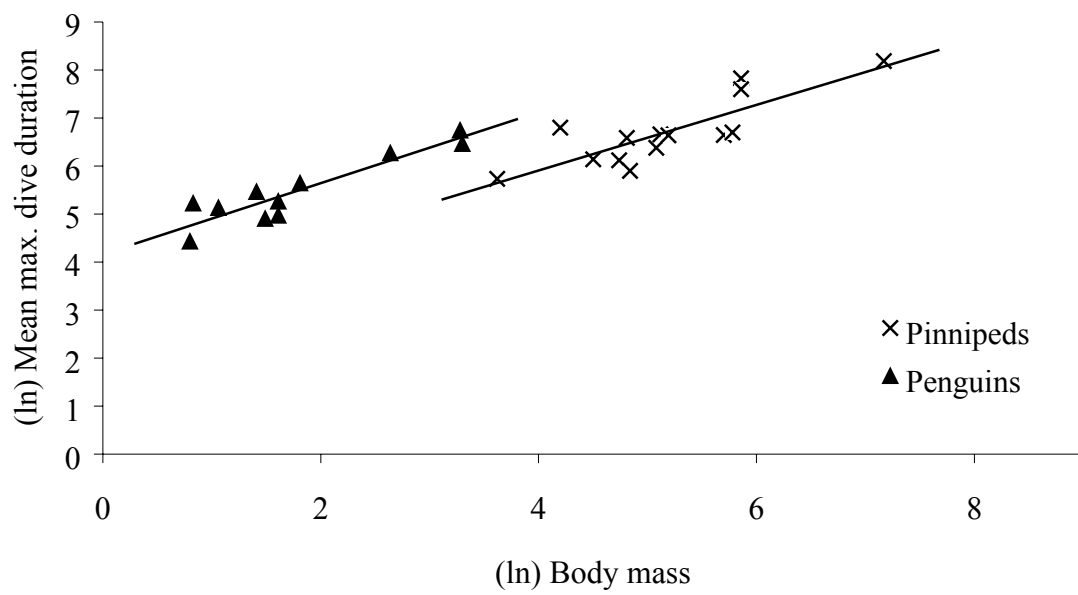
Figure VI–1

Allometric relationship on naperian log scales between recorded mean maximum dive durations of pinnipeds or estimated mean maximum dive durations of penguins, and mean body mass.
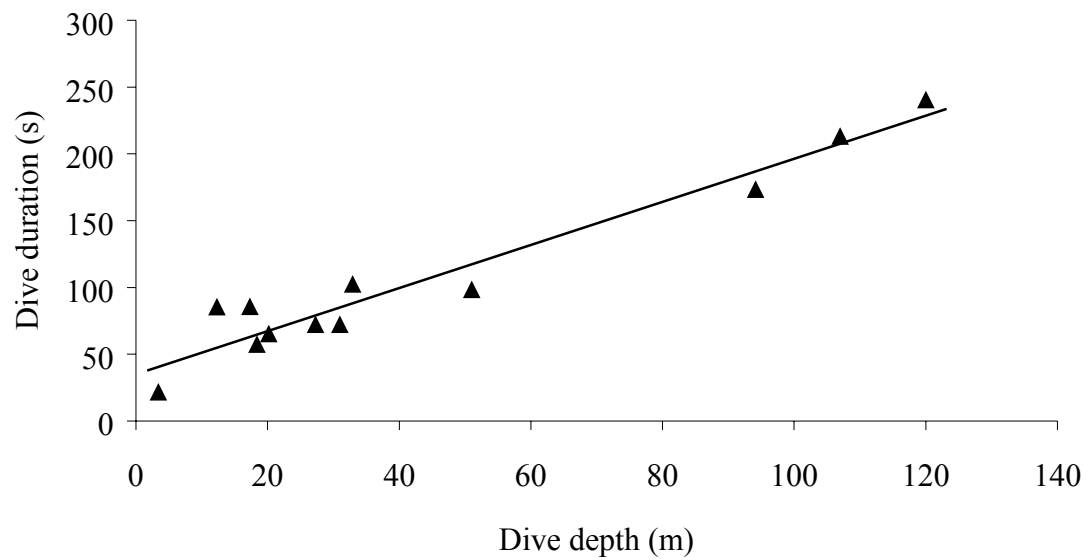
Figure VI–2

Simple linear relationship between mean dive durations and mean dive depths of penguin species.

VII. The Effect of Travel Distance on Diving Efficiency in Diving Foragers

The optimal breathing model (Kramer, 1988) generates a number of predictions about diving behaviour based on the assumption that a diver experiences diminishing returns of oxygen uptake during surface periods between dives. One prediction is that surface duration (t_s) will increase more rapidly than dive duration (t_d) as travel distance to the food source increases because, due to the diminishing returns of oxygen uptake, oxygen acquired by divers after undertaking longer dives is gained at a lower average rate (Kramer, 1988; Houston and Carbone, 1992). The present study tests this prediction by comparing diving behaviour across species, within two distinct taxonomic groups of species with varying body masses (M), namely the pinnipeds and the cormorants. The pinnipeds showed positive linear and allometric relationships between the dive to pause ratio (a measure of diving efficiency; d:p), and dive depth (d_d) and t_d. These trends contradict the model prediction. The cormorants, in contrast, showed an exponential relationship between t_s and t_d, supporting the model prediction. Mass is likely to be a confounding factor that could counteract and thus mask the effect of the diminishing returns of oxygen uptake while at the surface, and therefore may explain the allometric relationships of the pinnipeds. Because pinnipeds that dive for longer also tend to be larger and so have M conferred advantages on diving efficiency (since metabolic rate scales to M raised to approximately 0.75; e.g. Lasiewski and Dawson, 1967; Schmid et al., 1995; see also *Chapter VI*), they require less oxygen per unit M. They are therefore likely to spend less time recovering at the surface after a dive of equal duration compared to smaller divers. However, since the range of M for cormorants is much smaller than for pinnipeds, M specific advantages might be much less, leaving the slower rate of average oxygen accumulation of longer surfacing species as the main influence on diving efficiency in this taxon.

Introduction

Optimal foraging models generate testable predictions, making them an attractive tool for ethologists and evolutionary biologists. Kramer's optimal breathing model (1988) is an optimal foraging model adapted for diving animals, which assumes that they are trying to maximise rate of energy gain (Fig. VII-1). The model predicts that where the rate of food gain during a diving bout increases as the percentage time of the diving bout spent at the foraging site increases, divers should therefore attempt to maximise the proportion of time spent foraging. Kramer argued that this is equivalent to maximising the net rate of oxygen gain from each trip to the surface. Kramer's model offers a number of predictions of diving behaviour, which are often supported by trends in field data. For example, dive duration (t_d) and surface duration (t_s) are predicted to increase as dive depth (d_d) increases and this has been observed in a number of field studies (e.g. Dewar, 1924; Cairns et al., 1992; Kato et al., 2003). Furthermore, *Chapter II* has demonstrated quantitative predictive success of the optimal breathing model for tufted ducks at the species level.

However, one of the predictions of the optimal breathing model has yet to be specifically tested and discussed. Both Kramer (1988) and Houston and Carbon (1992) argue that as d_d increases, time at the surface and therefore the oxygen stores between dives should increase, while the amount of oxygen remaining at the end of the dive should be unaffected by distance. Thus oxygen acquired by divers after undertaking longer dives is gained at a lower average rate across the whole dive cycle (Kramer, 1988). Kramer's model therefore predicts that both t_s and t_d will increase with the travel distance of a dive, however t_s is expected to increase more rapidly. Consequently, the proportion of time during diving bouts spent at the surface will increase while the proportion of time submerged will decrease, as the travel distance increases i.e. the dive:pause ratio (t_d / t_s; $d{:}p$), which is a measure of diving efficiency (Dewar, 1924), will decrease. More complex models of diving behaviour have been developed since the optimal breathing model (e.g. Ydenberg and Clark, 1989; Houston and Carbone, 1992; Carbone and Houston, 1996; Mori, 1998; Mori et al.,

2002), however the former model offers simple and parsimonious predictions that justify sufficient testing before more complicated theories, which build upon this seminal model, are explored.

Direct and indirect evidence both supporting and refuting the predicted decrease in diving efficiency as travel duration increases, have been recorded in a number of different taxonomic groups. In support of Kramer's assumption, grey whales were found to spend a higher percentage of time at the surface when diving to greater depths (Wursig, 1986), as were humpback whales (Dolphin, 1988), and Wanless et al. (1993) found an accelerating relationship between t_s and t_d in shags. In contrast, Lea et al. (1996) reported several cormorant species diving more efficiently with increases in d_d, which is presumably correlated with t_d. Dewar (1924) and Stonehouse (1967) reported observing percentage time at the surface decreasing with d_d and then increasing towards maximum d_d in some species. Boyd and Croxall (1996) found no relationship between d_d and $d{:}p$ in penguins.

One possible confound to the relationship between diving efficiency and t_d is the M of different species, or even individuals of a species. This may, at least in part, explain some of the field observations that do not support the model prediction. The M specific metabolic advantage that larger animals have during diving (Schreer and Kovacs, 1997, see *Chapter V* and *Chapter VI*) allows them to dive for longer on their oxygen stores and may therefore increase their diving efficiency compared to smaller divers, despite diving for longer and surfacing for longer. The present study investigates the effect of travel distance and M on diving efficiency by comparing diving behaviour across species within two taxonomic groups to test the model assumption.

Methods

This study utilises data from the database utilised in *Chapter V* (*Appendix II*, Table AII-1). The development of the database is discussed in *Chapter V*. Relationships were tested for in taxonomic groups where sample size was greater than 10 and where the species in the group probably use anaerobic metabolism minimally during submergence, since the optimal breathing model assumes that all dives are purely aerobic. The only groups that satisfied these two conditions were the pinnipeds and the cormorants. All species from these two groups that were considered likely to frequently undertake anaerobic dives (e.g. Weddell seals, Kooyman et al., 1980) were excluded from the analyses. Relationships were only tested for in a particular taxonomic group when the sample size was greater than 10 for both variables, thus not all relationships were tested for in both taxonomic groups.

The mean of each diving parameter are the measures of central tendency used in the present study. The parameter mean maximum t_d, while often stated and not affected by bimodality, was not utilised as a variable since it is often likely to incorporate elements of anaerobic metabolism. The dive:pause ratio was calculated when a mean value for both t_d and t_s were available.

Bivariate statistical analyses

To test the assumption of Kramer's optimal breathing model that as distance to the foraging site increases, percentage time at the surface should increase while percentage time spent diving should decrease, a number of simple linear relationships and allometric relationships (using naperian log transformed data) were fitted across species using linear least squares regression. Allometric relationships were only tested for if the r^2 value was higher than in the linear analyses. Statistical analyses were considered invalid and were not included in the results if r^2 was less than 0.25. Phylogenetic relatedness was not accounted for in these regressions to maximise the

number of species in each regression and therefore maximise the power of each analysis.

As direct tests of the assumption, the dive:pause ratio was regressed against both mean t_d and mean d_d in pinnipeds and the mean t_d exponents of mean t_s were also calculated, both for the pinnipeds and the cormorants. To investigate the effect of M on diving efficiency, allometric analyses were conducted on M against d:p and mean d_d in pinnipeds, while the mean t_s exponents of M were calculated both for pinnipeds and for cormorants. All statistical analyses were conducted using SPSS (*v*. 10).

Results

The mean surface durations of pinnipeds were positively related to mean t_d:

$$t_s = 3.1\, t_{d\,\text{mean}}^{0.25} \qquad (SE_{\text{slope}} = 0.104,\ r^2 = 0.31,\ t_{16} = 2.41,\ P < 0.05) \quad (1)$$

The dive to pause ratios in pinnipeds were positively related to mean d_d and mean t_d (Fig. VII-2):

$$d{:}p = 0.015 d_d + 1.9 \qquad (SE_{\text{slope}} = 0.003,\ r^2 = 0.60,\ t_{16} = 4.74,\ P < 0.001) \quad (2)$$

$$d{:}p = 0.010 t_d + 1.3 \qquad (SE_{\text{slope}} = 0.001,\ r^2 = 0.70,\ t_{17} = 6.06,\ P < 0.001) \quad (3)$$

The mean dive depths of pinnipeds were also positively related to M:

$$d_d = -0.7 M^{1.01} \qquad (SE_{\text{slope}} = 0.276,\ r^2 = 0.47,\ t_{17} = 3.66,\ P < 0.01) \quad (4)$$

The dive to pause ratios in pinnipeds were positively related to M:

$$d{:}p = -2.1 M^{0.62} \qquad (SE_{\text{slope}} = 0.249,\ r^2 = 0.31,\ t_{17} = 2.50,\ P < 0.05) \quad (5)$$

The mean surface durations of cormorants were positively related to t_d:

$$t_s = -3.6\,t_{d\,\text{mean}}^{1.87} \qquad\qquad (SE_{\text{slope}} = 0.50,\ r^2 = 0.60,\ t_{11} = 3.70,\ P < 0.01) \quad (6)$$

The mean surface durations of pinnipeds were positively related to M (Fig. VII-3):

$$t_s = 3.4M^{0.37} \qquad\qquad (SE_{\text{slope}} = 0.152,\ r^2 = 0.28,\ t_{17} = 2.44,\ P < 0.05) \quad (7)$$

The mean surface durations of cormorants were positively related to M (Fig. VII-3):

$$t_s = 2.3M^{1.18} \qquad\qquad (SE_{\text{slope}} = 0.408,\ r^2 = 0.48,\ t_{11} = 2.90,\ P < 0.05) \quad (8)$$

Discussion

The optimal breathing model predicts that, as the depth to the feeding site increases, and therefore t_d increases, the proportion of time spent at the surface will increase and the proportion of time spent underwater will decrease. However, the power value of 0.25 in the allometric relationship between mean t_s and t_d in pinnipeds (Eq. 1) shows that as t_d increases, t_s increases less quickly. This is confirmed by the positive relationships between $d{:}p$, and d_d and t_d in the pinnipeds (Eqs. 2 and 3; Fig. VII-2). These trends do not support the model prediction but rather suggest that pinniped species that dive more deeply during a bout are more efficient (Eqs. 2 and 3; Fig. VII-2). Equation 4 shows that larger species tend to dive more deeply, as has been observed previously (e.g. Prince and Harris, 1988; Burger, 1991; Mori, 1998), and Eq. 5 confirms that therefore larger species are more efficient divers.

These relationships are derived from traditional, across-species analyses, which only indicate the presence of a correlation, not a causal evolutionary association between the variables. Nevertheless, hypotheses showing the correlations of diving parameters to M may still help us understand the effect of M on diving behaviour. For example, not only do large divers have a M specific metabolic advantage, but the diving costs in small-bodied divers are exacerbated because drag increases with M specific surface area (de Leeuw, 1996) and air trapped in the fur or feathers can generate positive

buoyancy (McIntyre et al., 2002). Evidence for these hypotheses is demonstrated in elephant seals, where smaller seals are estimated to use proportionally more of their oxygen reserves on dives than their larger counterparts (Hindell et al., 2000). These hypotheses predict that the increase in t_s, where t_s is assumed to be controlled by oxygen uptake, will be less than the increase in t_d, when t_d is positively correlated with M. This is supported by Eq. 1. Therefore, the effects of M conferred advantages on diving efficiency appear to work against the effects of diminishing returns of oxygen uptake in pinnipeds with larger M. In which case, the metabolic advantage received by heavier pinnipeds, in that they need to surface for less time after a given t_d (or conversely that they can dive for longer for a given t_s), appears to more than compensate for the slower average oxygen uptake they experience after their longer mean diving bouts, producing a greater net diving efficiency (Fig. VII-4a). In contrast, the exponential relationship between t_s and t_d in cormorant species (Eq. 6), showing that as t_d increases, t_s increases more quickly, supports Kramer's prediction. This trend between these diving parameters has also been observed in alcid species by Wanless et al. (1988).

Why should cormorants and pinnipeds show opposing trends in the relationships of travel duration against diving efficiency? Firstly, pinnipeds and cormorants have contrasting relationships between t_s and M (Eqs. 7 and 8). Fig. VII-3 illustrates the large difference in regression slopes, which suggests that there are different reasons for the correlations between the two taxonomic groups. The range of M for cormorants in this study (0.5 to 3.5 kg) is considerably smaller than that of the pinnipeds (37 to 1300 kg) so the advantage of larger M will be less significant in cormorants. Furthermore, since the ratio of the range of mean t_d compared to the range of M is much higher in cormorants (51:1) than in pinnipeds (1:1), this is likely to leave the slower average oxygen accumulation of longer t_s required for longer t_d as the main influence on diving efficiency in cormorants, with M being much less important (Fig. VII-4b). Secondly, according to Kramer (1988), the effects of diminishing returns of oxygen uptake are highest for shortest t_s. Since mean t_s of

cormorants is 24.6 ± 7.6 s compared to 131.10 ± 18.4 s for pinnipeds, this effect is likely to be more marked in the former taxonomic group.

The present study offers both supportive and contradictory findings for the tested prediction of the optimal breathing model depending on the taxonomic group investigated. The results therefore suggest, perhaps not surprisingly, that oxygen uptake rate is not the only factor affecting t_s and diving efficiency. In the present study, M specific metabolic rate has been considered as a confounding factor, scaling to M, which may therefore sometimes mask the effect of diminishing returns on oxygen uptake at the surface. Kramer's model does not account for the positive relationships often found between M, and d_d and t_d, and therefore that animals that dive for longer have metabolic advantages. Furthermore, evidence from diving studies of sexually dimorphic species suggests that M or a correlate may be important within species (e.g. Watanuki et al., 1996, Boyd and Croxall, 1996). Mass is therefore a variable strongly correlated with diving ability (see *Chapter V* for more information) except when a species or taxon has a negligible range of M. Therefore, the optimal breathing model may successfully predict certain diving patterns such as concurrent changes in t_s, t_d and travel durations between individuals, but is often too simplistic to predict changes in diving efficiency against d_d, where M is an influential variable.

The diving efficiency of air breathing species can rarely be calculated from data outside of studies on pinnipeds and cormorants, usually because t_s is not recorded. With technology for the collection of dive data developing all the time, such as remote sensing (Schreer and Kovacs, 1997), data-logging (Woakes et al., 1992; Falk et al., 2000) and satellite-linked dive recorders (Folkow, pers. comm.), an increase in the number of studies recording data on diving time budgets in the field can be expected. This will enable us to refine models such as the optimal breathing model (Kramer, 1988). Observations have been published qualitatively testing the optimal breathing model and later models, all considering within species effects (e.g. Houston and Carbone, 1996; Lea et al., 1996; Walton et al., 1998). However, they do not

enable us to better predict interspecies differences. A simple step towards a model making interspecies predictions has been made here by considering the net effects of diminishing returns of oxygen uptake and *M* specific metabolic rate on diving efficiency in two very different taxonomic groups.

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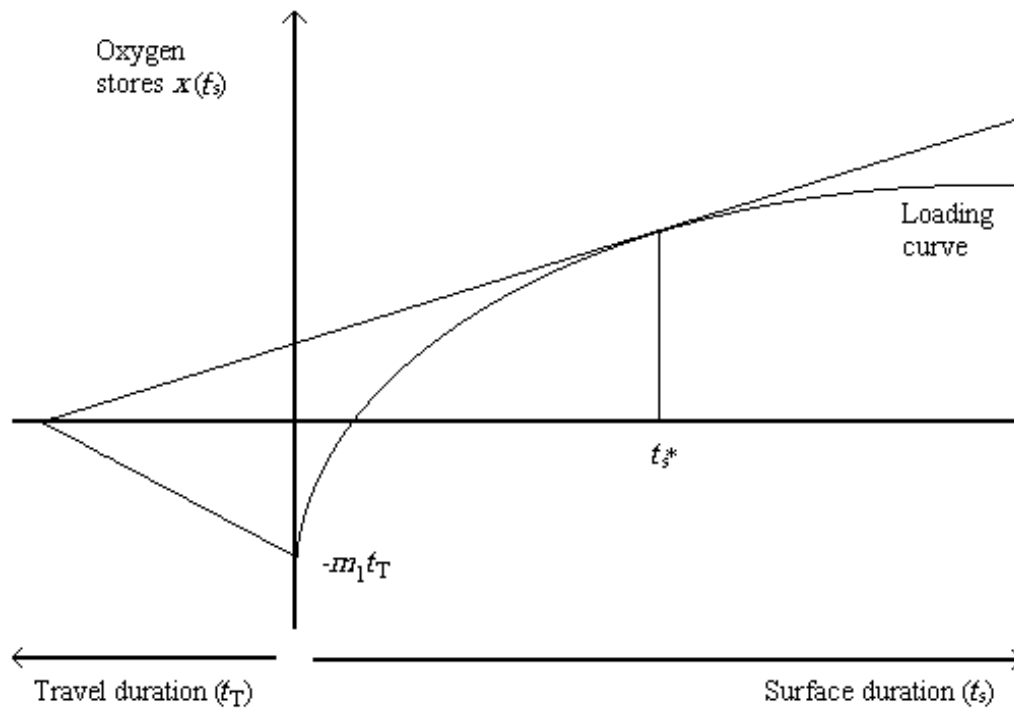
Figure VII–1

Graphical representation of the optimal breathing model (Kramer, 1988). The ordinate represents oxygen stores, $x(t_s)$. The line from t_T to $-m_1t_T$ represents a constant rate reduction of oxygen stores during travel time, t_T. The loading curve is the oxygen obtained over time at the surface (t_s), as a result of experiencing diminishing returns of oxygen accumulation over time. The value t_s*, found by constructing the tangent, represents the optimal surface duration for maximising the rate of delivery of oxygen to the foraging area and therefore the maximising the proportion of time spent foraging.
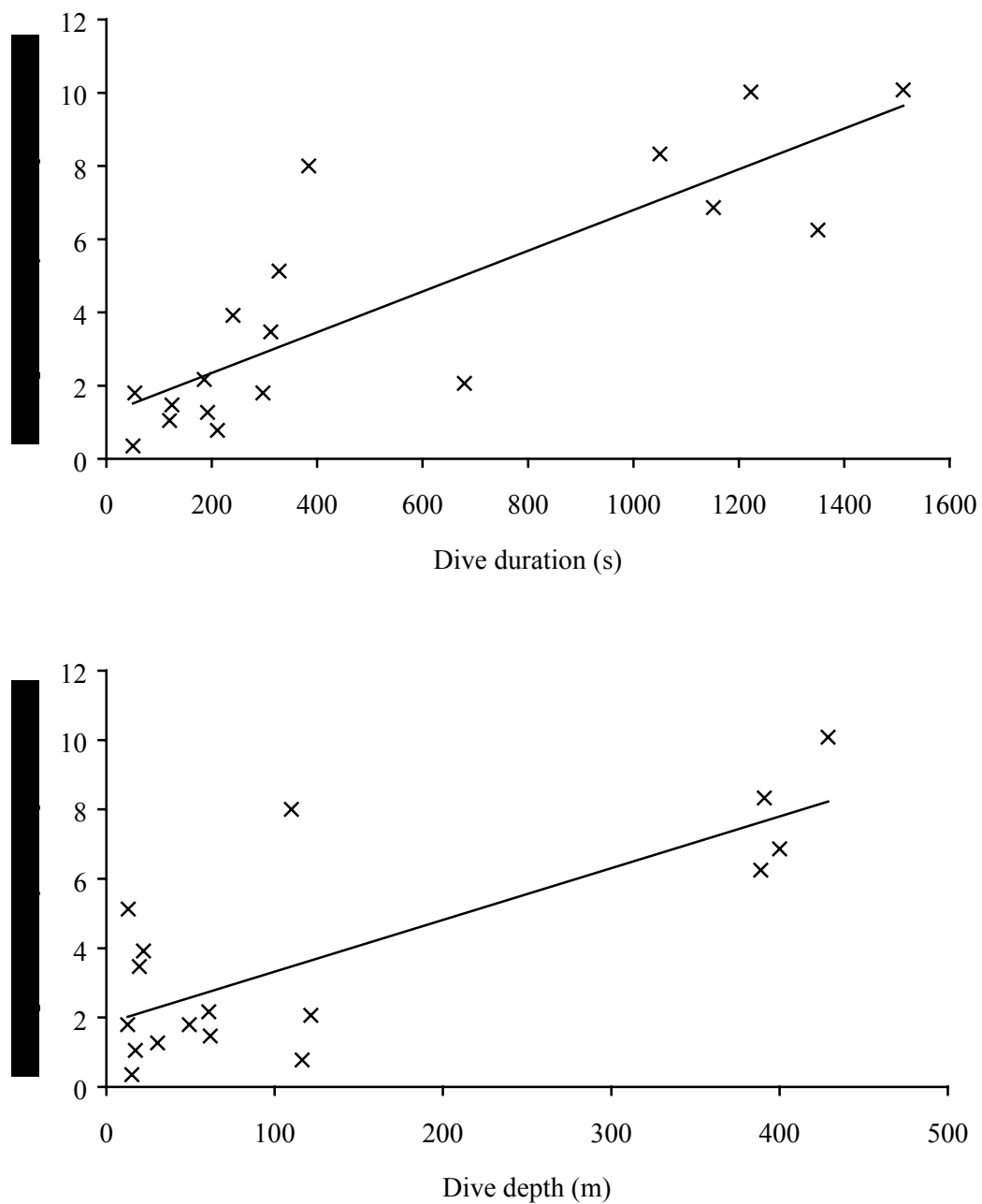
Figure VII-2

(a) Simple linear relationship between diving efficiency, represented by the dive:pause ratio (*d:p*), and mean dive duration in pinniped species.

(b) Simple linear relationship between diving efficiency, represented by *d:p*, and mean dive depth, in pinniped species.
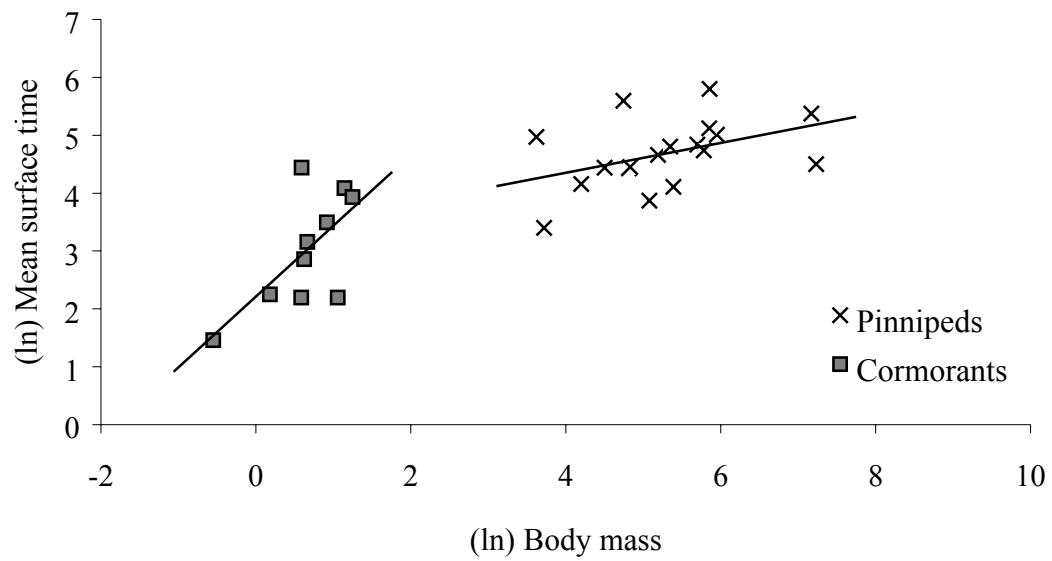
Figure VII-3

Allometric relationships on naperian log scales between mean surface dive duration and body mass of pinnipeds and cormorants.

Body mass, dive duration, dive depth

Body mass, dive duration, dive depth
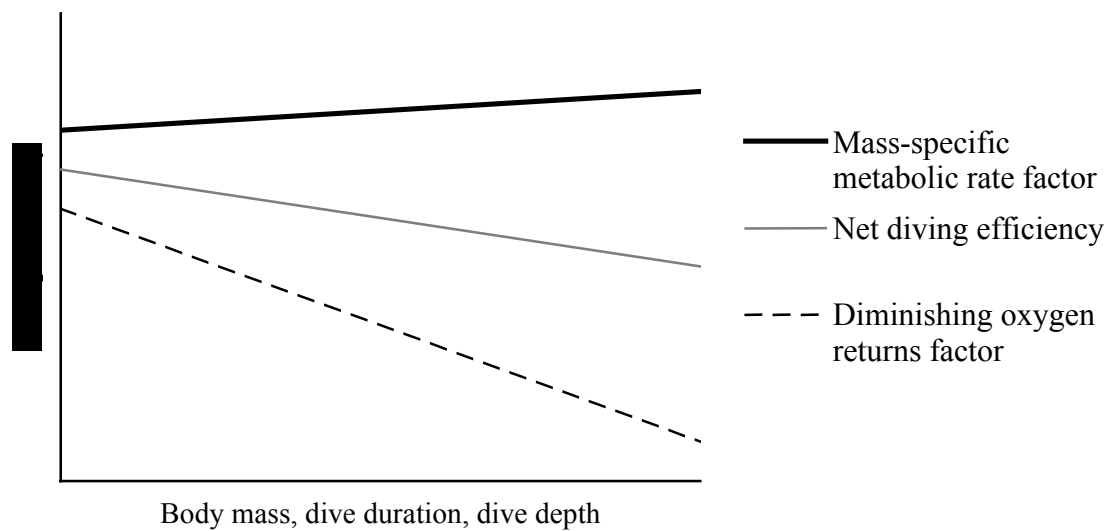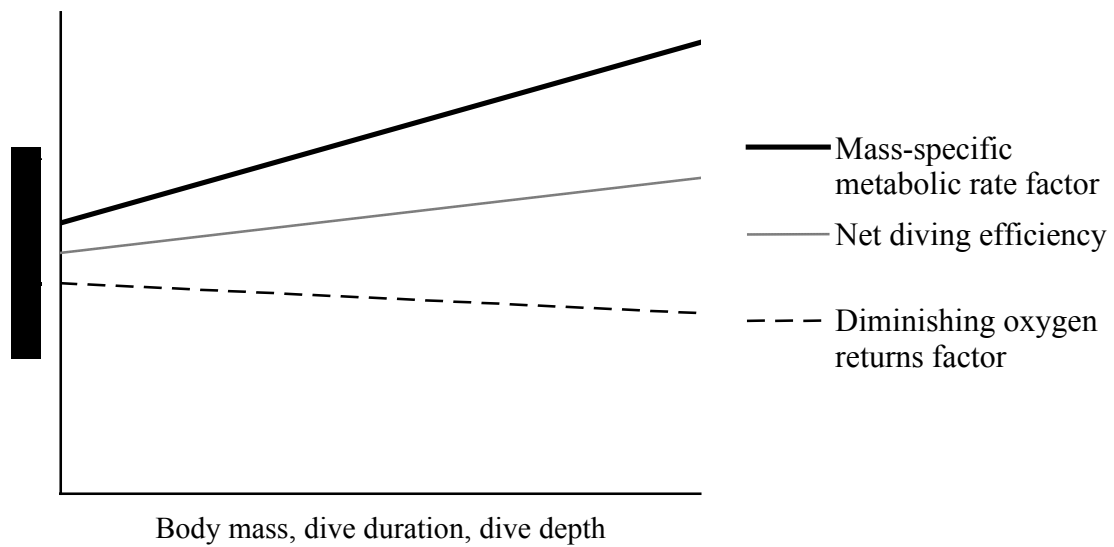
Figure VII-4

(a) Graphical representation of the possible net effects on diving efficiency (measured as the dive:pause ratio) in pinniped species of mass (*M*) specific metabolic rate combined with the diminishing returns of oxygen uptake as mean dive duration increases. Larger pinnipeds tend to dive deeper and for longer and are therefore disadvantaged by a slower average oxygen uptake rate at the surface, according to the optimal breathing model (bottom line). However this is more than compensated for by the advantage of a lower relative metabolic rate, which allows them to dive for longer relative to their surface duration (top line). This results in a net increase in diving efficiency in larger pinnipeds (middle line).

(b) Graphical representation of the possible net effects on diving efficiency in cormorant species of *M* specific metabolic rate combined with the diminishing returns of oxygen uptake as mean dive duration increases. The range of *M* in cormorant species are relatively small, however they still exhibit a large range of diving durations. Thus the advantage of a lower relative metabolic rate in larger species is small and is more than compensated for by the disadvantage of a slower average oxygen uptake rate at the surface. This results in a net decrease in diving efficiency in larger cormorants.

VIII. General Discussion

The primary objectives of this thesis were to measure the behaviour and respiratory gas exchange of tufted ducks in the context of investigating optimal diving models. The breadth of the investigation was extended with theoretical comparisons of the diving behaviours of a wide variety of other diving species. All the principle research objectives stated in *Chapter I* have been addressed and as such, this study has improved our understanding of the diving behaviour and respiratory physiology of diving ducks through the quantitative testing of optimal diving models, while widening the research with theoretical comparisons of other vertebrate divers.

Perhaps *Chapter VI* best underlines our current lack of understanding about some of the physiological mechanisms that affect the diving ability of different vertebrates. Furthermore, *Chapter V* suggests that a number of relationships assumed to be present between diving parameters and mass may in fact be spurious. If this conclusion is correct, then the physiological reasons for the differences found when comparing diving behaviour and capability across species within taxa are likely to be associated mostly with adaptations that are only broadly related to mass, or not related to mass at all, which is often in contrast to what has previously been presumed. However, the experimental studies in this thesis (*Chapters II-IV*) have taken our understanding of the structure and function of diving animals a step forward, in particular regarding tufted ducks.

Chapters II-IV use a methodology based on a respirometry technique developed by Woakes and Butler (1983), which treated the respirometer chamber as both an open and closed system. By measuring differences in gas concentrations with a mass spectrometer, Woakes and Butler (1983) were able to calculate gas exchange over

periods as short as 2 s despite the system having a much longer response time. R. Parkes recorded oxygen uptake in tufted ducks with a fast response oxygen analyser using this respirometry technique at a resolution of 0.25 s – a higher frequency than the fastest respiratory rate for this species (Butler and Woakes, 1979). Because the oxygen concentration in the respirometer box measured by the oxygen analyser was recorded, using software, automatically onto a computer from which calculations of oxygen uptake could be quickly calculated, the *n* value of this experiment (the number of dives recorded) was greatly increased from that of Woakes and Butler (1983). Thus, although the changes in oxygen concentration in the respirometer every 0.25 s were very small and often within the error of the oxygen analyser, creating a low ratio of measurement signal to signal noise, by averaging the data points the signal to noise ratio was greatly increased. As such, precise cumulative oxygen uptake curves were produced for tufted ducks in between dives. Indeed, the accuracy of the data collected by R. Parkes was such that the first few breaths taken by the ducks after emerging from a dive can be clearly seen in the averaged data. The custom designed program used in the present studies automates the data recording and acquisition process further (*Appendix IV*) and thus even larger *n* values have been obtained and more experimental conditions run. Because these studies, in particular *Chapter II*, develop the ideas and findings of the study by R. Parkes, I felt that it was appropriate to publish the results of this earlier work before my own work. In collaboration with R. Parkes, I therefore re-wrote this study (Parkes, 2002) as a manuscript for publication, which was accepted by the *Journal of Experimental Biology* (Parkes et al., 2002; *Appendix III*).

Chapter II builds upon the study by Parkes et al. (2002) by combining the quantification of the oxygen uptake curve for tufted ducks with estimates of power costs for this species during the dive in order to empirically test the predictive validity of the optimal breathing model (Kramer, 1988). Perhaps not surprisingly, given the large morphological and behavioural variation between the six tufted ducks used in this experiment (which is not a trait at all unique to tufted ducks but has been reported

by scientists in many, if not all, studied vertebrate species e.g. gentoo penguins, J. Green, pers. comm.; grey seals, J. Reed, pers. comm.) the model did not successfully predict the behaviour of individual birds. When studies focus on individual animals (*Chapter II*), it often becomes clear that present behavioural models are far too simplistic to incorporate all the important parameters affecting each unique individual's behavioural strategies. This conclusion parallels the conclusion of *Chapter VII* on interspecies variation, that the same model is too simplistic to be valid for predicting the average diving behaviour of a species for a wide variety of diving vertebrates, because of the vast array of different adaptations to diving. However, maybe even more surprisingly, the model did successfully predict the behaviour of all the subject ducks when combined, which is particularly unexpected given the omission from the model of the body carbon dioxide stores as a variable.

Within the sphere of research on human diving, the prominent influences of carbon dioxide on breath-hold duration has been appreciated for some considerable time (e.g. Otis et al., 1948; Rahn and Otis, 1949; Rahn et al., 1953). However, no optimal diving models incorporate body stores of carbon dioxide as a factor. Thus a reason for the limited quantitative predictive success in optimal diving models, and indeed qualitative success (Heithaus and Frid, 2003), may in part be explained by their failure to appreciate the importance of this respiratory gas. Evidence of the importance for tufted ducks of balancing the carbon dioxide stores was obtained in *Chapter III* and supports the conclusions of Boutilier et al. (2001) that readjustment of these stores is associated with the time a diver spends ventilating at the surface. *Chapter III* also alludes to evidence that oxygen stores do not appear to be limiting the dive durations of tufted ducks raising the question of whether carbon dioxide is influential in the element of the dive cycle as well. Results in *Chapter IV* support this evidence, demonstrating that even when the ducks were inspiring levels of oxygen half that in normal air in between dives, they were still capable of diving completely aerobically for far longer than they normally do during dives in natural conditions.

Despite this, however, the ducks still reduced their dive durations when diving from hypoxia. While this seems somewhat paradoxical, it may be highlighting a criticism of optimality theory, not discussed in the otherwise comprehensive condemnation of optimality modelling by Pierce and Ollason (1987) discussed in the *General Introduction*. Most animals simply may not be able to behave optimally for more than a fraction of the time they are active because of the stress, both physical and mental, that consistently striving to maximise efficiency would cause. We humans need only look at the consistently suboptimal behaviour in our own activities for evidence of this. Tufted ducks may therefore be diving well within their limits simply because there is no need, and therefore there is not the motivation, to put in more effort to work harder. In contrast, King penguins and Emperor penguins, notwithstanding their many morphological adaptations, are routinely pushed to their physiological limits when diving for food (Butler, 2000) and probably rely on anaerobic metabolism in the muscles as the oxygen stores deplete, enabling vital access to food sources in deep waters that would otherwise be unobtainable (Kooyman and Ponganis, 1998). Perhaps, then, the foraging patterns of these penguin species are more of a mystery than the apparent sub-optimal behaviour of diving ducks since it seems extraordinary that these Antarctic divers are able to cope with the stresses of consistently maximising effort during foraging bouts that often continue for days.

Not just tufted ducks but in fact the majority of diving species are believed by many to perform dives that are usually well within their aerobic diving capacity (e.g. Fedak and Thompson, 1993; Boyd, 1997; Butler and Jones, 1997; Butler, 2001). While it is accepted that certain species do rely on anaerobic metabolism during the latter part of some longer dives, such as Weddell seals (Kooyman et al., 1980), this may be the exception rather than the rule (Boyd, 1997). Further, species such as tufted ducks, which always perform dives within their calculated aerobic dive limit (*Chapter III*), are therefore considered never to use anaerobic pathways. Therefore, eighteen months ago, it would have been somewhat surprising to me to think that two paragraphs in the *General Discussion* of my thesis would be devoted to writing about anaerobiosis in

diving animals. However, evidence is mounting that anaerobic pathways are utilised by diving animals more often than previously considered (e.g. Butler and Jones, 1982). Mori (1999) suggests that natural selection would favour a diver that can vary the ratio of energy supplied anaerobically to total energy spent during the dive, while Ydenberg and Clark (1989) and Boyd (1997) argue that anaerobic metabolism can be more efficient when exploiting an ephemeral prey resource. That alcids in general can dive for longer than penguins in general, for their size, could not be explained by Watanuki and Burger (1999) given that their oxygen stores are similarly sized and their mass specific metabolic rates during diving are matched. They suggest that information on the use of anaerobic metabolism by alcids may help explain their remarkable diving capabilities (see also *Chapter V* and *Appendix III 'Ultimate Divers'*).

There is also evidence that some divers, particularly avian divers since they are small animals and so have relatively small oxygen stores in relation to their metabolic rate, routinely exceed their calculated aerobic dive limits (e.g. blue-eyed shags, Croxall et al., 1991; Brünnichs guillemots, Croll et al., 1992; shags, Wanless et al., 1993). This is also the case with King and Emperor penguins, of course. Now it appears that even tufted ducks may employ anaerobic metabolism in certain 'natural' situations. For example, when they are temporarily unable to surface from a voluntary dive they exhibit a profound bradycardia indicative of peripheral vasoconstriction and anaerobiosis (Stephenson et al., 1986). Their mean rate of oxygen consumption during a dive is reduced during longer dives, possibly producing a more severe vasoconstriction in non-active parts of the body that might suggest a move to employing anaerobic pathways (Bevan et al., 1992). Estimates of oxygen consumption during foraging in *Chapter IV* suggest a dramatic decrease in oxygen uptake during dives from hypoxia, which may simulate the latter part of the longer dives in tufted ducks reported by Bevan et al. (1992). This again indicates an increase in preferential perfusion of the active muscles and thus a possible move to anaerobic metabolism in other parts of the body. However, the physiological responses of diving

birds to breathing hypoxic gas mixes are unclear and certainly the implantation of heart rate monitors into ducks diving from hypoxia would afford very useful further information.

Chapters V-VII demonstrate that comparative biology is a powerful tool when striving to understand the behaviour of a diving species. The efficacy of comparative biology, specifically comparative physiology, to elucidate the principles of respiratory adaptations of diving animals was glimpsed in *Chapter III*. This study incorporated a section that compared rates of respiratory gas exchange between tufted ducks and grey seals to help understand what influences the termination of a ventilatory period and the start of the next dive. Work obtaining comparative data on the behaviour and physiology of further diving species using the respirometry technique incorporated in *Chapters II-IV* would be valuable further work building on the studies of the present thesis as it would undoubtedly begin to answer some further questions regarding the physiological basis of respiratory adaptations and behaviour. Furthermore, predictions about diving behaviour generated from such laboratory based studies, specifically designed to be testable in the field, would allow the behaviour of further species to be studied, in particular ones which cannot be researched using laboratory studies due to, for example, their size or their protected status.

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Appendices

Appendix I   Avian Anatomy

Avian air sacs

The air sacs promote the necessary tidal ventilation by way of volume changes but with only a negligible diffusion of gas across their walls (Magnussen et al., 1976). Thus, in contrast to the mammalian lung, the structures subserving ventilation and gas exchange are separated (Scheid, 1979). During respiration, the sternum rotates about its end close to the vertebral column, enlarging or compressing the thoracic cavity, which causes the volume changes in the air sacs (Duncker, 1974). These air sacs exist as four paired and one unpaired ventilatory bags. The pairs of cervical air sacs and cranial thoracic air sacs, along with the unpaired clavicular air sac, all connect to the medioventral secondary bronchi and are known as the cranial group air sacs, while the caudal group (the two caudal thoracic air sacs and the two abdominal air sacs) are directly connected to the mesobronchus (Scheid and Piiper, 1971).

The neopulmo parabronchi

The neopulmo extends from the main bronchus as well as the mediodorsal and medioventral secondary bronchi to the caudal air sacs. Unlike the paleopulmo, this network lies in series with the airflow from the primary bronchus into the cranial group of air sacs. The development of the neopulmo varies considerably between avian species, being highly developed in song bird species and fowl birds including the hen but absent in penguins (Scheid, 1979). Cormorant species constitute an early point and duck species a midway point of neopulmo development along this continuum, although in ducks it still only occupies a minor part of the total lung mass (Scheid and Piiper, 1971). The histology of the neopulmo parabronchi is very similar to that of the paleopulmo parabronchi, although in ducks the neopulmo has been recorded as being slightly better perfused than the rest of the lungs (Holle et al., 1978). The presence of the neopulmo correlates with an adjustment in lung shape and most importantly, the caudal primary bronchus connecting the abdominal air sacs is substituted by the neopulmonal network (Fitzgerald and Blais, 1993). Airflow within the neopulmo network is bi-directional and so dependent upon repiratory phases since it lies in series with the airflow from the primary bronchus to the cranial group of air sacs.

Rectification of flow and the controlling mechanisms

Brackenbury (1971) saw the airflow patterns in the avian respiratory system to be controlled by two related features, notably the aerodynamic properties of the lung denoted by the lung 'circuitry' architecture and the spatial relationships of the air sacs. The posterior and anterior air sacs were shown to deflate aphasically, thus conferring rectification, however more recent studies suggest that the circuitry itself is more influential in the rectification process.

When the volume of the air sacs increase, causing a decrease in air pressure, the inspired air stream divides at the opening of the medial dorsal secondary bronchi into that which passes through the bronchi and into the paleopulmo and that which enters the caudal air sacs (Torre-Bueno et al., 1980). There is a concurrent airflow into the cranial group of air sacs from the medioventral secondary bronchii, which is supplied in turn by an airflow from the parabronchi. In turn, the parabronchi are flooded with ambient air supplied from the primary bronchus via the mediodorsal secondary bronchi. Thus, during inspiration, the cranial group of air sacs receive air that has been involved in gaseous exchange while the caudal group sacs receive fresh air.

Expiration then forces air from the sacs and perhaps partly due to the dorsal curvature of the primary bronchus, the airflow from the abdominal air sac is directed into the openings of the mediodorsal secondary bronchi. Scheid et al. (1972) suggested that the openings of the secondary bronchi into the mesobronchus offer a direction dependent resistance to air flow cranially, due to detachment of flow at sites of sharp edges, which would also influence the expired air to enter the mediodorsal secondary bronchi. This air then passes back through the parabronchi, back through the medioventral secondary bronchi and back into the anterior primary bronchus. Here, it is supplemented with air flowing caudally from the laterobronchus, which originates from the ventral side of the primary bronchus. The expired air from the cranial air sacs streams into the primary bronchus together with the parabronchial air from the caudal air sacs (Duncker, 1974).

Consequently, the paleopulmonal parabronchi are ventilated unidirectionally. According to Duncker (1974), the flow in the parabronchi during the expiratory phase

of respiration is partly due to the assembly of the mediodorsal secondary bronchial openings on the curved part of the primary bronchus in combination with the laterobronchus. He suggests that the cranial air sacs, with their connections to the medioventral secondary bronchi, establish the inspiration phase airflow through the parabronchi. However, since the connections of the medioventral secondary bronchi to these air sacs are limited, this theory does not adequately identify all the mechanisms responsible for maintaining unidirectional flow.

With a deficiency of evidence for mechanical valves as the mechanism controlling unidirectional flow (e.g. Hazelhoff, 1943; Jones et al., 1981) such as valve leaflets (Brown et al., 1995) further testable theories have been lacking. Wang et al. (1992) points out that the pivotal question concerns how the flow of air during inspiration bypasses the medioventral secondary bronchi and continues caudally to the mediodorsal secondary bronchi and the caudal group air sacs. Wang et al. (1988) assessed various aerodynamic factors potentially influencing valve function during inspiration by introducing steady inspiratory flows into models of bifurcation. They found that the flow rate and geometry upstream of the bifurcation did play roles, however that the branching angles of the medioventral secondary bronchi did not.

Hazelhoff (1943), Brackenbury (1971), Scheid et al. (1978) and Kuethe (1987) have offered various speculations concerning aerodynamic valves. However, not until studies by Banzett et al. (1987), Banzett et al. (1991) and Wang et al. (1992) has there been evidence that muscular constriction at the medioventral secondary bronchial junction in the primary bronchus, which increases the velocity of the inspired air stream, functions as an inspiratory aerodynamic valve during resting conditions (Butler et al., 1988; Wang et al., 1988). This constriction is termed the segmentum accelerans (Wang et al., 1992; see *Figure AI- 1*). When airflow rates are high the segmentum accelerans is dilated since gas velocity is high enough to maintain valve effectiveness without the need of constriction at the caudal end of the primary bronchus. With lower airflow rates, the segmentum accelerans is constricted to maintain valving. The air is accelerated into the mesobronchus due to the constriction to ensure that it still bypasses the medioventral secondary bronchial openings. These findings agree with the predictions of Butler et al. (1988) and Wang et al. (1988) that

convective inertia of the inspiratory gas flow promotes preferential axial flow and is likely to be the principal mechanism for inspiratory aerodynamic valving.

Brown et al. (1995) investigated the presence of a mechanism controlling expiratory valving to direct the gas exiting the caudal air sacs to flow through the mediodorsal secondary bronchi with no flow cranially along the primary bronchus. Powell et al. (1981) reported that only 12 % of caudal sac gas bypassed the lung during expiration at rest in ducks while Bouverot and Dejours (1971) reported a 10 % bypass in chickens. Furthermore, the study by Brown et al. (1995) indicated that 'valving' became even more efficient at higher flow rates such as during exercise. They offer two possibilities to explain their findings, both of which rely on changes in airway calibre. Either the expiratory valving is a mechanism governed by connective inertia or dynamic compression of the mesobronchus during expiration could produce a velocity sensitive expiratory valve.

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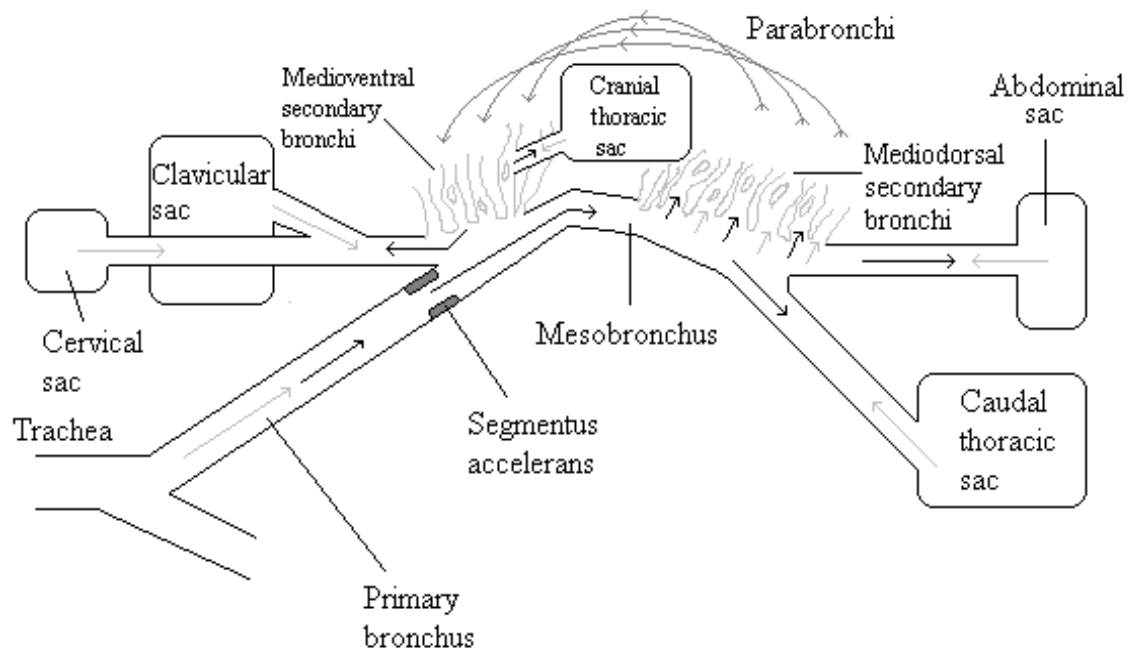
Figure AI-1

Schematic of an avian right lung, airways and bronchial tree, viewed from the medial aspect. Black arrows represent air flow direction during inspiration while light grey arrows represent air flow direction during expiration. The dark grey arrows represent the unidirectional flow of ventilatory air through the parabronchi. The segmentus accelerans is also depicted (Wang et al., 1995). The neopulmo has been omitted to aid clarity.

Appendix II Data on Air Breathing Aquatic Vertebrates

Table AII-1. Database of aquatic air breathing vertebrates: Diving parameters, body mass and number of observations

Common Name	Scientific Name	Mass, M (kg)	Submergence duration		Surface interval			Diving extent		Subject sample size, N	Dive sample size, n	Sources
			Mean dive duration, t_d (s)	Mean max. dive duration, t_{dmax} (s)	Mean Surface duration, t_s (s)	Modal Surface duration (s)	Dive : Pause ratio, d:p	Mean dive depth, d_d (m)	Mean max. dive depth, d_{dmax} (m)			
Main taxonomic groups (placed in regression analyses)												
Alcids (Family: Alcidae)												
Rhinoceros Auklet	*Cerorhinca monocerata*	0.5	45	30	11	-	4.18	-	-	16	75	Burger et al. (1993)
Black Guillemot	*Cepphus grylle*	0.4	71	-	31	12	2.3	21	35	-	-	Bradstreet and Brown (1985), Burger (1991) and Cairns (1992)
Guillemot / Common murre	*Uria aalge*	0.97	45	-	11	10	3.98	8	60	-	-	Bradstreet and Brown (1985), Croll and McLaren (1993) and Wanless et al. (1988)

Brünnichs guillemot / Thick-billed murre	*Uria lomvia*	0.8	51	-	15	-	3.40	13	80	-	-	Bradstreet and Brown (1985), Croll and McLaren (1993), Croll et al. (1992)
Razorbill	*Alca torda*	0.6	29	-	36	-	0.97	5	10	-	-	Bradstreet and Brown (1985) and Wanless et al. (1988)
Atlantic puffin	*Fratercula arctica*	0.4	28	-	30	-	.93	8	-	-	462	Bradstreet and Brown (1985) and Wanless et al. (1998)
Pigeon guillemot	*Cepphus columba*	0.6	67	-	98	-	.68	20	42	20	21	Clowater and Burger (1994)
Dovekie	*Alle alle*	0.2	-	-	-	-	-	-	28	34	-	Falk et al. (2000)
Cassins's auklet	*Ptychoramphus aleuticus*	0.2	50	-	-	-	-	28	43	22	-	Burger and Powell (1990)

Marbled murrelet	*Brachyramphus marmoratus*	0.2	26	-	18	-	1.44	-	-	14	6179	Jodice and Collopy (1999)
Cormorants (Family: Phalacrocoracidae)[2]												
Shag	*Phalacrocorax aristotelis*	1.8	62	-	84	50	0.73	-	-	31	4171	Wanless et al. (1993)
Blue-eyed shag	*Phalacrocorax atriceps*	2.7	169	-	-	96	-	30		2	674	Croxall et al. (1991)
Maquarie cormorant	*Phalacrocorax albiventer*	2.7		-	-	13	-	-	-	6	15779	Parkes (2002)
Termminck's cormorant	*Phalacrocorax filamentosus*	2.8	-	-	-	8	-	-	-	11	2684	Parkes (2002)
Rock shag	*Phalacrocorax magellanicus*	1.2	28	-	9	-	2.97	4.6	-	-	-	Wanless and Harris (1991)
Heard Island shag	*Phalacrocorax nivalis*	2.6	-	-	-	-	-	-	37	11	-	Green and Williams (1997)
Japanese cormorant (male)	*Phalacrocorax capillatus*	3.1	37	-	59	-	0.62	15	39	6	4617	Watanuki et al. (1996)

Japanese cormorant (female)	*Phalacrocorax capillatus*	2.5	24	-	33	-	0.74	7	26	7	7242	Watanuki et al. (1996)
Pelagic cormorant	*Phalacrocorax pelagicus*	1.9	37	-	17	-	2.11	-	-	-	1911	Cooper (1986)
Bank cormorant	*Phalacrocorax neglectus*	2.0	37	-	24	-	1.57	-	-	-	171	Cooper (1986)
Reed cormorant	*Phalacrocorax africanus*	0.6	15	-	4	-	3.40	-	-	-	64	Cooper (1986)
Crowned cormorant	*Phalacrocorax melanoleucos*	0.8	22	-	8	-	2.86	-	-	-	-	Cooper (1986) and Lea et al. (1996)
White-breasted cormorant	*Phalacrocorax lucidus*	2.9	39	-	9	-	4.36	-	-	-	54	Cooper (1986)
European cormorant	*Phalacrocorax carbo*	3.5	51	-	14	-	3.67	-	-	-	34	Cooper (1986)
Guanay cormorant	*Phalacrocorax bougainvillii*	1.8	-	-	-	-	-	-	34	27	-	Zavalaga and Paredes (1999) and Cooper (1986)
Pied shag	*Phalacrocorax varius*	1.8	27	-	9	-	2.94	-	-	-	-	Lea et al. (1996) and Cooper (1986)

Seals, fur seals and sea lions (Suborder: Pinnipedia)[1,2]

Grey seal	*Halichoerus grypus*	179.6	297	-	106	30	2.82	49	-	-	-	Thompson and Fedak (1993) and Beck et al. (2000)
Antarctic fur seal	*Arctocephalus gazella*	41.2	54	284	30	45	1.80	13	-	11	62210	Boyd and Croxall (1991)
Southern elephant seal	*Mirounga leonine*	300.0	1050	775	126	105	8.33	391	-	1	1939	Boyd and Arnbom (1991)
Southern elephant seal (female)	*Mirounga leonine*	383.0	1512	-	150	-	10.08	429	1629	13	50000	Hindell et al. (1991)
Northern elephant seal	*Mirounga angustirostris*	1300.0	1350	3600	216	198	6.25	389	1100	6	36233	DeLong and Stewart (1991)
Northern elephant seal (female)	*Mirounga angustirostris*	350.0	1152	2520	168	-	6.86	400	860	8	10036	Le Bouef et al. (1988)

New Zealand sea lion	*Phocarctos hookeri*	115.0	211	457	269	75	0.78	116	294	15	19720	Gales and Mattlin (1997)
Bearded seal	*Erignathus barbatus*	325.0	120	812	114	-	1.05	17.2	184	4	15077	Krafft et al. (2000)
Harp seal	*Phoca groenlandica*	123.0	192	730	84	-	1.27	30	95	4	3701	Lydersen and Kovacs (1993)
California sea lions	*Zalophus californicus*	90.0	124	463	85	-	1.47	62	224	10	8900	Feldkamp et al (1988)
Southern sea lions	*Otaria flavescens*	126.0	186	366	86	-	2.17	61	117	6	18057	Werner and Campagna (1995)
New Zealand fur seal (female)	*Arctocephalus fosteri*	37.0	66	284	144	-	0.35	23	146	-	-	Harcourt et al. (1995) and Harcourt and Davis (1997)
Ross seal	*Ommatophora rossii*	160.0	384	588	48	-	8.00	110	212	1	97	Bengston and Stewart (1997)
Ringed seal	*Phoca hispida*	60.0	351	-	-	45	-	53	-	7	100872	Gjertz et al. (2000)

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Saimaa Ringed seal	*Phoca hispida saimensis Nordq*	67.0	328	900	64	-	5.13	13	-	4	17000	Hyvarinen et al (1995) and Kunnasranta (2001)
Hooded seal	*Cystophora cristata*	210.0	1223	-	122	-	10.02	-	700	16	120000	Folkow and Blix (1995) and Folkow and Blix (1999)
Crabeater seal	*Lobodon carcinophagus*	220.0	240	-	61	45	3.92	22	106	6	7753	Bengston and Stewart (1992) and unauthored web page
Weddell seal (immature)	*Leptonychotes weddelli*	167.0	529	780	-	-	-	195	290	4	-	Kooyman et al. (1983)
Weddell seal (adult)	*Leptonychotes weddelli*	350.0	680	2000	330	-	2.06	122	438	69	24199	Castellini et al. (1991)
Atlantic Walrus	*Odobenus rosmarus rosmarus*	1386.0	312	-	90	-	3.47	20	69	1	1693	Wiig et al. (1993)

Penguins (Family: Spheniscidae)[*1]

Macaroni penguin	*Eudyptes chyrsolophus*	3.6	85	(136)	30	28	2.86	17	60	13	2555	Green (pers. comm.)

Royal penguin	*Eudyptes schlegeli*	5.0	102	(193)	-	-	-	33	95	29	42382	Hull (2000)
Eastern rockhopper penguin	*Eudyptes chryscome filholi*	2.9	72	(169)	-	-	-	27	80	29	57130	Hull (2000)
Northern rockhoppper penguin	*Eudyptes chryscome moseleyi*	2.3	57	(185)	21	17	2.71	18	90	14	16572	Cherel et al. (1999)
Little penguin	*Eudyptula minor*	1.2	21	(84)	-	-	-	3	27	12	6000	Bethge et al. (1997)
Adélie penguin	*Pygoscelis adeliae*	4.5	85	(135)	42	-	2.02	12	59	-	26444	Watanuki et al (1993) and Watanuki (2001, unpub. data)
Gentoo penguin	*Pygoscelis papua*	6.1	98	(282)	98	-	1.00	51	150	-	15841	Williams et al (1992) and Croxall et al. (1988)
Chinstrap penguin	*Pygoscelis antarctica*	4.1	72	(235)	-	-	-	31	121	4	10525	Bengston et al. (1993)
Emperor penguin	*Aptenodytes forsteri*	26.6	212	(845)	-	120	-	107	845	5	15938	Kooyman and Kooyman (1995)

Emperor penguin (female)	*Aptenodytes forsteri*	27.0	240	(637)	-	-	-	120	637	14	30645	Kirkwood and Robertson (1997)
King penguin	*Aptenodytes patagonicus*	14.0	171	(529)	102	-	1.68	94	529	23	11874	Kooyman et al. (1992)
Magellanic penguin	*Spheniscus magellanicus*	5.0	65	(145)	-	-	-	20	65	-	6030	Peters et al. (1998); Radl and Culik (1999), Scolaro and Suburo (1991) and Reilly (1994)
Yellow-eyed penguin	*Megadyptes antipodes*	5.5	-	-	-	-	-	-	34	24	-	Seddon et al. (1990)
Ducks and Grebes (Families: Anatidae and Podicipedidae)[†]												
Tufted duck	*Aythya fuligula*	0.8	18	-	-	8	-	-	-	30	488	Parkes (2002) and Magnusdottir and Einarsson (1990)
Ruddy duck	*Oxyura jamaicensis*	0.7	-	-	-	10	-	-	-	300	7914	Parkes (2002)
Harlequin duck	*Histrionicus histrionicus*	0.8	22	-	14	-	1.57	-	-	-	-	Rodway (1998)

Scaup	*Aythya marila*	1.1	-	-	-	-	-	-	6	-	-	Dewar (1924)
Common Scoter	*Melanitta nigra*	1.0	30	-	-	-	-	-	6	-	-	Dewar (1924) and Magnusdottir and Einarsson (1990)
Common Eider	*Somateria mollissima*	2.1	-	-	-	-	-	-	11	-	-	Dewar (1924)
Great crested grebe	*Podiceps cristatus*	1.0	20	-	16	11	1.25	-	6	6	334	Parkes (2002) and Dewar (1924)
Western grebe	*Aechmophorus occidentalis*	0.9	38	-	225	-	0.15	-	8	-	153	Forbes and Sealy (1988)

Taxonomic groups for 2^0 signal species

Albatrosses (Family: Diomedeidae)

Shy albatross	*Diomedea cauta*	3.9	6	-	-	-	-	2	9	15	52	Hedd et al (1997) and Wilson (pers. comm.)
Grey-headed albatross	*Diomedea chrysostoma*	3.5	4	-	1200	300	-	1	7	4	485	Huin and Prince (1997)

Marine Turtles (Family: Cheloniidae)

Green turtle	*Chelonia mydas*	200.0	1938	-	117	-	16.56	13	20	2	368	Hays et al. (2000)

Toothed Whales (Suborder: Odontoceti)

Northern bottlenose whale	*Hyperoodon ampullatus*	6500.0	1995	-	-	-	-	-	956	2	23	Hooker and Baird (1999) and Macdonald (1995)
Sperm whale (male)	*Physeter macrocephalus*	45000	1938	-	-	600	-	-	-	136	142	Jacquet et al (2000)
Harbour porpoise	*Phocoena phocoena*	44.6	65	-	-	-	-	24	151	7	8167	Westgate et al (1994)
Pantropical spotted dolphins	*Stenella attenuata*	80.0	36	-	-	-	-	15	200	4	-	Baird et al. (2001)

Sea Otters (Family: Mustelidae)

Cape clawless otter	*Aonyx capensis*	13.0	21	-	8	-	2.73	-	-	106	1696	Somers (2000)

* Values in parentheses are estimates (see text, *Chapter V* for details).

[†] The families anatidae and podicipedidae were combined into one group in order to increase the number of data points in analyses. This amalgamation is deemed acceptable because these families have very similar diving behaviours and body masses. Both are generally inland water dwelling, foot propelled, non-plunge divers which perform flat-bottom rather than bounce dives (Wilson, 1991).

[1] Taxonomic groups included in *Chapter VI*.

[2] Taxonomic groups included in *Chapter VII*.

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Phylogenetic relationships of the main taxonomic groups
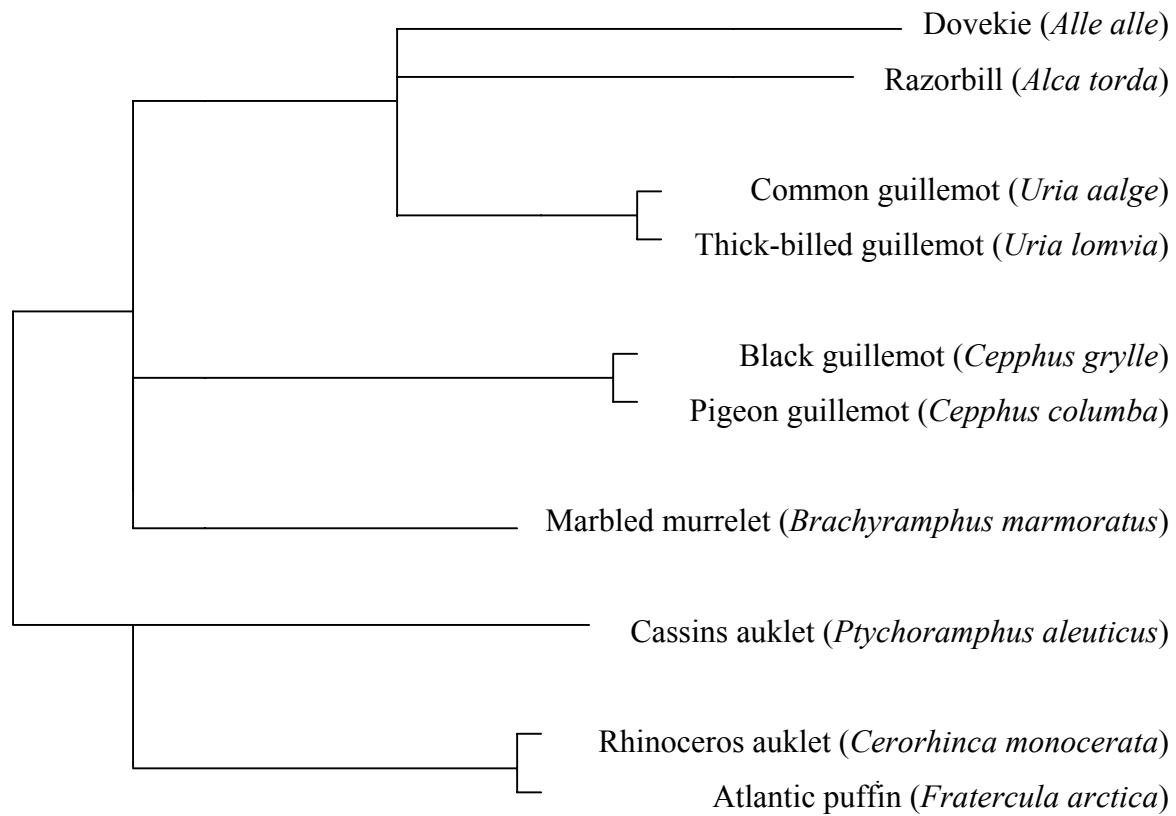
Figure AII-1

Phylogenetic tree of some species of the family Alcidae. Based on data from Friesen et al. (1996) and Strauch (1985).
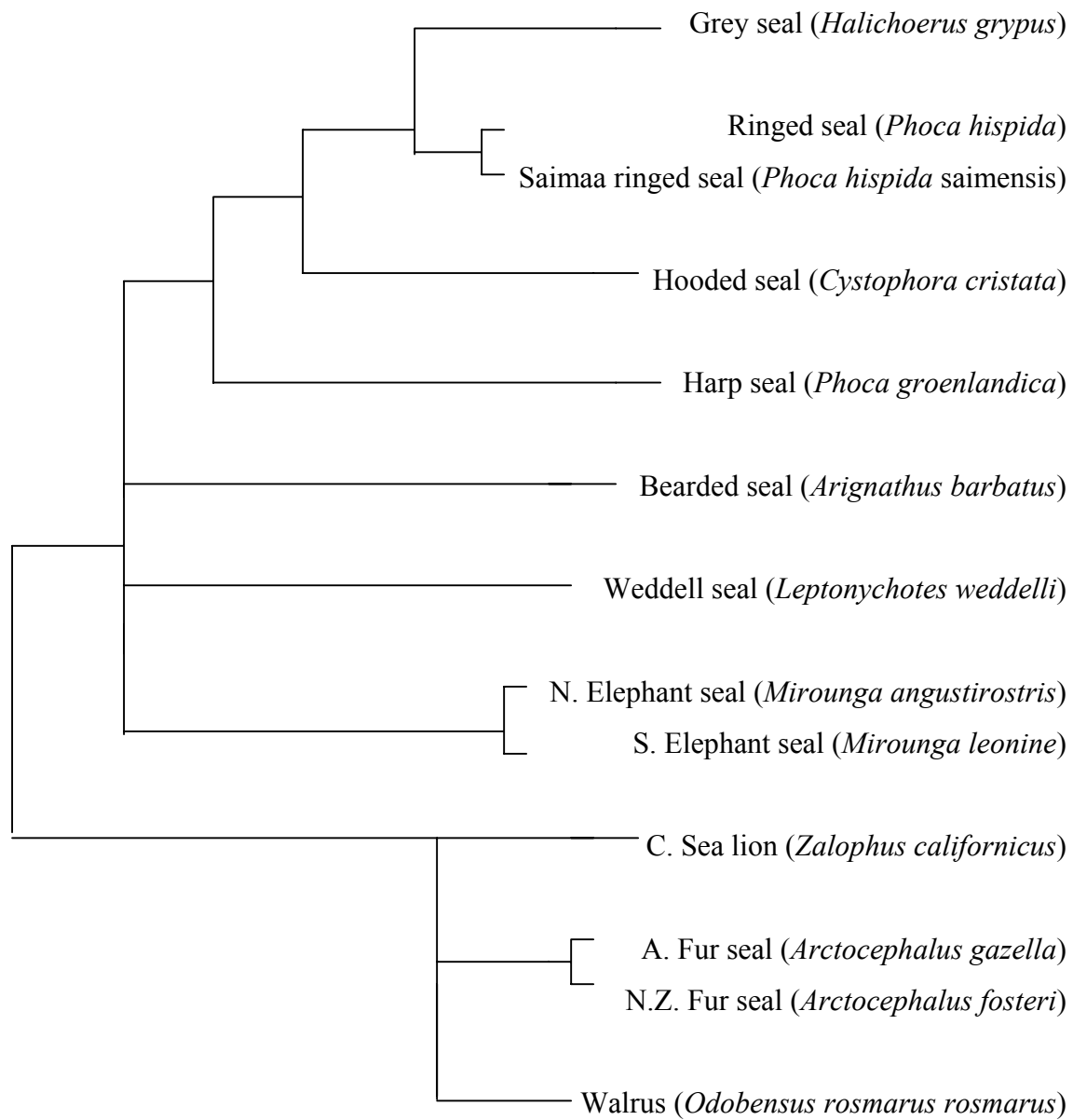
Figure AII-2

Phylogenetic tree of some species of the suborder Pinnipedia. Based on data from Arnason et al. (1995), Slade et al. (1994), King (1983) and Bininda-Edmonds (2000).
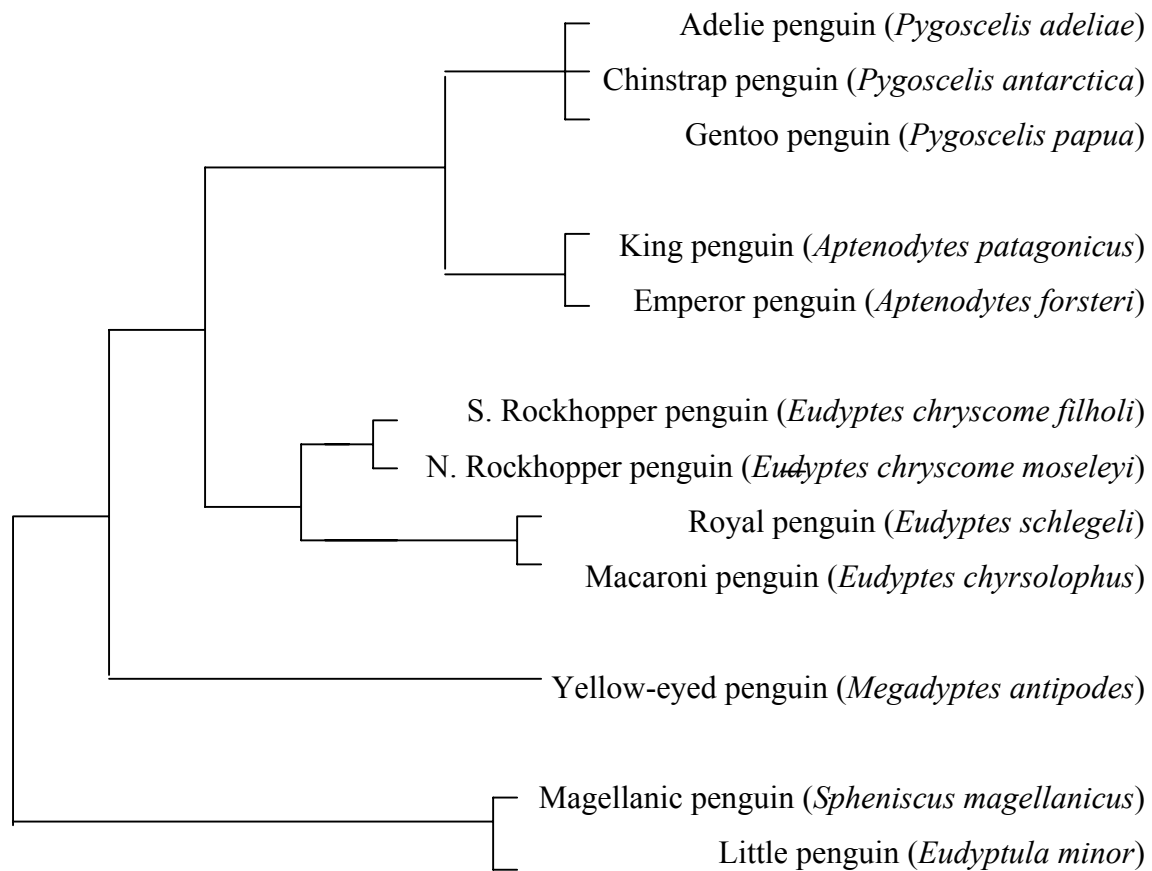
Figure AII-3

Phylogenetic tree of some species of the family Spheniscidae. Based on data from Paterson et al. (1995), Williams (1995) and O'Hara (1989).

Figure AII-4

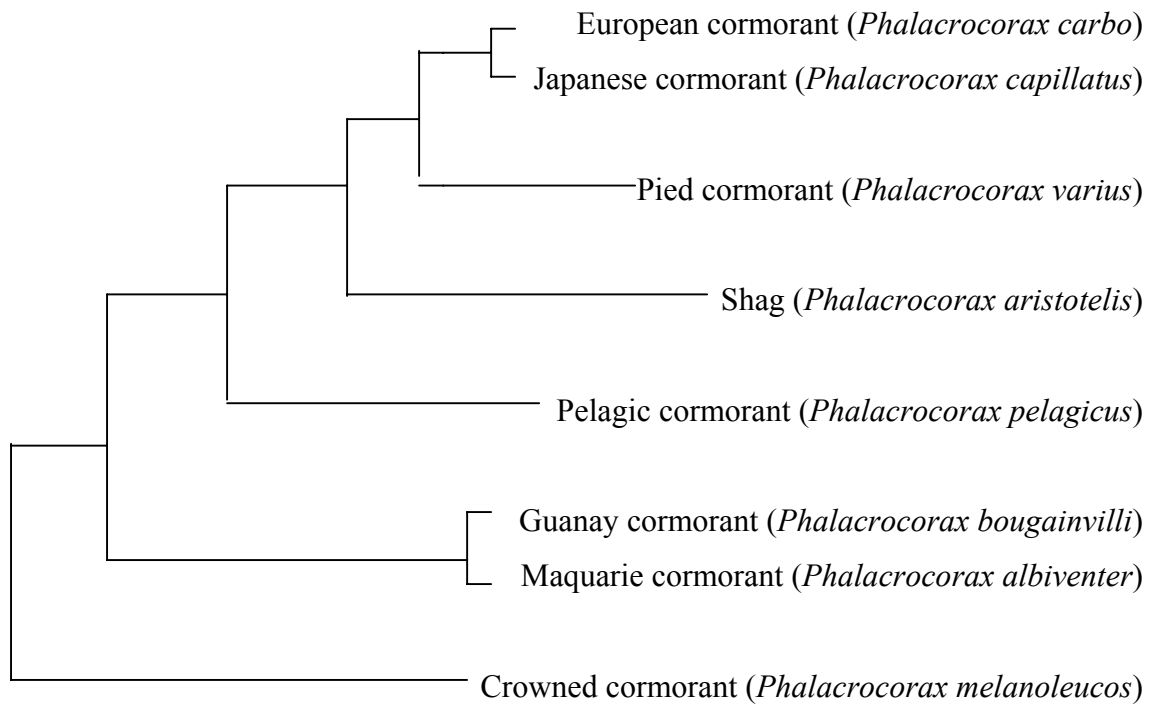Phylogenetic tree of some species of the family Phalacrocoracidae. Based on data from Kennedy et al. (2000) and Siegel-Causey (1988).

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Appendix III   Other Publications

Abstract Publication

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A4 – General Respiration – Posters

A4.10 – Can diving optimality models predict adjustments in the diving behaviour of tufted ducks?

L.G. Halsey, P.J. Butler and A.J. Woakes

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The effects of differing foraging costs on dive time and rate of oxygen uptake at the surface were measured in six tufted ducks during bouts of voluntary dives to a feeding tray. The birds were trained to surface into a respirometer after each dive, so that changes in the rate of oxygen uptake could be measured. The tray either held just food, or held closely packed stones on top of the food to make foraging energetically more costly. In contrast to predictions from the optimal foraging model of Houston and Carbone (1992), foraging time increased after dives incorporating higher foraging energy costs while surface time remained the same. However, the rate of oxygen uptake was significantly higher during the surface period after energetically more costly dives. This compensated for the increased energy consumption of the dive without the need to increase surface time. The 'optimal breathing model' by Kramer (1988) predicts the amount of energy metabolised during foraging as well as the optimal surface time. This model was tested with data on time budgets and oxygen uptake curves from the present study, and estimates of power costs during diving from Lovvorn et al. (1991). The model failed to predict the surface time of the ducks or oxygen consumption during foraging. This suggests that the ducks were not diving to maximise time spent at the feeding tray. However, the precision of the model may have been affected by inaccurate values of power cost estimates.

Abstract Publication

F07 – Nutrition, foraging and energetics – Poster Abstracts
Can diving optimality models predict adjustments in the diving behaviour of tufted ducks?

Lewis G. Halsey, Roland Parkes, Patrick J. Butler and Anthony J. Woakes

Keywords: Tufted ducks, optimal foraging, energetics

Optimal foraging models predicting the behaviour of air breathing divers have successfully anticipated some qualitative behavioural trends (e.g. Kramer, 1988; Houston and Carbone, 1992; Mori, 1998). However, the shape of the oxygen uptake curve, which is a key defining aspect of these models, has not been quantified and it is likely that its details have a critical effect on the gross model predictions (Ruxton et al., 2000). For example, Walton et al. (1998) suggest that the curve for birds is biphasic due to their cardio-respiratory physiology, and the resultant 'kink' in the curve would effect optimal surface times. The effects of increased dive duration and increased energy demands of foraging on the shape of the oxygen uptake curve were quantified for six tufted ducks *Aythya fuligula*. The birds were trained to surface into a respirometer after each dive to a feeding tray, so that changes in the rate of oxygen uptake could be measured. The tray either held just food, or held closely packed stones on top of the food to make foraging energetically more costly. The oxygen uptake curve after longer dives was statistically biphasic. However, this was probably due to an increase in respiratory frequency for the first few seconds after the dive and the kink did not affect predicted optimal surface times. The rate of oxygen uptake was significantly higher after energetically more costly dives i.e. of longer duration or foraging among stones. The 'optimal breathing model' (Kramer, 1988) predicts the amount of oxygen consumed during foraging and the optimal surface time. This model was tested with data on time budgets and oxygen uptake curves from the present study, and estimates of power costs during diving from Lovvorn et al. (1991). The model successfully predicted surface time and oxygen consumption during foraging for the mean of all ducks, suggesting that the ducks were diving optimally. However, the model did not consistently predict these variables for individuals.

Abstract Publication

24.0 – Diving: Where have we been and where are we going? - Posters

24.13 – **Can diving optimality models predict adjustments in the diving behaviour of tufted ducks?**

Lewis Halsey, Pat Butler, Tony Woakes

We measured the effects of differing foraging costs on dive time and rate of oxygen uptake at the surface in tufted ducks during bouts of voluntary dives to a feeding tray at 1.1 m depth. The birds were trained to surface into a respirometer so that changes in the rate of oxygen uptake could be measured. The tray either held just food, or held closely packed stones on top of the food to make foraging energetically more costly. In contrast to predictions from the optimal foraging model of Houston and Carbone (1992), foraging time increased after energetically more costly dives. However, the rate of oxygen uptake was significantly higher during the surface period after these dives. This compensated for the increased energy consumption of the dive without the need to increase surface time. The 'optimal breathing model' by Kramer (1988) predicts the energy metabolised during foraging and surface time. This model was tested with data on time budgets and oxygen uptake curves from the present study, and estimates of power costs during diving from Lovvorn et al. (1991). The model successfully predicted surface time and oxygen consumption during foraging for the mean of all ducks. However, the model did not consistently predict surface time or oxygen consumption during foraging using data for individual birds. The precision of the model may have been affected by inaccurate values of power cost estimates. This work was supported by a NERC studentship.

Essay Publication

Ultimate divers

Lewis Halsey

An extraordinary variety of mammals, birds, amphibians and reptiles are adapted to life in the oceans. Many of them spend their entire lives there without being able to breathe underwater. But just how do they exploit this hostile environment?

Whales, dolphins, marine turtles, seals and sea lions, penguins and many other species are heavily constrained by the necessity to periodically break the water surface in order to obtain oxygen. Their obligation to surface carries many disadvantages, including limiting time available to explore and forage, and increasing the risk of attack from predators. Yet the existence of all these species shows that they have successfully adapted to exploiting the aquatic environment. Of vital importance has been the evolution of physiological and biochemical adaptations that increase the amount of time these species can remain under water. Many of them have also adapted to exploit resources in particularly deep parts of the ocean. Not only does this require the ability to function without ventilating the lungs for long periods but it also necessitates tolerance to very high hydrostatic pressures.

Inevitably, some air breathing, diving species are capable of performing dives of much longer duration or to much greater depths than other species, and some achieve astonishing feats of underwater endurance. In a recent article in *Biologist*, Bill Milsom (2000) described many of the physiological and biochemical adaptations of air breathing divers that enable them to prolong their time beneath the surface. In this article, I debate which species are the world's elite divers, in terms of depth of dive and duration of dive. Present understanding of the adaptations that allow such extreme diving behaviour in these particular animals is also discussed.

Deep divers

So which air breathing divers are able to travel deepest into ocean waters? Emperor penguins are famous for their deep diving ability since they are able to dive deeper than any other bird species and have been recorded at depths of up to 500 metres. However, marine mammals are generally deeper divers than marine birds and many of the large pinniped species (seals, sea lions and walruses) frequently dive to several hundred metres. Northern elephant seal bulls, the largest and deepest diving of all the pinnipeds, have been recorded at depths of around 1000 m. The cetaceans (dolphins, porpoises and whales) vary considerably in terms of maximum diving depth. However, many of the larger whale species are known to dive very deeply, often up to around 1000 m. The apparent depth barrier of one kilometre for deep diving pinniped and cetacean species may well be related to the depth that sunlight penetrates the ocean. Beyond 1000 m depth, the ocean environment is in complete darkness making foraging impossible for visual predators.

Only one air breathing species has been recorded diving noticeably deeper than one kilometre, and indeed to depths far in excess of any other air breathing diver. The Sperm whale is a huge animal, up to 18.5 m in length and weighing up to 50 tonnes. The deepest recorded dive is 2.25 km (1.4 miles) although the stomach contents of one individual included a species of dogfish found only on the seafloor, suggesting that they may dive to over three kilometres. Their ability to dive to incredible depths is not only reliant upon adaptations to tolerate immense hydrostatic pressures but also upon their ability to maintain a neutral buoyancy in the water at any depth.

Their head accounts for one quarter of their length and one third of their body weight. It contains massive organs filled with waxes, known as spermaceti oils. The density of these oils can be altered, changing the whale's buoyancy, so that it can hang motionless within the water locating its prey while using up very little oxygen. The wax organs have a second function: they act as an acoustic lens for focusing sound by refraction through the concentric layers of wax. This allows the whale to hunt in partial or complete darkness by using echolocation to search for prey. Echolocation enables the size, direction and distance of prey to be determined. This sense is highly efficient and blind Sperm whales have been captured in perfect health with food in

their stomachs. Their most common prey are giant squid weighing up to 300 kg and bottom-dwelling sharks. Sperm whales also consume cuttlefish and octopuses. They spend 90 % of their time in deep water, only resting at the surface intermittently.

Adaptations for deep diving

Although many air breathing divers exhale before they submerge, they still retain some air in their lungs and often in their respiratory tracts, in order to supply oxygen to their bodies during a dive. The problems that deep divers must overcome develop as the animal propels itself deeper into the water column. This is due to compression of these air-filled cavities.

Mechanical effects

As humans descend through the water column, even short vertical descents cause large volumes of blood to shift into the thoracic cavity. This ensures that pressure in the lungs remains at equilibrium with the increasing external pressure. Compression of the lung is thus seriously depth limiting in humans. However, whales appear not to be restricted in this way. It has been conjectured that their lungs and chests are designed to collapse as pressure increases with depth. The diaphragm is set obliquely and as the abdominal viscera presses on one side, the lungs crumple on the other. The lungs collapse to a fraction of their original size and force the air into the windpipe and the extensive nasal passages.

Increased hydrostatic pressure also causes 'high pressure neurological syndrome' which is exhibited as tremors and eventually a complete seizure in a number of terrestrial vertebrates including man. It has been presumed that deep divers are not affected since otherwise they would be unsuccessful as predators. Only very recently has an understanding developed concerning some of the adaptations in such species to combat this phenomenon. Studies on toothed whales suggest that they have an increased inhibitory feedback available in their central nervous system, which provides protection against the hyperexcitability of nerves that is induced by high pressure at extreme depths.

Nitrogen tensions

Human divers enter a state of stupor and then a terminal loss of consciousness if they dive too deeply for too long. During descent, nitrogen partial pressures increase in the lungs and nitrogen is absorbed into the arteries and tissues. Nitrogen within the tissues can cause an anaesthetic 'nitrogen narcosis', leading to reduced mental and motor capabilities, euphoria, coma and, ultimately death. These symptoms are due to the toxic effect of high nitrogen pressure on nerve conduction.

After periods of high pressure, decompression causes the gases in the body to expand. Oxygen is quickly absorbed but, if decompression is too rapid, rising nitrogen concentrations in the arteries can cause nitrogen bubbles to form in the bloodstream and then death may ensue (in human divers this condition is known as 'The Bends'). Bubbles develop in the capillaries of laboratory animals at nitrogen concentrations around 330 kPa. Nitrogen tensions in the cetaceans seem to remain lower than this. Nitrogen concentrations are probably limited before tensions in the blood and tissues reach this critical level by total lung collapse as the cetacean dives, preventing further nitrogen absorption during the dive. Some of the air from the lungs is forced into the windpipes where it cannot diffuse into the haemoglobin and myoglobin. Similarly, air pushed into the large nasal cavities cannot diffuse further due to the thick membrane lining.

Latent hypoxia

Deep dives by humans expose them to the risk of brain anoxia. This refers to a lack of oxygen within the brain tissues, which can cause unconsciousness. At depth, high partial pressures of oxygen within the lung can cause rapid diffusion into the bloodstream, raising the pressure of arterial oxygen while lowering the pressure of oxygen in the lungs. During ascent from depth, lung expansion causes a further decrease in lung oxygen tension, which could crucially drop to a level lower than that of the venous blood causing oxygen to diffuse into the lungs. In turn, this can cause arterial oxygen pressure to decline to intolerable levels within the brain. This reversal in oxygen gradient will occur during the diver's ascent through the upper 10 m of the water column. This is where the greatest expansion of the lung occurs as it doubles in volume.

The adaptations of deep divers to cope with this hazard are poorly understood. It is likely that many species counteract this risk through the reduction of lung-blood gas exchange rates at depth. Again, cetaceans would achieve this through lung collapse, nullifying the problem. Furthermore, measurements made in some mammalian divers have shown them to have much lower arterial partial pressures of oxygen in their brains than terrestrial animals, enabling them to cope with low oxygen tensions during ascent.

Long duration dives

For many taxonomic groups of air breathing divers, positive correlations exist between depth and duration of dives, since dives to greater depths require more travelling time. Nevertheless, certain species may spend considerable amounts of time submerged without descending to particularly great depths. This is because their prey inhabits the upper level of the pelagic zone, or they are only physiologically capable of withstanding relatively low hydrostatic pressures. Therefore, longer dives do not necessarily rely on the ability to dive deeply, but do require the diver to remain active underwater despite not having the opportunity to restock their oxygen stores. (Certain amphibians and reptiles can stay underwater for exceptionally long periods of time; Box 1).

Size advantage

The two overriding factors influencing how long an animal can dive for are considered to be their oxygen storage capacity and their efficiency at consuming oxygen. Allometric studies have demonstrated positive trends between body size and dive duration across many taxa of air breathing divers. Larger diving species have a body mass advantage because, as body size increases, blood volume increases more rapidly than resting metabolic rate. Therefore, larger divers store a greater supply of oxygen, and metabolise that oxygen more slowly, in relation to their body mass. Sperm whales are not only the deepest divers but also one of the longest divers, taking advantage of their great mass. They spend long periods travelling to and from their deep feeding sites as well as considerable time locating prey. Sperm whales have additional adaptations that enable them to dive for longer than similar sized cetaceans,

for instance they have significantly higher myoglobin stores than baleen whales, allowing them to store more oxygen in their muscles.

However, the majority of larger species, including cetaceans, do not tend to remain submerged for very long relative to their body mass. The Pantropical spotted dolphin, weighing about the same as a man, averages only half a minute underwater. This may be due to the relatively low myoglobin concentrations in their skeletal muscles. Furthermore, some smaller species regularly endure submergence for incredibly long periods of time when considering their low oxygen storage capacities and high metabolic rates relative to their body mass. This suggests that they possess particularly specialised behavioural and / or physiological adaptations.

Endurance divers

While many aquatic birds are considered relatively poor divers, such as tufted ducks which weigh 0.8 kg and only average dives of 12 seconds, penguin species demonstrate more impressive breath-holding capabilities. The Emperor penguin, at only 30 kg, can dive for well over 10 minutes. However, when comparing body size against dive duration, the family of divers that remain submerged the longest are the Alcidae, a taxon of small Atlantic seabirds including guillemots and razorbills.

The champion endurance diver, according to current data, is the Black guillemot. They weigh less than 0.5 kg and can travel through the water column for over 2 minutes. The Black guillemot is a ubiquitous species across the North Atlantic, breeding in all marine waters north of the cool subtropical zone, including the British Isles. There they breed in small groups or pairs along rocky coasts, mainly in Scotland and Ireland. They have a wider prey spectrum than the other alcid species that includes fish, jellyfish, sponges and crustaceans. Their diet during the breeding season reflects their coastal habitat where they feed on animals found on the sea bottom in shallow water. However, during the rest of the year they feed further out to sea in deeper waters, often around icebergs or pack ice.

So, what makes alcids such effective divers? Unfortunately, answers to this question are unclear because, in contrast to the Sperm whale, their diving prowess is rather

paradoxical. Since alcids are adapted for flying, they are not particularly specialised divers. They have not been able to increase their body density, and so reduce their buoyancy to the extent of flightless diving birds, such as penguins. Their energy demands in shallow waters are therefore higher.

Furthermore, alcids do not seem to possess unique physiological or biochemical adaptations which could enable them to stay underwater for longer. For their size, they seem to have similar oxygen storing capacities to other marine birds and their metabolic rates are also comparable. Nevertheless, alcids have been recorded regularly diving for longer periods than expected according to their oxygen storage capacity and the rate at which they utilise that oxygen while diving.

Behavioural adaptations in alcids

Alcids may be able to use their oxygen stores efficiently by getting deep into the water column quickly. Unlike the majority of diving species, alcids have a distinct advantage when propelling themselves downwards through the water. Rather than commencing the dive from a 'standing start' on the water surface, they take advantage of their flying capability and plummet towards the water, which provides them with considerable momentum as they hit the surface. Their streamlined shape means that they efficiently use this momentum to descend a considerable distance through the water. By the time they start to utilise their oxygen stores to continue descending by wing propulsion, they have already reached depths where the hydrostatic pressure has compressed their internal air cavities and reduced their buoyancy. This results in less energy, and oxygen, being used to maintain their level in the water column; the same trick employed by the Sperm whale but by very different means. A possible further adaptation in these birds is the large-scale employment of anaerobic metabolic pathways as the oxygen store depletes (Box 2). For such species, predictions of dive duration based on oxygen stores and metabolic rate will never fully account for their diving endurance.

Concluding Remarks

The record for the deepest human dive is 73 m, where descent and ascent was aided by a rope. The longest breath-hold is seven minutes and 38 seconds, although this

record only requires that the face is immersed in water and is by no means comparable to an active dive. While these feats are impressive, they serve to underline the incredible degree of specialism that many aquatic air breathing divers have to their water environments. Just as extraordinary is the vast array of these air breathing divers, representing the evolution of such an enormous diversity of adaptive solutions for exploiting the oceans. Consequently, the history of investigation of diving animals is extensive but, even today, our understanding of these expert divers is limited and their appeal is unabated. Furthermore, recent misuse of the ocean's resources has made preservation of these species a major stimulus for future research. Ongoing investigation will help to explain poorly understood physiological adaptations for diving to depth. Most importantly, in light of current environmental concerns, it will develop our appreciation of how diving species interact with their environments, both aquatic and terrestrial, so that we can ensure their continued and enchanting presence in the world's oceans.

Box 1. Inactive submergence

Certain reptiles and amphibians can stay submerged for weeks at a time at low temperatures if they remain inactive, such as the snapping turtle and the green turtle. During cooler seasons, these animals can reduce their metabolic rates to well below their normal resting levels. The oxygen concentration of the water also affects their submergence times since they are capable of limited gaseous exchange across their skin. Reptiles other than turtles do not tend to voluntarily dive for longer than an hour in the laboratory; for example, the Nile monitor lizard submerges for around 15 minutes at 25 ° C. The elephant-trunk snake dives for up to 30 minutes during night time foraging bouts. However, field observations have recorded startled Iguana remaining submerged and inactive for over 4 hours at 25 ° C. Perhaps the most impressive recordings are from observations of the Lake Titicaca frog, which lives on the bottom of the lake at an altitude of 3800 m and at an average temperature of 10 ° C. They have never been observed surfacing in their natural environment but do so in laboratory conditions if the partial pressure of oxygen is sufficiently reduced.

Box 2. Optimal foraging

Dives that are long enough to incorporate significant periods of anaerobic metabolism are energetically less efficient than aerobic dives. For this reason, many species always surface before their oxygen stores have run out. However, for some underwater foragers, longer duration dives involving a significant element of anaerobic metabolism are likely to be optimal under certain circumstances. When they do surface, they minimise this phase of the dive cycle by tolerating lactic acid build up in their muscles rather than metabolising it. This allows them to concentrate simply on re-saturating their lungs and blood with oxygen. Some alcids feed on shoaling fish that are mobile and camouflaged and so difficult to initially locate. When they find a school, it is important that they maintain contact because resurfacing would allow their prey to escape. Many alcid species feed on fish that inhabit deep waters, so maintaining pursuit will sometimes require particularly lengthy and deep dives. Such dives may involve anaerobic metabolism, along with the ability to tolerate the presence of considerable lactic acid, while remaining highly active. Bouts of anaerobic dives cannot continue indefinitely because this form of respiration causes lactate fermentation in the muscle cells, which produces fatigue if it is allowed to accumulate. As such, this diving strategy is usually terminated by an extended surface period enabling removal of the lactate.

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I would like to thank Hannah Bishop for her consistent advice and encouragement.

Author Biography

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Reply to comment on *Ultimate divers*

Letterbox. Plunge-diving alcids?

Lewis Halsey

My statement in the August issue about alcids plunge-diving from the air was certainly too bold. Undoubtedly, not all alcid species dive-bomb the water and the 'general' alcid behaviour is to commence a dive from a standing start on the water surface. Nevertheless, certain alcids have been observed starting a dive from the air. For example, little auks have been observed diving from the air for food (Greenwood, pers comm). Furthermore, some puffin populations have been seen crashing to the water from the air and diving in one motion (Bourne, 1976). There is also anecdotal evidence of black guillemots diving into the water from the air at the sight of boats and other alcids diving into the water to escape kleptoparasitic Arctic skuas (Walton, pers comm).

These facts aside, it remains true that the wording in my article implied that plunge-diving was general alcid behaviour and the reader is right to point out that it is not.

Essay Publication

Take a deep breath…

Lewis G. Halsey

Published in   *Planet Earth (Winter, 2002): 16-19*

and   *The Marine Observer (2003) 73: 116-119*

How do creatures such as whales, marine turtles, seals, and penguins spend almost their entire lives submerged without being able to breathe underwater? NERC Ph. D student Lewis Halsey explains.

Well, of course the simple answer is that they come up for air. But this means they have to break off whatever they are doing and risk attacks from predators. So how do air breathing creatures keep surfacing to a minimum? Who dives deepest? Who stays down longest, and how do they cope with the rigours of diving?

Going deep

Emperor penguins are famously deep divers, reaching 500 m – deeper than any other bird. But mammals beat birds when it comes to depth. Some seals dive to several hundred metres while northern elephant seals can get down to 1 500 m. Certain whale species routinely dive to around 1000 m, but not many air breathers go deeper. No light penetrates below around 1000 m, so for sight hunters there's not much point in going further.

The sperm whale, a huge animal up to 18.5 m long and weighing up to 50 tonnes, is the exception. Sperm whales have been recorded 2.25 km (1.4 miles) down in the ocean. A dogfish that only lives on the deep seafloor was found in the stomach of one sperm whale, suggesting they may dive to over 3 km. Sperm whales largely use sound (or echolocation) to hunt, so light is largely irrelevant – perfectly healthy but blind sperm whales have been captured with food in their stomachs. A sperm whale's head is one quarter of its length and one third of its weight. It contains massive organs

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filled with concentric layers of waxes, known as spermaceti oils, which refract and focus sound, allowing sperm whales to find their prey of giant squid (weighing up to 300 kg), bottom-dwelling sharks, cuttlefish and octopuses. Sperm whales spend 90 % of their time in deep water, only occasionally resting at the surface. By altering the density of their spermaceti oils and adjusting their buoyancy to match the surrounding water, they can hang motionless 'listening' for prey while using very little oxygen.

Under pressure

Conserving oxygen isn't the only challenge for deep divers. Water pressure increases by one atmosphere (the pressure we experience at the surface) every 10 m, building up to a crushing weight on the lungs and leading to several other problems.

Lung collapse

Without pressurised air, we humans can't dive far before our lungs collapse. The deepest human dive aided by a rope is 73 m. Whales don't appear to suffer this restriction. It seems whale lungs and chests are designed to collapse without injury. Increasing pressure on the intestines forces the diaphragm against the lungs, which crumple, pushing the air into the windpipe and extensive nasal passages.

The tremors

Increased pressure also causes 'high pressure neurological syndrome' in a number of land vertebrates, including humans. The increased pressure probably causes the nerves to fire more often and intensely, causing tremors and eventually a complete seizure. It's only very recently that we have reached some understanding of how deep diving species combat the tremors. Studies on toothed whales suggest that their central nervous systems probably have a way of stopping the nerves getting over-excited.

Nitrogen tensions

Human divers go into a stupor and then lose consciousness if they dive too deeply for too long. During a dive, the increased pressure pushes nitrogen through the lung walls

and it is absorbed into the bloodstream. Nitrogen is an anaesthetic gas, especially at higher pressures, so it reduces one's ability to think and move, then causes euphoria, coma and death.

On the way back up the gases in the body expand. If decompression is too rapid, nitrogen bubbles form in the bloodstream and can cause paralysis and death (known as 'the bends' in human divers). But cetaceans' lungs probably collapse totally before nitrogen reaches a critical level in the blood, preventing further nitrogen being absorbed. Also nitrogen cannot get into their bloodstream from the air forced into the windpipes and large nasal cavities as their lungs collapse.

Latent hypoxia

Human divers can also suffer brain anoxia, a lack of oxygen in the brain, which causes unconsciousness. Under high pressure, oxygen rushes from the lungs into the bloodstream causing oxygen pressure to rise in the arteries and lower in the lungs. As the diver comes back up through the top 10 m of water, the lungs double in volume. Then oxygen rushes back into the lungs, and starves the brain of oxygen.

Once again, the cetaceans' collapsible lungs avoid this problem by reducing the rate at which oxygen leaves the lungs when they are diving deeply. Other diving mammals can cope because they have much lower oxygen pressure in their brain arteries than land animals.

Long dives

Certain species stay underwater for a long time without diving particularly deeply. The longest a human has held their breath for underwater is seven minutes and 38 seconds, although this was only a face immersion and not an active dive. Certain reptiles and amphibians, such as the snapping turtle and the green turtle, can stay submerged for weeks during cooler seasons by reducing their metabolic rates to well below their normal resting levels and remaining inactive. In the laboratory, reptiles other than turtles do not tend to dive for longer than an hour. For example, the Nile monitor lizard submerges for around 15 minutes at 25 º C, and the elephant-trunk snake dives for up to 30 minutes. However, in the wild, startled iguana have remained

submerged and inactive for over four hours at 25 º C. Most impressive of all is the Lake Titicaca frog, which lives on the bottom of the lake and has never been observed surfacing in the wild. It does so in the lab if the oxygen pressure is sufficiently reduced.

Size matters

How long an animal can dive for depends on its how much oxygen it can store and its efficiency at consuming oxygen. Larger divers store more oxygen, and use it up more slowly, in relation to their body mass. Sperm whales, the deepest divers, take advantage of their great mass and therefore their ability to store large quantities of oxygen, spending long periods travelling to and from their deep feeding sites and locating prey. They are further adapted to dive for longer than the similar sized baleen whale by having significantly more myoglobin (a red iron-containing protein pigment similar to haemoglobin) in their muscles in which to store oxygen.

However, most large species, including cetaceans, don't stay underwater for long. The pantropical spotted dolphin, weighing about the same as a man, averages only 30 seconds underwater, perhaps because of the low myoglobin concentrations in its skeletal muscles

Endurance divers

Some smaller species with low oxygen storage capacities and high metabolic rates for their size regularly submerge for incredibly long periods of time. This suggests that they possess particularly specialised behavioural and/or physiological adaptations. Penguins can really hold their breath. The Emperor penguin, at only 30 kg, can dive for well over 10 minutes.

But when comparing body size against dive duration, the diving kings are the Alcidae, a family of small Atlantic seabirds including guillemots and razorbills. The champion endurance diver is the black guillemot. Weighing less than 500 g, it can travel underwater for over two minutes. The black guillemot is found throughout the North Atlantic, including the British Isles, and feeds in both shallow and deep waters.

We're not sure what makes alcids such effective divers. Because they fly as well as swim, they have not increased their body density to reduce their buoyancy to the extent of flightless diving birds. They therefore use more energy in shallow waters. For their size, they seem to have similar oxygen storing capacities and metabolic rates to other marine birds. Therefore, alcids regularly dive for longer than one would think possible.

Behavioural adaptations in alcids

Alcids may use oxygen efficiently by getting into deep water quickly. They propel themselves down with their wings (and some also use their feet) at the start of a dive, enabling them to overcome their buoyancy. Once they reach a depth where their internal air cavities are compressed and their buoyancy reduced, they can use less energy and oxygen to maintain their depth. These birds might also use anaerobic respiration as the oxygen store depletes (see below). For such species, their oxygen stores and metabolic rate will never fully account for their diving endurance.

Anaerobic respiration

Many species always surface before they run out of oxygen. However, some underwater foragers can respire anaerobically under certain circumstances, such as when hunting shoals of quick-moving camouflaged fish. These dives cannot continue indefinitely because anaerobic respiration causes lactate fermentation in the muscles, which produces fatigue if it accumulates. When they do surface, the divers tolerate lactic acid build up in their muscles rather than metabolising it. This allows them to concentrate simply on re-saturating their lungs and blood with oxygen. Anaerobic diving usually ends with a long rest on the surface to get rid of the lactate.

To finish off

A vast array of aquatic, diving creatures have evolved to exploit the oceans. Even today, our understanding of these creatures is limited. Further research will develop our appreciation of how they interact with their environments, both aquatic and terrestrial, so that we can ensure their continued and enchanting presence in the world's oceans.

Thank you to Pat Butler for his advice on this essay.

Contact

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Manuscript Publication

Oxygen uptake during post dive recovery in a diving bird, *Aythya fuligula*: Implications for optimal foraging models

R. Parkes, L.G. Halsey, A.J. Woakes, R.L. Holder, P.J. Butler

Appendix IV   Custom made programme in LabVIEW 5.0

Appendix V   Conference Posters