CARDIAC PHYSIOLOGY AND DIVING BEHAVIOUR OF DOUBLE-
CRESTED CORMORANTS (*PHALACROCORAX AURITUS*)

by

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Abstract

Heart rate and dive behaviour were monitored in adult double-crested cormorants during shallow and deep diving and after exposure to different breathing gas mixtures to investigate the role of intravascular chemoreceptors in cardiac and behavioural control during voluntary diving. A data logger was used to record heart rate and dive behaviour of cormorants diving within a shallow (1 m) and deep (13 m) dive tank. Pre-dive heart rate in both shallow and deep diving birds was about three times the resting heart rate, falling abruptly upon submersion to around 200 - 250 beats$\cdot$min^{-1}. During shallow diving most birds showed a secondary drop in heart rate after 5 - 10 s into the dive to around the resting level. In contrast, during deep diving heart rate stabilised at the initial bradycardic level or decreased further only very slightly. Mean dive heart rate ($\pm$ S.D.) was significantly lower during shallow diving (163.2 $\pm$ 14.0 beats$\cdot$min^{-1}) compared to deep diving (216.4 $\pm$ 7.7 beats$\cdot$min^{-1}), but in both cases was significantly above the resting value (137.9 $\pm$ 17.5 beats$\cdot$min^{-1}). Exposure to a hyperoxic gas mixture before shallow diving significantly increased mean dive heart rate, while exposure to a hypoxic gas mixture in both the shallow and deep dive tank significantly reduced mean dive heart rate. In contrast, hypercapnic gas before diving had no significant effect on dive heart rate. These results suggest that the cardiac response to voluntary diving in double-crested cormorants is strongly influenced by changes in arterial oxygen tension (Pa_{O_2}) throughout the dive. Dive duration was unaffected by alterations in inspired gases, but surface interval duration decreased after hyperoxia and increased after hypoxia. The most efficient dive pattern (highest dive/pause ratio) was observed after hyperoxic exposure.

To investigate the ontogeny of the cardiac response to voluntary diving, heart rate was also recorded from naive double-crested cormorant chicks. The cardiac response to first ever and subsequent voluntary submergence was similar to the response observed in adult cormorants. Heart rate was also monitored in a separate group of chicks in which the first exposure to water was during whole body forced submergence. Again, the observed response was similar to the adult forced submergence response, although the cardiac

response of chicks to forced submergence was more extreme than in voluntary submergence. These results do not support the hypothesis that cormorants 'learn' the appropriate cardiac response to voluntary diving via habituation or conditioning of the 'classical dive response'.

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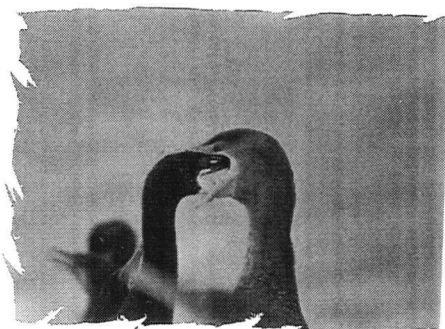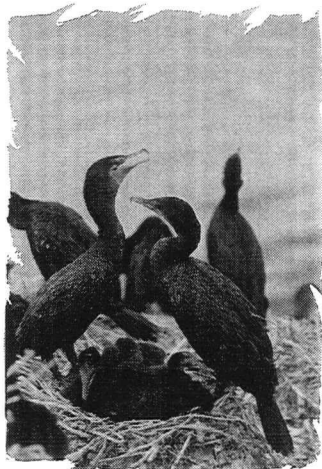
Chapter 1: General Introduction

Forced submergence versus voluntary diving

In the past, many physiologists investigating the physiological responses to diving in vertebrates focused their studies on restrained animals. Marked cardiovascular adjustments during forced submergence have been described for many vertebrates (for review see Andersen, 1966; Butler and Jones, 1982 and 1997; Blix and Folkow, 1983; Jones and Furilla, 1987). These adjustments are believed to facilitate the conservation of the limited oxygen stores for oxygen dependent tissues, namely the heart and the brain. The major components of this 'classical dive response' (Scholander, 1940) are apnoea, bradycardia, and peripheral vasoconstriction.

One central component of the 'classical dive response', the bradycardia, is commonly used as an indicator for the overall response. Forced submergence experiments are, however, fundamentally different from natural diving. First, animals are usually inactive when forcibly submerged, while under natural conditions diving is associated with exercise and an increase in ambient pressure (depth). Second, forced submergence studies impose psychogenic stress on the animals, and consequently, the results obtained from these studies may have little relevance to freely diving animals (see Kanwisher *et al.* 1981).

With the ingenuity of some investigators and recent advances in biotelemetry and microprocessors, it has become possible to study cardiovascular variables in unrestrained, voluntarily diving animals in the laboratory or even in the field. What is more, the

simultaneous employment of instruments recording behavioural data allows researchers to combine physiological measurements (*e.g.* heart rate, body temperature, etc.) with data of the animals' natural foraging behaviour (*e.g.* dive depth, dive time, surface time, swim velocity, etc.).

Results from research on voluntarily diving birds reveal different cardiac responses from animals forcefully submerged in the laboratory. Although all avian divers show a decline in heart rate when diving voluntarily, the degree of that decline is usually less pronounced than during forced submergence. This is not surprising, since forcibly submerged animals have no control over their situation and, consequently, might activate the maximum defensive response possible to conserve oxygen. Furthermore, it has become clear that the cardiac responses during voluntary diving are not 'clear-cut' (*i.e.* a 'classical dive response'), but are rather complex, depending on many factors, such as dive duration, type of dive (*e.g.* shallow vs. deep dives), and differ between species.

Shallow versus deep diving

Most studies on voluntary diving birds have been restricted to small tanks, where birds performed short and shallow dives, a setup that most closely simulates natural conditions for diving ducks (Woakes and Butler, 1983). It is not surprising, therefore, that most of these studies were in fact done on diving ducks, *e.g.* the tufted duck (*Aythya fuligula*) and the lesser scaup (*A. affinis*). Butler and co-workers found moderate cardiac responses in these ducks during short and shallow dives, with dive heart rates well above the resting level (Butler and Woakes, 1976, 1979, 1982a, 1982b; Woakes and Butler, 1983; Stephenson *et al.* 1986; Bevan and Butler, 1992).

Similar results were reported for double-crested cormorants (*Phalacrocorax auritus*; Kanwisher *et al*. 1981), Humboldt penguins (*Spheniscus humboldti*; Butler and Woakes, 1984), and Adélie penguins (*Pygoscelis adeliae*; Culik, 1992) performing short dives in shallow tanks or ponds. In contrast, when birds performed long and deep dives in natural or semi-natural settings the cardiac responses were profound, resembling the 'classical dive response' and suggesting the utilisation of anaerobic metabolism during these dives (for gentoo [*Pygoscelis papua*] and Adélie penguins see Millard *et al*. 1973; for emperor penguins [*Aptenodytes forsteri*] see Kooyman *et al*. 1992b; for South Georgian shags [*Phalacrocorax georgianus*] see Bevan *et al*. 1997). The same response was observed in tufted ducks performing long ('extended') dives in a shallow tank (Stephenson *et al*. 1986).

Cormorant foraging behaviour

Cormorants are foot-propelled pursuit divers (Ashmole, 1971) that actively chase their prey underwater. Most species feed on benthic fish and invertebrates. Hence, they are inshore feeders, rarely seen out of sight of land (Cooper, 1986). Double-crested cormorants in British Columbia are opportunistic foragers, with the majority of their prey being taken in the littoral-benthic zone (Robertson, 1974). Foraging activity is organised in bouts, with birds diving continuously from the water surface, interspersed with only brief periods between dives at the surface. Foraging activity of cormorants varies considerably in intensity throughout the day, but most species seem to spend about 4 - 6 hrs. per day foraging (Cooper, 1985; Tindle, 1984; Croxall *et al*. 1991; Watanuki *et al*. 1996). From data available on dive duration and dive depth of different cormorant species

(Cooper, 1986; Johnsgard, 1993) it appears that most dives of cormorants are rather shallow, and in most cases last less than one minute. Larger species generally appear to be capable of both longer and deeper dives (Johnsgard, 1993). Furthermore, based on the correlation between dive to pause ratio and body mass, Cooper (1986) suggested that larger species are more 'efficient' than smaller ones, since they spend relatively more time underwater (the dive to pause ratio is generally used as an index of dive performance in birds; Dewar, 1924).

Recent studies recording dive variables directly, however, show that some species are capable of extended and deep dives: blue-eyed shags (*Phalacrocorax atriceps*) have been shown to dive for up to 380 s (Wanless *et al.* 1992) and to a maximum depth of 116 m (Croxall *et al.* 1991). European shags (*P. aristotelis*) have been reported to forage in water depths varying from 7 - 50 m, with a mean dive time of 62 s and a maximum dive duration of 163 s (Wanless *et al.* 1993). In a different study, Wanless *et al.* (1991) utilising miniature depth gauges, reported a maximum dive depth of 42 m for European shags. For the double-crested cormorant mean dive times of 25.1 s (Ross, 1974), 20.7 s (Duffy, cited in: Cooper, 1986) and 25.0 s (Ainley *et al.* 1990) and a maximum dive time of 70 s (Munro, 1927) have been reported. In a more recent study, King *et al.* (1995) observed double-crested cormorants diving in shallow fish ponds (mean depth: 1.4 m). Not surprisingly, the mean dive time reported was short, 11.9 s. No direct measurements of dive depth are available, but Ross (1974) observed double-crested cormorants diving in water 1.5 - 7.9 m deep (mean depth: 4.7 m). Ainley *et al.* (1990) reported double-crested cormorants diving in water about 20 m deep. If double-crested cormorants predominantly forage on benthic prey, depth reached during the dive should be strongly reflected in the

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observed dive duration. Hence, the maximum dive duration of 70 s reported by Munro (1927) suggests that double-crested cormorants are in fact capable of submerging to much greater depth than previously reported.

Diving physiology facilitating foraging behaviour

Pursuing fast moving prey (as opposed to stationary feeding) requires flexibility in locomotion (manoeuvrability, ability to quickly accelerate and decelerate, rapidly change depth, etc.) and pursuit divers probably switch from a slow search mode to a very rapid (and energetically more expensive) chase mode, once they have detected prey underwater (Ydenberg and Forbes, 1988). Chasing prey for potentially extended periods after an already long search period will push the predator towards its physiological limits. Kooyman and Ponganis (1998), among others, proposed that deep diving avian pursuers (*e.g.* emperor and king penguins [*Aptenodytes patagonicus*]) might therefore frequently rely on anaerobic metabolism, allowing them to exploit prey-rich depths otherwise unavailable to them. Burger (1991) envisioned anaerobic diving as a profitable option for seabirds under certain circumstances. When foraging on prey at considerable depth, prolonging dives past the aerobic capacity may increase overall foraging efficiency by reducing the proportion of time spent travelling underwater. Similarly, Ydenberg (1988) suggested that divers feeding on mobile prey items may postpone complete physiological recovery from a dive until a series of dives has been completed. Significant anaerobic contributions to diving metabolism, however, seem problematic in most cases. First, the energetic gain per molecule substrate used is small compared to the aerobic pathway. Second, the build up of lactate, associated with anaerobic metabolism, could force

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animals to extend their post-dive surface intervals, thereby reducing the proportion of the dive cycle spent underwater and hence, potential foraging time. Not surprisingly, most diving animals seem to show a preference for aerobic metabolic pathways during voluntary diving (Butler and Jones, 1997).

It is still poorly understood exactly how animals achieve prolonged dive times. Oxygen is the main, short-term resource available to the animal during a dive and its utilisation and depletion strongly influences dive duration. Diving animals store oxygen to a varying degree in the lungs, blood and muscles. Upon submergence the animal becomes a 'closed system' with respect to the oxygen stores and their economic utilisation during the dive is essential for maximising underwater foraging time. Besides the general necessity to maintain and regulate aerobic metabolism during submergence, sufficient oxygen has to be kept in the oxygen stores to maintain physiological integrity of those tissues (*i.e.* heart and brain) that cannot survive without it. Modulation of the aerobic metabolic rate will influence diving performance, so that the higher the rate of aerobic metabolism, the shorter will be the dive time (Butler and Jones, 1997). Cardiac output and tissue perfusion are important regulators of aerobic metabolism: a reduction in the perfusion of specific tissues and organs (*e.g.* splanchnic and peripheral vascular beds) will lead to a suppression of the aerobic metabolism. Hence, circulation becomes the proximate regulator of aerobic metabolism during diving, modulating dive time (Butler and Jones, 1997). Clearly, cardiovascular mechanisms, facilitating the economic utilisation of limited oxygen stores during dives, might be of great importance to active pursuit divers.

Studying the physiological 'strategies' of double-crested cormorants that facilitate the displayed foraging behaviour is rewarding for many reasons. First, double-crested

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cormorants are capable of deep and long dives and will employ both modes of diving (short and shallow vs. long and deep) during their natural foraging behaviour. The forces acting upon an animal during shallow and during deep diving are fundamentally different, and this might have consequences for cardiac control and hence the cardiac adjustments during these dives. Double-crested cormorants can serve as a good model to contrast these two different modes of diving and the associated cardiac responses. Second, if one looks at the different foraging strategies employed by aquatic birds and the morphological and physiological adaptations that facilitate these modes of life, it becomes obvious that cormorants occupy an intermediate position in a spectrum of diving birds that ranges from dabbling and diving ducks on the one end to alcids and penguins on the other. While certain features are similar to diving ducks (*e.g.* foot propulsion, mostly benthic foraging) others are closer to penguins (*e.g.* overall reduced buoyancy [Lovvorn and Jones, 1991, 1994], active pursuit of mobile prey). Still others are unique to them, such as wettable feathers (Owre, 1967; Rijke *et al.* 1989). The study of the physiological 'strategies' that accommodate the different forces acting upon these birds during diving might contribute to a better understanding of the foraging ecology of aquatic birds in general.

Ontogeny of the cardiac response to diving

In adult diving ducks and cormorants, voluntary diving is associated with abrupt changes in cardiac rhythm, *i.e.* heart rate increases before the first dive in a series followed by a steep decline upon submersion (Butler and Woakes, 1979; Kanwisher *et al.* 1981; Jones *et al.* 1988; Bevan *et al.* 1997). Elevated heart rates before or after a dive support the quick loading of the body oxygen stores, while a heart rate decline during

diving, as part of the overall cardio-respiratory response, is believed to conserve oxygen. I already mentioned the differences in the cardiac response of diving animals to forced submergence and to voluntary diving. While heart rate of double-crested cormorants during voluntary diving ranges between 180 and 220 beats·min^{-1} (Kanwisher *et al.* 1981), it falls to a level of 50 beats·min^{-1} within the first 10 - 15 s of forced submergence (Mangalam and Jones, 1984; Jones and Larigakis, 1988). The difference between these two responses is not easily explained. While it has been suggested that the 'classical dive response' during forced submergence is a fear-induced artefact of the experimental conditions (Kanwisher *et al.* 1981; Kanwisher and Gabrielsen, 1985), it has been demonstrated that the cardiac response to forced submergence is present even in decerebrate dabbling ducks, supposedly incapable of emotions (Andersen, 1963b; Gabbott and Jones, 1985; Gabbott and Jones, 1991). Hence, Gabbott and Jones (1985) suggested that the cardiac response during forced submergence is largely reflexogenic in nature. They acknowledged, however, that psychogenic influences might be important for the cardiac adjustments associated with voluntary diving. An alternative hypothesis is that the oxygen-conserving 'classical dive response' is the basic reflex response to submersion, which is suppressed during voluntary diving, possibly by habituation or conditioning (see Stephenson and Butler, 1987).

What are habituation and conditioning and what is their potential in the context of cardiac control during voluntary diving? Gabrielsen *et al.* (1985) described habituation as the decrease in intensity and gradual disappearance of a behavioural/physiological response upon repeated stimulation. According to Dworkin (1993), conditioned reflexes

(classical conditioning) develop by repeated association in time of two stimuli: one a sensory or 'conditioned' stimulus that is detectable by the nervous system but has little reflex physiological effect, and the other a more potent 'unconditioned' stimulus that effectively irritates the afferent receptive field of a physiological reflex. With accumulating associations, with the sensory stimulus always preceding the physiological stimulus, the sensory stimulus itself gradually acquires the power to produce a reaction closely resembling the physiological reflex. Another form of learning, named 'instrumental learning', 'trial and error learning' or 'operant conditioning', has in common with classical conditioning, that it too depends on a repeated temporal relationship to strengthen a response. In contrast to classical conditioning, however, the relationship is between the response and a subsequent, rather general consequence (Dworkin, 1993).

How then can we imagine the phenomena of habituation and conditioning in the context of cardiac control during voluntary diving? To investigate these 'simple' forms of learning and their potential influence on the cardiac response during voluntary diving, it would seem best to focus on juvenile animals that are on the verge of taking up their diving existence. If diving animals have to 'learn' how to adjust their cardiovascular system during diving, this process should be detectable during this phase. What cardiac response would we expect to see in these animals during their initial dives if they in fact have to 'learn' the appropriate cardiac response?

In the case of habituation one would expect diving animals to display a more profound cardiac response during their first ever and subsequent early dives. Over time, however, the intensity of the cardiac response would decrease. It has been shown that the

bradycardia evoked by forced head submersion is susceptible to habituation in Pekin ducks (Gabrielsen, 1985; Gabbott and Jones, 1985). The finding that voluntary diving tufted ducks are able to 'switch' to a full bradycardia when trapped underwater was interpreted as dishabituation of the 'normal' (habituated) cardiac response to voluntary diving (Stephenson et al. 1986; Stephenson and Butler, 1987). The only study designed to investigate habituation of the cardiac response to voluntary diving, however, produced no evidence in support of the 'habituation hypothesis' (Keijer et al. 1988). In tufted ducklings (Aythya fuligula), the response to first ever and subsequent voluntary head or whole body submergence was variable but, in the majority of birds, similar to the adult response.

If classical conditioning is involved, animals would learn to associate a sensory stimulus (e.g. pre-dive posture) with the succeeding physiological stimulus (e.g. stimulation of trigeminal receptors upon submersion), so that over time the conditioned sensory stimulus would be sufficient in eliciting the cardiac response. In case of operant conditioning, animals would learn to associate the physiological response with a subsequent consequence (e.g. animals might learn that a bradycardia makes the breath-hold easier or shortens the recovery period), again over time producing a conditioned response that precedes the physiological response. Ridgway et al. (1975) demonstrated that both the rate and degree of the bradycardia displayed by California sea lions (Zalophus californianus) during trained breath-holds in air can be increased by classical and operant conditioning. There are a number of observations on voluntary diving animals that support the 'conditioning hypothesis'. Tufted ducks can display the whole gamut of cardiac adjustments to diving (pre-dive tachycardia followed by a rapid fall in

heart rate) without actually submerging (Woakes and Butler, 1983) or before submersion occurs (Butler and Woakes, 1976). Similarly, a heart rate decline in anticipation of submersion has been reported for harbour (*Phoca vitulina*) and harp seals (*Phoca groenlandica*; Jones *et al.* 1973; Casson and Ronald, 1975).

However, if the hypothesis that the 'classic dive response' (observed during forced submergence) is the basic reflex response to submersion and is suppressed during voluntary diving by habituation or conditioning is correct, then the cardiac response of diving animals during their early dives should follow a similar pattern, regardless of whether habituation or conditioning is shaping the development of the response. In both cases (habituation or conditioning) the intensity of the heart rate decline during voluntary diving should decrease over time. Hence, by simply observing the cardiac response of 'naive' animals (never exposed to water) during their first ever and subsequent dives, it would not be possible to distinguish between the mechanisms (habituation or conditioning) shaping the response. It would, however, shed some light on the question of whether the cardiac response during voluntary diving is in fact 'only' a modified 'forced submergence response'.

Given that most diving animals in the wild will rarely encounter a 'forced submergence situation' one could turn the reasoning around and argue that the cardiac response observed during voluntary diving is in fact the 'normal' or 'basic response' that is modified under certain circumstances (*e.g.* escape dive, forced submergence). Even in this scenario, assuming that 'naive' divers during their first ever and subsequent voluntary dives do not display a strong bradycardia (as during forced submergence) that gradually declines over time but rather have a stable moderate response, it would not be possible to

demonstrate conditioning by simple observation of the response. As Butler and Jones (1997) pointed out, there can be no expectation that the reflex response to submergence (*e.g.* via stimulation of nasal receptors) should be different from the conditioned response. Hence, an experimental design to investigate conditioning of the cardiac response to voluntary diving would necessitate the blocking of receptor groups (*e.g.* cutting branches of the trigeminal nerve) in 'naive' animals.

Habituation, on the other hand, can be investigated by observing the cardiac response to diving in initially 'naive' animals over time. Regardless of the absolute level of the cardiac response during their initial dives (strong or moderate bradycardia as during forced submergence or voluntary diving, respectively), the response should decrease in intensity over time as animals habituate to the new situation. Keijer *et al.* (1988) found no evidence that tufted ducklings might 'learn' the appropriate cardiac response to voluntary diving via habituation of the 'classical dive response'. Their ducklings had been held on land for about 2 weeks to allow implantation of heart rate transmitters and subsequent post-operative recovery. Unfortunately, of necessity, the ducks were offered water to drink during this period and repeated beak wetting may have triggered and reinforced heart rate responses displayed in first ever dives. In fact, ducklings did not dive immediately after being exposed to deep water, but performed beak dips and head submersions which precipitated marked falls in heart rate compared with the rate preceding these activities.

In nature, precocial chicks of diving ducks can walk, swim, or dive shortly after hatching (Matthews and Evans, 1974), whereas altricial young of cormorants remain in proximity of the nest for up to 6 or 7 weeks, before venturing onto water to take up their

diving habit (Lewis, 1929). Preceding these marked differences at hatching are distinctly different developmental patterns of cardiovascular and metabolic function during embryology (Tazawa and Hou, 1997). For precocial ducklings a mature cardiac response to diving at hatching is essential to obtain food, escape from predators, etc. Many altricial birds, on the other hand, live on food that must be located and captured with adult strength and skill. Hence, for these chicks, dependence on parents during a period of growth, maturation, and learning is fundamental before they can feed themselves (Gill, 1990). Given that altricial cormorant chicks are still being fed by their parents when starting to dive, the time available for maturation of the cardiac response is greater in these chicks than in precocial ducklings. Hence it should not be surprising to find differences in the ontogeny of the cardiac response to voluntary diving between these two groups.

Objectives of thesis research

The objectives of the research conducted for this thesis were:

(1) To contrast the cardiac and behavioural responses of double-crested cormorants during voluntary shallow and deep diving and to investigate the role of intravascular chemoreceptors in the mediation of the observed responses (Chapter 2).

(2) To investigate the ontogeny of the cardiac response of double-crested cormorants to voluntary submergence and to contrast the cardiac responses during first ever and subsequent voluntary and forced submergences (Chapter 3).

Chapter 2: Cardiac and behavioural responses of double-crested cormorants during voluntary shallow and deep diving

Introduction

<u>Diving to depth</u>

Diving to depth is challenging for animals in various ways, yet many descend to great depth. Within the group of avian divers it is the largest birds, namely emperor and king penguins that have been shown to dive to great depth (max. depth recorded: 534 m and 304 m, respectively) and for extended time periods (15.8 min and 7.5 min, respectively; Kooyman and Kooyman, 1995; Kooyman *et al.* 1992a).

Besides these 'expert deep divers', many other aquatic birds dive to considerable depth. The heaviest species within the *Alcidae*, the common murre (*Uria aalge*), has been found entangled in fishing nets set at 180 m (Piatt and Nettleship, 1985). The similarly sized thick-billed murre (*Uria lomvia*) dives to a maximum depth of 210 m (Croll *et al.* 1992). Blue-eyed shags, one of the heaviest species within the *Phalacrocoracidae*, submerge to 116 m in pursuit of their prey in dives lasting up to 6.3 min (Croxall *et al.* 1991; Wanless *et al.* 1992). Even within the group of diving ducks, which is generally considered to contain rather shallow diving species, there are some notable exceptions. Schorger (1947) reported oldsquaws (*Clangula hyemalis*) found entangled in fishing nets set at 60 m, while king eiders (*Somateria spectabilis*) have been found to forage on bivalves at similar depths (Cottam, 1939). Other sea ducks such as the white-winged scoter (*Melanitta fusca*)

and the surf scoter (*M. perspicillata*) are reportedly foraging at 9 - 13 m of depth (Cottam, 1939; Savard *et al.* 1998).

The forces acting upon these divers, limiting the depth range that can be utilised, are manifold. One aspect when considering depth is concerned with the fact that birds have to return to the water surface for gas exchange. Oxygen stores are limited (so is the tolerance for the storage of accumulating metabolic endproducts, *e.g.* CO_2) and even their most economical use will limit dive duration and, hence, dive depths that can be reached. Another important aspect is the pressure experienced when diving to depth. Beside the problems related to the increased absorption of gases (*e.g.* nitrogen) into the tissues of divers that might cause problems during rapid ascent, the physiological control system that facilitates the efficient management of the limited oxygen during these deep dives remains unclear. During descent overall pressure will increase by one atmosphere for every 10 m of depth. As the volume of the respiratory system is compressed during descent, the partial pressure of the gases within the respiratory system will rise in proportion to the total pressure (Dalton's law). Lanphier and Rahn (1963), investigating the alveolar gas exchange of humans during surface breath-holds and simulated dives to 10 m, found a marked increase in the alveolar partial pressure of oxygen (PA_{O_2}) to approximately twice the corresponding surface values during the descent phase of dives. PA_{O_2} dropped rapidly during the breath-holds at the surface to values at which the arterial blood was no longer saturated. During simulated deep diving, however, PA_{O_2} remained relatively high until ascent. Similar results were found in other studies on human diving

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during simulated and even unassisted dives to depth (Linér *et al.* 1993; Craig and Harley, 1968).

How much of a gas will diffuse into the blood and tissues of divers depends on the partial pressure of the particular gas. As the partial pressure of a gas increases during descent, the amount that dissolves in the blood will rise proportionately (Henry's law). It was suggested, therefore, that the increase in PA_{O_2} at depth may augment blood oxygenation (Lanphier and Rahn, 1963). Kooyman *et al.* (1973) found that the arterial oxygen tension (Pa_{O_2}) during the early phases of simulated dives of Adélie and gentoo penguins to 30 and 68 m was considerably above pre-dive values. Ventilating these birds with 100 % oxygen before their 5 minute dives increased the pre-dive Pa_{O_2} about 4.5 times. After compression, blood oxygen tensions went off-scale (> 800 mm Hg) and remained off-scale for the entire time of the compression (4 min). Similarly, Qvist *et al.* (1986), when investigating blood gas tensions in freely diving Weddell seals (*Leptonychotes weddelli*), found a drastic increase in Pa_{O_2} in the early descent phase of dives.

The vast majority of oxygen molecules in vertebrate blood are bound to haemoglobin. The contribution of oxygen in physical solution to the overall oxygen content is minuscule. If an oxygen saturation of greater than 90 % at the start of a dive is assumed, then the increase in PA_{O_2} during descent will not add any significant amount of oxygen to the blood but it will lead to an increase of oxygen in physical solution and thereby increase Pa_{O_2} early in the dive. This, in turn, might have consequences for the development of the cardiac response during deep diving. During the dive, when oxygen is

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consumed by the tissues, the elevated PA_{O_2} will support blood oxygenation and help to keep the arterial blood at a high saturation level.

Control of heart rate during diving

Our understanding of the various components of the cardiovascular responses to submergence and their interactions has to a large degree been derived from studies on forcibly submerged animals. Based on these studies two distinct phases of a reflexogenic response have been proposed. In an initial response to submergence, wetting of nasal or upper respiratory tract receptors leads to an immediate suppression of the respiratory centre via trigeminal and glossopharyngeal afferents, resulting in apnoea. Inhibition of the respiratory centre, in turn, stimulates the cardio-inhibitory centre. The increased parasympathetic output via the vagus nerve on the efferent side facilitates the slowing of the heart (Andersen, 1963a, 1963b; Butler and Jones, 1968; Jones and Butler, 1982; Blix and Folkow, 1983). During the dive, central and peripheral chemoreceptors are stimulated by the progressive increase of CO_2 and decrease of O_2 in the blood. Thus, the increased chemoreceptor drive reinforces the initial dive response and maintains the dive bradycardia (Jones and Purves, 1970; Jones et al. 1982; Lillo and Jones, 1982; Butler and Woakes, 1982a).

The carotid body chemoreceptors in birds

The tissue associated with intravascular chemoreception in vertebrates is formed by clusters of cells (lobules, glomoids), surrounded by a dense capillary network and

penetrated by sensory nerve endings. The clusters are formed by two cell types, chemoreceptor or type I cells, and sustentacular or type II cells (Fidone *et al.* 1997). In birds, the largest aggregation of chemoreceptive tissue, the carotid bodies, are found in the central cardiovascular area close to the parathyroid and thyroid glands (Jones and Purves, 1970; Abdel-Magied and King, 1978). The avian carotid body is innervated by one or more vagal branches from the nodose ganglion.

The carotid bodies, most importantly, detect changes in the chemical composition of their environment. Sensory discharges increase in frequency when the O_2 tension (P_{O_2}) or pH of the arterial blood falls or when CO_2 tension (P_{CO_2}) increases. Conversely, the discharge frequency decreases with increasing P_{O_2}, low P_{CO_2} or alkalinity (Acker, 1989).

With respect to the oxygen sensing mechanism, the chemoreceptors are active, even at normal arterial oxygen tensions and bring about ventilatory responses to environmental stress, such as hypoxia. When there are no accompanying changes in breathing, such as during diving in aquatic birds, then carotid body chemoreceptor activation is expressed on the cardiovascular system (Jones and Milsom, 1982). In domestic Pekin ducks (*Anas platyrhynchos*) for example, nearly all of the bradycardia and at least half of the increase in total peripheral resistance which accompanies forced submergence are prevented by carotid body deafferentation (Jones and Purves, 1970; Jones *et al.* 1982).

P_{O_2} chemoreception is generally defined as a P_{O_2}-dependent release of neurotransmitter from type I cells that generates action potentials in the postsynaptic afferent nerve endings. The involvement of calcium as an effector in this process has been demonstrated (Acker, 1989). The exact mechanism of O_2 sensing, however, is still controversial. Fidone

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et al. (1997) in a recent review summarise three models for the low-P_{O_2}-transduction cascade in carotid body chemoreceptor cells. In a 'membrane model', a haemeprotein, located in the plasma membrane of chemoreceptor cells, acts as the O_2 sensor that becomes unsaturated upon a lowered P_{O_2} and decreases the opening probability of O_2-sensitive K^+ channels. The reduced K^+ conductance will depolarise these cells, resulting in increased firing of the chemoreceptor cells, with a consequent entry of Ca^{2+} and the release of neurotransmitter. Other models are the 'metabolic hypothesis' and the 'NAD(P)H oxidase model'.

Although a change in P_{O_2} is generally believed to be the specific stimulus for O_2-sensitive chemoreceptors, changes in the oxygen content have been suggested as the more appropriate stimulus (Milsom, 1990, 1993, 1997). Direct evidence, however, is lacking.

The role of chemoreceptors in the development of the dive bradycardia

The importance of peripheral and central chemoreceptors for the cardiac responses during forced submergence has been clearly demonstrated for dabbling Pekin ducks (Jones and Purves, 1970; Jones *et al.* 1982; Lillo and Jones, 1982; Butler and Woakes, 1982a; Mangalam and Jones, 1984). Jones *et al.* (1982), in their attempt to assess the contribution of central and peripheral chemoreceptors to the bradycardia displayed by forcefully submerged Pekin ducks, found that the peripheral chemoreceptors (carotid bodies) caused virtually all the bradycardia (85 %) during the later stages of forced submergence. The strongest contribution to the cardiac response accounted for by the carotid bodies came from Pa_{O_2} ($\sim$ 65 %), while Pa_{CO_2} contributed little ($\sim$ 20 %). For

diving ducks, such as the redhead duck (*Aythya americana*) and the tufted duck, it seems clear that chemoreceptors are of little importance during the early phase of forced submergence or voluntary dives, but rather reinforce the initial cardiac response later in the dive (Butler and Woakes, 1982a; Furilla and Jones, 1986, Butler and Stephenson, 1988).

The role of chemoreceptors in the development of cardiac responses in cormorants is unclear. Two studies on forcibly submerged double-crested cormorants produced different results. Mangalam and Jones (1984) found that the bradycardia during forced submergence was largely unaffected by different levels of O_2 and CO_2 breathed beforehand. It was noted, however, that the average heart rate of the cormorants during forced submergence was consistently (but not significantly) higher after breathing 50 % O_2 before submersion. Jones and Larigakis (1988), on the other hand, found that breathing 100 % O_2 before submersion significantly elevated heart rates of double-crested cormorants during forced submergence. This difference in cardiac response was established early but not progressively reinforced during the rest of forced submergence.

The importance of chemoreceptors as a feedback system, modulating the cardiac responses to voluntary shallow and deep diving has never been investigated in cormorants. If cormorants depend on chemoreceptors, sensing blood gas tensions (most importantly Pa_{O_2}) to adjust their cardiac performance during diving, then this should become most obvious during shallow diving. Since shallow diving birds dive at an ambient pressure only slightly elevated above the surface pressure, Pa_{O_2} will start to decrease (and Pa_{CO_2} will increase) early in the dive, as O_2 is consumed by the tissues. This

will lead to an increase in the discharge frequency of the chemoreceptors and in turn reduce heart rate. During the early descent phase of deep dives, however, chemoreceptors are not likely to be of great importance for the slowing of the heart. As the total pressure experienced by the birds increases during descent, the blood gas tensions sensed by the chemoreceptors (most importantly Pa_{O_2}) will increase, reducing the chemoreceptor discharge frequency.

It can be hypothesised then, if double-crested cormorants depend on P_{O_2} sensing chemoreceptors to reinforce and maintain their initial reduction in heart rate during diving, then this reinforcement should be delayed in deep vertical dives, compared with shallow horizontal dives. In deep dives of moderate duration (18 - 22 s) the overall chemosensory drive should be reduced, and hence, the reduction in heart rate will be less pronounced. Furthermore, manipulation of blood gas tensions of birds before diving should produce a corresponding change in the displayed cardiac response during diving via the altered discharge frequency of chemoreceptors.

Blood gases as regulators of dive behaviour

Beside the effects on the cardiovascular performance of diving animals, blood gases are important regulators of ventilation. Adjustment of dive duration and the length of the succeeding post-dive surface interval is an important behavioural component in the regulation of ventilation (Craig and Påsche, 1980). Hence, blood gases might play an important role in controlling dive behaviour. Both O_2 and CO_2 have been shown to affect dive duration in pinnipeds (Kooyman et al. 1971; Påsche, 1976a; Påsche, 1976b; Craig

and Påsche, 1980). Decreasing O_2 concentrations and increasing CO_2 concentrations in the inspired air generally decrease dive duration. The effect of changes in blood gases during diving on the length of the succeeding post-dive interval, however, is less clear. While in some animals exposure to low O_2 concentrations or high CO_2 concentrations increases the time spent at the surface breathing, it has no effect on others (Påsche, 1976a; Påsche, 1976b; Craig and Påsche, 1980). Butler and Stephenson (1988) showed that blood gases affect the dive behaviour of tufted ducks. While hypoxia mainly decreased dive duration, hypercapnia increased the duration of the post-dive surface interval and decreased dive duration.

To investigate the cardiac and behavioural responses of double-crested cormorants to different modes of diving (shallow vs. deep), birds were acclimatised to dive in different setups, while heart rate and behaviour were monitored. Furthermore, to investigate the role of blood gases, and hence, intravascular chemoreceptors in cardiac and behavioural control during these dives, birds were exposed to different gas mixtures before diving.

Materials and methods

<u>Birds</u>

Nine adult or sub-adult double-crested cormorants (minimum age: 2 years) with a mass of 2.36 ± 0.17 kg (range: 2.17 - 2.58 kg) were used in this study. Data were collected in both experimental conditions (shallow vs. deep diving) in only five individuals. The birds were captured as chicks (5 - 6 weeks of age) from the Mandarte Island breeding colony (Haro Strait, B.C.) in 1991 and from the Chain Islet breeding colony (Oak Bay, B.C.) in

1994. They were housed in two sheltered outdoor pens (8 m long, 4 m wide, 5 m high) with water tank access (1.90 m in diameter, 0.90 m deep) at the South Campus Animal Care Facility at U.B.C., Vancouver. Birds were fed about 10 percent of their body weight daily with a mixed diet consisting of pacific herring (*Clupea harengus*) and rainbow smelt (*Osmerus mordax*), supplemented with vitamin B1 tablets (thiamine hydrochloride, Stanley Pharmaceuticals Ltd., North Vancouver, B.C.).

Training protocol

The cormorants were introduced into the shallow dive tank (9 m long, 3 m wide, 1 m deep) within their first 3 months of captivity. After the first few weeks of introduction birds were rotated between the shallow dive tank and the outdoor holding pens. Three birds at a time were kept inside the shallow dive tank for up to 3 months. The surface of the shallow dive tank was progressively covered during the training sessions until only a small section (1 m x 1.5 m) at one end of the tank remained open. Birds would submerge here, swim to the opposite end of the tank where chopped herring pieces and smelt had been placed, pick up a fish piece and return to the opening to swallow their prey (Fig. 2.1a). The opening was enclosed by a fine mesh net that prevented the birds from escaping during the trials and a perch was provided so birds could rest outside the water once their foraging bouts were finished. At the end of the diving trials, birds were either released into the open area of the tank or returned to their outdoor pens. Before data collection, birds were held inside their outdoor pens, captured on a daily basis, equipped with a harness holding a dummy data logger and introduced into the shallow dive tank. After one complete foraging bout, birds were recaptured for removal of the harness and

returned to their pens. After 3 to 4 weeks of training, each bird underwent surgical implantation of electrocardiogram (ECG) electrodes.

Five of the nine cormorants were introduced into the deep dive tank (5 m in diameter, 13 m high) where they were trained to pick up chopped herring pieces from a feeding platform suspended within the water column (Fig. 2.1b). The water surface was covered with the exception of one segment (2.27 m^2). Over the course of two weeks the feeding platform was gradually lowered until the birds were diving down to the bottom of the tank (12 m water depth). Birds were then captured, equipped with harness and a dummy data logger as described above for 4 to 5 days before data collection started.

Instrumentation

To record heart rate from the cormorants a purpose built data logging system was developed (Andrews, 1998). The data logger assembly consisted of a modified Tattletale Lite computer (Onset Computer Corp., Pocasset, MA, USA) connected to two ECG electrodes (to record the electrocardiogram) and a small liquid level sensor (Model LL105100, Microswitch, Freeport, IL, USA) used as an event marker (*i.e.* to sense submersion and emergence). The response time of the optical event marker was about 0.5 s in water. The data logger was programmed to sample the ECG at 100 Hz and the submergence sensor at 2 Hz. It was powered by two 3.6 V lithium coin cells (TL-5186, Tadiran, Port Washington, NY, USA), mounted slightly above the computer board. The entire data logger assembly was cast in Sealtronics epoxy (Sealtronic, Industrial formulators of Canada Ltd., Burnaby, B.C.) and silicone rubber (RTV 118, GE, Waterford, NY, USA) provided strain relief where the ECG lead assembly exited the data

logger. The 7.0 cm lead assembly terminated in a miniature 2 pin waterproof plug to allow connection to the implanted ECG leads. The ECG was conducted through two fine wire electrodes to a modified Polar heart rate detector/transmitter board (Polar Electro, Port Washington, NY, USA), incorporated into the data logger, which served as an ECG amplifier. The amplified ECG signal was fed into one of the data logger analog channels. The data logger design (size, mass, shape, etc.) attempted to minimise potential effects of the instrumentation on the birds. The size of the data logger used in this study (8 cm long, 5 cm wide, 1 cm high) amounted to less than 5 % of the cross-sectional area of a double-crested cormorant (4.27 ± 0.24 %, mean ± S.D., N = 4). Its mass (75 g) represented about 3.20 ± 0.23 % (N = 9) of the birds' body weight. Before experimental application the data logger was glued onto a harness, made of rubber neoprene and velcro straps. The harness and data logger were extremely well tolerated by the birds and no changes in their swimming or diving behaviour were detectable. The data logger and its position on the birds during deployment is shown in Fig. 2.2.

The ECG electrodes intended for implantation were prepared by stripping the insulation from a 5 cm section of Teflon-insulated multistrand stainless steel wire and coiling this section into a 0.5 cm diameter loop. The insulated sections of the two ECG leads were additionally encased in silastic tubing (Bolab Inc., Lake Havase City, AZ, USA) filled with latex rubber, for protection from bodily fluids. Both ECG electrode leads extended past the silastic tubing so that one uninsulated ECG electrode was 0.5 cm distal to the tubing end and the other electrode lay 4.5 cm from the tubing end. All connections were sealed with silicone and heat shrink tubing.

For lead implantation, a 500 ml mask was placed over the bird's head and anaesthesia was induced with 1.5 - 2.5 % halothane (Fluothane, Wyeth-Ayerst, Montreal, PQ) in oxygen. The bird was then intubated (Softtech endotracheal tube, O.D. 7.0 mm, Hudson RCI, Temecula, CA, USA) and artificially ventilated (Tidal volume $[V_T]$ = 50 ml, Respiratory frequency $[f_R]$ = 17 min^{-1}). Anaesthesia was maintained with 0.75 - 1.5 % halothane. The site of the incision (midventral abdominal wall, just posterior to the caudal end of the sternum) was exposed by working a mixture of Betadine (Purdue Fredrick, Pickering, ON) and KY-gel (Johnson & Johnson Inc., Bramalea, ON) into the plumage and combing the feathers to the side. Through the 5 - 6 cm opening of the incision the lead assembly was passed towards the heart until one electrode lay near the apex and the other electrode near the base of the ventral side of the heart, a separation of approximately 4 cm. The lead assembly was sutured to the abdominal wall, lead out of the peritoneum and tunnelled subcutaneously to the exit site on the midline of the dorsal surface, about 4 cm cranial to the caudal end of the synsacrum. At the point of externalisation the lead assembly was connected to a miniature 2 pin water-proof connector. The connector was fixed in place by embedding it in five minute epoxy (ITW Devcon, Danvers, MA, USA) onto a small neoprene patch that was mounted on the bird's feathers with cyanoacrylate adhesive (Loctite Quick Set 404 industrial adhesive, Loctite Corporation, Rocky Hill, CT, USA). This provided for an easy and reliable connection of the data logger's ECG electrode leads with the implanted ECG leads. Birds tolerated the glued neoprene patches extremely well and data could be collected for up to 5 months before the patches finally fell off when birds were going through the moult. Birds were given a single i.m. injection

of antibiotics (Baytril, 12.5 mg·kg^{-1} body weight; Haver, Bayvet Division, Chemagro Ltd., Etobicoke, ON) at the end of the surgical procedure.

Experimental trials

Birds were given at least one week to recover from surgery before the experimental trials started. Prior to a trial a cormorant was caught in its holding pen and the harness with data logger was placed on its back. The data logger's ECG-electrode leads were connected to the implanted leads and the sampling mode of the data logger was triggered. The handling time of the birds was kept to a minimum and usually did not exceed five minutes. Immediately afterwards birds were introduced into the shallow dive tank where they would start diving spontaneously. In case of the deep dive tank birds were housed inside the setup during the entire duration of the experimental period and had unrestricted access to the water. When catching a bird in the deep dive tank, water access was denied until the feeding platform had been lowered to the bottom of the tank, marking the beginning of the trial. In both shallow and deep dive trials, birds would start diving immediately after the experimenter had left the setup. The diving birds were watched by a hidden observer and/or filmed by a video camera and submersion/emergence times were noted as a backup for the data logger's submergence sensor. At the end of an experimental trial, which generally lasted 20 - 30 min, birds were recaptured to unplug the ECG leads and remove the harness. Birds were released into their holding pens (shallow dive tank) or into the setup (deep dive tank) and the data were downloaded from the data logger into a personal computer.

Altered breathing gas trials

Blood gas levels (O_2 and CO_2) of cormorants were manipulated before the onset of a dive bout by exposing birds to different breathing gas mixtures. On both the shallow and deep dive tank a transparent PVC cage (0.8 m long, 0.6 m wide, 0.6 m high), that enclosed the opening of the tank's surface cover was filled with the desired gas mixture from a gas bottle via PVC tubing. The PVC cage was kept airtight by immersing its open bottom part, however, 2 small holes had to be introduced for a trapdoor and allowed some gas to escape the cage. Gas samples were drawn continuously from the cage during the entire trial and analysed for their O_2 and CO_2 contents (Beckman O_2-analyser OM11 and Beckman CO_2-analyser LB-2, Beckman Instruments Inc., Schiller Park, IL, USA) to ensure the desired mixture was maintained. After the introduction of a bird into the cage, the gas flow was readjusted until the desired gas concentration stabilised. Water access was denied through a trapdoor at the bottom of the cage. Birds were exposed to the stabilised gas mixture for 10 min to allow for equilibration with the cardio-respiratory system, before the trapdoor was opened and the dive bout could start. During the trial the gas flow into the cage was kept at a minimum but sufficient rate to keep the gas concentration stable. All birds were familiarised with the cage during earlier training sessions.

In the shallow dive tank birds were exposed to the following gas mixtures: a) normal air (control); b) hyperoxic air mixture (> 80 % O_2; designed to unload peripheral chemoreceptor drive via increased Pa_{O_2}); c) hypoxic air mixture (12 % O_2; designed to stimulate peripheral chemoreceptors via decreased Pa_{O_2}); d) hypercapnic/normoxic

mixture (3 % CO_2 and air; designed to stimulate both central and peripheral chemoreceptors via increased Pa_{CO_2}); e) hypoxic/hypercapnic mixture (12 % O_2 and 3 % CO_2; designed to stimulate both central [Pa_{CO_2}] and peripheral chemoreceptors [Pa_{O_2} and Pa_{CO_2}]).

Preliminary trials showed that hypercapnia had little effect on the cardiac response during voluntary diving in the shallow dive situation. Hence, the hypercapnic trials were discontinued, so that data are available for the shallow dive tank only. In the deep dive situation birds were exposed to the normoxic (control) and hypoxic air mixture (here: 8 - 9 % O_2) alone. The lower oxygen concentration for the hypoxic mixture in the deep dive situation was chosen because the compression experienced by the birds during descent and the accompanying increase in PA_{O_2} and therefore in Pa_{O_2} might have potentially masked the chemoreceptor response at the level of hypoxia chosen for the shallow dive situation (12 % O_2). Preliminary data analysis from one bird seemed to confirm this expectation, so that a more severe level of hypoxia was chosen. All gas mixtures were administered in random order.

Resting heart rate

For comparison with diving trials, resting heart rates were obtained from five birds while they were in their outdoor holding pens. Birds were equipped with a data logger and harness as described above but kept inside their holding pens. Birds would perch immediately after release and return to their routine shortly after the handler departed. Heart rate was recorded during these trials and the birds were either observed from a

blind or filmed by videocamera. Trials lasted for approximately one hour and an effort was made to choose similar periods during the daily cycle of birds for all trials. All trials were done during daylight hours, with postabsorptive birds that were awake and perched in an upright position.

Dive behaviour

Dive duration, surface interval duration, and dive/pause ratios were computed for five birds. For each cormorant in each different treatment ten dives lasting between 15 and 30 s and the subsequent surface intervals were selected at random from diving bouts in which birds performed at least three successive feeding dives.

Data analysis and statistics

Dive trials

To allow comparison between the different experimental situations and reduce the influence of dive duration on the expression of the cardiac response to voluntary diving, only dives between 18 and 22 s in duration and only dives with an obvious foraging intention (*i.e.* birds picked up fish pieces or at least checked them) were selected for analysis of heart rate. In addition, to investigate the effect of dive duration on the expressed cardiac response during voluntary diving, all shallow dives performed by three individuals (dive duration: 3 - 28 s) were included in a separate analysis.

Submergence and emergence times were determined from the record of the data logger's submergence sensor. These times were checked against the times noted from direct observation or video filming and usually matched to the nearest second. The point

of submersion was generally obvious from the ECG-trace as well, with a much longer interbeat interval signalling the start of a dive. Cardiac interbeat intervals were derived from the ECG trace after identifying the QRS peaks by eye. A mean value for the interbeat intervals of each dive that was included in further analysis was calculated and subsequently converted into beats per minute (beats·min^{-1}). For each cormorant in each different treatment six dives were analysed. A mean value for each bird was calculated from the six individual dives per treatment. For each treatment a grand mean was calculated from the individual bird means. To compute heart rate profiles for the different experimental treatments, heart rate data were divided into 3-s-intervals, starting 9 s before a dive and ending 9 s after its completion. Mean values for these intervals were calculated for all dives and used to generate grand means as described above.

Resting trials

Instantaneous heart rate over the entire trial period was plotted against time. Heart rate was elevated due to the handling at the beginning of the trial but fell to a baseline value within 10 min in all birds. After heart rate had reached a stable level, a section of 20 min was chosen for the calculation of a single resting heart rate value. A mean value was calculated from all interbeat intervals during that selected period and converted into beats·min^{-1}.

Statistical analysis

Multiple comparisons among different experimental conditions during shallow diving (air, hyperoxia, hypoxia, etc.) and among different phases of the dive (pre-dive, dive, post-dive) were performed using one-way repeated-measures analysis of variance (ANOVA) with Student-Newman-Keuls pairwise multiple comparisons. When single comparisons were made, as in comparing values obtained from the two experimental conditions during deep diving (normoxia and hypoxia), Student's paired t-test was used. Significance was accepted at the level of $P < 0.05$. The average relationship between mean dive heart rate and dive duration that takes into account variability between subjects was determined using repeated measures multiple linear regression with each cormorant being assigned a unique index variable. All mean values are presented with standard deviation ($\pm$ S.D.).

Results

Cardiac responses to shallow and deep diving

The grand mean for resting heart rate from five birds was 137.9 ± 17.5 beats·min^{-1}. Table 2.1 summarises heart rates of individual birds resting and during dive trials.

Shallow diving

Before the first dive in a series, when birds floated quietly on the surface, heart rate was moderately high (200 - 250 beats·min^{-1}) and increased just before submersion. Immediately upon submersion heart rate dropped from a pre-dive rate of 380.6 ± 12.6

beats·min^{-1} to a level of about 200 beats·min^{-1} (Fig. 2.3). Heart rate stabilised at this level

for a few seconds before there was a second drop after about 5 - 10 s into the dive to a

heart rate around or even below the resting level. Towards the end of the dive heart rate

increased in anticipation of surfacing, leading to a post-dive heart rate of 397.2 ± 19.6

beats·min^{-1}. Mean dive heart rate (163.2 ± 14.0 beats·min^{-1}) was well above and

significantly different from the resting heart rate (Table 2.1). Minimum heart rate during

diving, however, was significantly below resting in all birds (88.4 ± 16.1 beats·min^{-1}).

The cardiac responses to voluntary diving in the shallow dive tank are shown in Fig. 2.3

and 2.4 (for better comparison the figures include the response in the deep dive situation).

The degree of the decline in heart rate during shallow diving was dependent on the dive

duration. Mean dive heart rate was higher during short dives and lower during long dives

(Fig. 2.5).

Deep diving

When diving beyond 1 m, cormorants displayed a strikingly different cardiac response

(Fig. 2.3 and 2.4). Pre- and post-dive tachycardia were comparable to the shallow dive

situation (Table 2.1) and even the initial drop in heart rate upon submersion was similar

(Fig. 2.3). During the dive, however, heart rate stayed at the initially established level or

decreased at a much slower rate (compared to shallow diving), and no distinct secondary

decrease in heart rate was evident (Fig. 2.3). Mean dive heart rate (216.4 ± 7.7

beats·min^{-1}) and the minimum heart rate during deep diving (159.9 ± 11.5 beats·min^{-1})

were well above the resting heart rate and significantly different from the shallow dive situation (Table 2.1).

Cardiac responses to altered breathing gases

Manipulating oxygen content in the inspired air before diving produced the strongest effects on heart rate during diving (Fig. 2.6). Exposure to the hyperoxic gas mixture in the shallow dive tank increased the mean dive heart rate significantly (195.4 $\pm$ 13.0 beats·min^{-1}) compared to the normoxic control situation (174.3 $\pm$ 13.4 beats·min^{-1}). Upon submersion heart rate fell to a virtually identical level but did not decline appreciably during the rest of the dive (Fig. 2.7).

Exposure to the hypoxic gas mixture reduced the mean dive heart rate significantly in both the shallow (154.0 $\pm$ 11.5 beats·min^{-1}) and the deep dive situation (153.3 $\pm$ 17.1 beats·min^{-1}) compared to normoxic dives (Fig. 2.6). The course of the heart rate response during shallow and deep diving was almost identical. Heart rate stayed well below the control level throughout the dive (Fig. 2.7 and 2.8). During deep diving a secondary drop in heart rate after about 5 - 10 s was detectable (Fig. 2.9). Furthermore, hypoxia effectively reduced pre- and post-dive values, with a more pronounced reduction in the deep dive situation, where the level of hypoxia was more severe.

Breathing elevated levels of CO_2 before diving in the shallow dive tank had little effect on mean dive heart rate or the time course of the heart rate response (Fig. 2.6 and 2.10). In case of the hypercapnic hypoxic exposure no further reduction in dive heart rate beyond the response after hypoxic exposure was detectable.

To ensure that diving inside the PVC cage *per se* had no effect on the displayed cardiac response before or during diving, mean pre-dive and dive heart rates of five birds (for which data in both dive tanks were available) diving inside the cage after exposure to air (control situation) were compared to the voluntary dive situation (no cage). In both dive regimes diving inside the cage or without the cage had no effect on the mean pre-dive heart rate (shallow diving: 385.5 ± 29.2 vs. 386.8 ± 11.8 beats·min^{-1}; deep diving: 387.9 ± 20.5 vs. 390.5 ± 11.6 beats·min^{-1}) or the mean dive heart rate (shallow diving: 172.4 ± 14.1 vs. 170.0 ± 11.6 beats·min^{-1}; deep diving: 217.6 ± 16.2 vs. 216.4 ± 7.7 beats·min^{-1}).

Dive behaviour

There was no significant difference in the mean dive duration of normoxic (control) birds whether diving in the shallow or the deep dive setup (20.43 ± 0.83 s and 20.29 ± 1.37 s respectively; Fig. 2.11). The duration of the surface interval following a dive was significantly longer after deep dives, however (shallow: 9.08 ± 1.45 s, deep: 15.05 ± 3.37 s), resulting in a higher dive/pause ratio during shallow diving (2.54 ± 0.33 vs. 1.48 ± 0.38).

Manipulation of breathing gases had no significant effect on the dive duration of birds in all experimental trials (Fig. 2.11). Surface interval duration and the resulting dive/pause ratio, however, were strongly and significantly affected after different gas exposures (Fig 2.11). Hyperoxia in the shallow dive tank reduced the time spent at the surface between dives, thereby increasing the proportion of the dive cycle spent underwater. This was reflected in the highest dive/pause ratio observed in this study (4.02

± 0.57). Hypoxia produced the opposite effects, increasing the surface interval duration and hence reducing dive efficiency, which was particularly true for the deep dive situation. Exposure to elevated levels of CO_2 in the shallow dive tank increased the post-dive surface interval when compared to the control situation. This increase was especially remarkable in the hypercapnic/normoxic exposure, since changes in the hypercapnic/hypoxic exposure were of the same magnitude as in the hypoxic exposure alone.

Discussion

<u>Cardiac responses</u>

Resting, voluntary shallow and deep diving

Resting heart rates reported in this study are comparable to predicted resting rates for a 2.36 kg bird (127.9 beats·min^{-1}), based on allometric equations derived by Calder (1968) and Lasiewski and Calder (1971). They are, however, lower than the 'resting' heart rates of restrained double-crested cormorants (168 beats·min^{-1}, prior to forced submergence) reported by Mangalam and Jones (1984). They are in between 'basal' heart rates (recorded at night) reported for double-crested cormorants (100-120 beats·min^{-1}; Kanwisher *et al.* 1981) and South Georgian shags (104.0 beats·min^{-1}; Bevan *et al.* 1997) and the heart rates of 'moderately active' double-crested cormorants (swimming slowly or standing on the dock while drying their wings; 170-230 beats·min^{-1}) reported by Kanwisher *et al.* (1981).

Double-crested cormorants, like many other diving vertebrates, undergo marked cardiac and, if blood pressure is to be maintained, vascular changes during their daily foraging activities. In preparation for a dive and in response to a terminated dive, heart rates are elevated to about three times the resting value, presumably facilitating the quick loading of O_2 and unloading of CO_2. At the onset of dives lasting between 18 and 22 s, heart rate drops immediately, followed by a secondary decline after about 5 - 10 s (shallow diving) or a relatively stable (or much slower declining) heart rate during the rest of the dive (deep diving). Heart rate increases towards the end of the dive, reaching pre-dive levels upon surfacing (Fig. 2.3 and 2.4, Table 2.1). The heart rate changes of voluntary diving cormorants reported in this study are strikingly different from heart rate changes associated with forced submergence, as reported by Mangalam and Jones (1984) and Jones and Larigakis (1988). During forced submergence experiments heart rate fell from pre-submergence values of 170 - 200 beats·min^{-1} to a level of 50 beats·min^{-1} within the first 10 - 15 s of submergence. Heart rates that low were not observed in this study of voluntary diving cormorants. Even the much stronger heart rate responses that were observed in occasional 'chased dives' (birds would 'escape dive' when presented with a threatening stimulus) were not of the same magnitude as reported for forced submergence, $i.e.$ heart rate stayed well above 50 beats·min^{-1} (even though single minimum heart rates around 50 beats·min^{-1} did occur). These findings are not surprising given the artificial setting of forced submergence studies (see $Chapter\ 1$ this thesis) and similar results were obtained by Kanwisher $et\ al.$ (1981), contrasting 'free and forced diving' in double-crested cormorants.

The cardiac responses observed in voluntary shallow and deep diving cormorants in this study consisted of a marked decrease in heart rate during diving when compared with pre-dive heart rates (57.03 ± 3.05 % decline in shallow dives; 44.42 ± 1.60 % decline in deep dives). When compared with the resting heart rates, however, the cardiac changes associated with voluntary diving should perhaps be described as a pre- and post-dive tachycardia rather than a diving bradycardia. Sub-resting heart rates were rarely achieved in shallow dives (heart rate was typically around the resting level towards the end of shallow dives) and never in deep dives (Fig. 2.4). The cardiac responses of double-crested cormorants during deep diving were comparable to cardiac responses displayed by diving ducks (tufted ducks, lesser scaups, pochards [*Aythya ferina*], redhead ducks), Humboldt and Adélie penguins during shallow diving: moderate heart rate changes occurred, however, heart rate did not drop below the resting level (Stephenson *et al.* 1986; Furilla and Jones, 1986, 1987; Butler and Woakes, 1976, 1979, 1984; Culik, 1992). They were also similar to heart rates reported for 2 freely diving double-crested cormorants (180 - 220 beats·min^{-1}) in a study by Kanwisher *et al.* (1981). The authors did not report dive depth or dive duration. Presumably dive depths were shallow while, judging from the published heart rate traces, dive duration was similar to the present study. The degree of the tachycardia between dives in their birds (280 - 340 beats·min^{-1}), however, was less than in this study (Table 2.1, Fig. 2.3). Kanwisher *et al.* (1981) found that dive heart rates were similar to heart rates during 'moderate activity' (*e.g.* swimming on the surface) and concluded, therefore, that diving was not accompanied by bradycardia in double-crested cormorants. Support for the legitimacy of this comparison comes from Stephenson *et al.*

(1986) who define 'bradycardia' as a reduction in heart rate below the value which is 'normal' for a given level of activity. These authors suggest that surface swimming is probably the closest approximation to diving exercise, at least in ducks. If this definition is used, however, then heart rates during voluntary diving in their study object, the tufted duck, represent a bradycardia, since they are significantly lower than during surface swimming. Heart rates during 'moderate activity' were not systematically recorded in the present study. Occasional recordings during slow surface swimming revealed heart rates in the range of 200 - 250 beats·min^{-1}, *i.e.* similar to the heart rates reported by Kanwisher *et al.* (1981). When viewing the present results in this light, the cardiac responses of double-crested cormorants during deep diving do not seem to comprise a bradycardia. During shallow diving, however, a bradycardia is evoked.

The greater cardiac response during shallow diving closely resembles the response shown by tufted ducks making 'extended' horizontal dives in a 2.8 m deep tank (Stephenson *et al.* 1986). In these dives, lasting 41.4 s, heart rate of ducks declined steadily after about 7.5 s, while actively swimming to the feeding spot, reaching sub-resting levels after about 27.5 s. It should be mentioned here that birds in the present study had to swim actively towards and away from the feeding spot during shallow diving (as in the tufted duck study), whereas birds in the deep dive tank returned to the surface more or less passively. When leaving the bottom of the deep dive tank birds usually propelled themselves upward by a single kick and used the increasing buoyant force to advance towards the surface without further locomotor effort. Birds kept their neck in a ventrally bent position (shortened overall body length) when steering towards the surface,

making extensive use of their tail. Just before reaching the surface birds stretched out and resumed their typical swim position. The similarity of the experimental setups and reported results in the present study and in the study of tufted ducks making 'extended' dives (Stephenson *et al.* 1986) is very intriguing. If oxygen is used up at a faster rate during shallow horizontal diving, depleting Pa_{O_2} more rapidly, then a stronger cardiac response will be evoked via intravascular chemoreceptors. It is conceivable that the energetic costs of 'extended' horizontal dives might be increased, compared to deep, vertical dives. During diving, birds have to overcome three forces: buoyancy, inertia and drag. Buoyancy is the dominant factor determining dive costs in lesser scaups (Stephenson, 1994). Shallow diving ducks, once they reach their stationary feeding position mainly have to counteract buoyant forces during this phase of the dive (with little mechanical costs added from drag but none from inertia) and can surface passively, once they stop feeding. In lesser scaups the mechanical power output during the bottom phase is reduced by 58 % when compared to the descent phase (Stephenson, 1994). Deep diving cormorants, searching along the sea bottom, still have to overcome inertial forces and drag but work against buoyancy is reduced. Hence, ducks and cormorants performing shallow horizontal dives face the worst situation from an energetic point of view. Furthermore, swimming close to the surface will increase drag and add to the energetic cost of these dives (Hertel, 1969; Blake, 1983).

The difference in the heart rate response to shallow and deep diving might be further accounted for by the effects of pressure changes associated with deep diving on the cardio-pulmonary system. Birds diving to depth will experience a compression hyperoxia

during descent (Lanphier and Rahn, 1963; Kooyman *et al.* 1973; Qvist *et al.* 1986). If Pa_{O_2} stays elevated during this phase of the dive (Qvist *et al.* 1993), any chemoreceptor mediated reinforcement of the initial heart rate drop will be delayed as a consequence (de Burgh Daly, 1997). Energetic savings, due to the reduced locomotor effort during the bottom (birds in this study did not chase prey) and ascent phase of deep diving cormorants will help to maintain a relatively high Pa_{O_2}, further delaying any chemoreceptor contribution to the cardiac response. Hence, heart rate stays well above the resting level during these relatively short dives (18 - 22 s). It is conceivable that in longer dives heart rate will fall more drastically, as Pa_{O_2} will drop, provoking a chemoreceptor driven cardiac inhibition. Severe cardiac responses were observed in much longer (range: 140 - 287 s) and deeper dives (range: 35 - 101 m) of South-Georgian shags diving at sea (Bevan *et al.* 1997). While heart rate fell to a level near the resting value in the early phase of a dive (after 30 - 60 s), sub-resting levels were reached later in the dive. Unfortunately, a closer comparison with the present study is not possible due to the coarse time resolution of the shag study (heart rate was averaged over 15s-periods by the data logger). In contrast to this deep diving scenario, shallow diving birds will not experience a compression hyperoxia, hence Pa_{O_2} will decrease early in the dive (especially if shallow diving is in fact associated with increased energetic costs), accelerating the chemoreceptor mediated cardiac inhibition.

During the ascent phase of deep dives PA_{O_2} will fall rapidly (Lanphier and Rahn, 1963), potentially reversing the direction of oxygen transport (Olszowka and Rahn, 1987). Accordingly, there should be a drop in Pa_{O_2}, which would increase the chemoreceptor

drive and in turn reduce heart rate. In the present study, however, heart rate did not decrease further during ascent and even increased just before birds reached the surface. Obviously, other neurological inputs must override the chemoreceptor contribution during this phase of deep dives. In addition to possible influences of higher brain centres, anticipating the return to the surface, re-expansion of the respiratory system might activate pulmonary stretch receptors, which would in turn increase heart rate (Kooyman, 1989). Increasing heart rate in anticipation of surfacing seems to be a usual feature of the cardiac response to voluntary diving in birds and mammals (Butler and Jones, 1997). For ringed seals (*Phoca hispida*) it has been shown that visual orientation is important in facilitating the anticipatory heart rate increase during ascent (Elsner *et al.* 1989), which stresses the influence of components of the central nervous system (CNS) above the reflex level.

Results obtained from shallow diving cormorants seem to point towards intravascular chemoreceptors as an important mediator of the cardiac responses during voluntary diving in two ways: First, the secondary fall in heart rate observed 5 - 10 s after initiation of a dive (Fig. 2.3) might reflect an increase in chemoreceptor discharge frequency - due to a reduction in Pa_{O_2} - which in turn is expressed on the cardiovascular system. Second, the significant linear relationship between mean dive heart rate and dive duration found for three birds during shallow diving (Fig. 2.5) suggests that a gradual mechanism facilitates the reduction in heart rate, again, pointing at chemoreceptors. Further support comes from the observed cardiac responses of deep diving birds (Fig. 2.3). In these dives, where Pa_{O_2} will be elevated initially due to the experienced compression, heart rate stayed

relatively stable throughout the dive (or declined slowly) and no secondary drop in heart rate was detectable.

Alteration of breathing gases

The results obtained from experimental manipulation of the oxygen content in the inspired air before diving strongly support the hypothesis that intravascular chemoreceptors are an important component in the cascade of events that produces the cardiac responses observed during voluntary diving in double-crested cormorants. Further support comes from the following two considerations: if intravascular chemoreceptors are accountable for the observed difference in heart rate responses during the altered breathing gas trials then (i) 'in hypoxic shallow dives, the second drop in heart rate should be detectable earlier in the dive compared with control dives.' This early secondary drop in heart rate became detectable in individual dive traces during hypoxic shallow diving. Heart rate also dropped at a faster rate during hypoxic diving (Fig. 2.7), so that after 3 - 6 s of submergence heart rate was already below the values reached during control dives. (ii) 'In hypoxic deep dives, a second drop in heart rate should become apparent that is absent in control dives.' The reappearance of this second heart rate drop is illustrated in Fig. 2.9.

The findings of this study are in basic agreement with results reported for voluntary diving tufted and redhead ducks. Butler and Woakes (1982b) found that chronic bilateral denervation of the carotid bodies had no effect on the immediate reduction in heart rate upon submersion. Heart rate was, however, significantly elevated towards the end of spontaneous dives. Similarly, Furilla and Jones (1986) found that altering the level of O_2

breathed by redhead ducks before voluntary submersion had no effect on heart rate early in the dive (after 2 - 5 s of submergence). Although Butler and Stephenson (1988) found that heart rate of tufted ducks during diving was unaffected by the inspired gas composition in control and carotid body denervated ducks, dive heart rate was increased in carotid body denervated ducks, regardless of the gas mixture breathed beforehand. From these studies it was generally concluded that carotid body chemoreceptors might play only a minor role in the cardiac control of diving, at least under the circumstances investigated (short and shallow dives). In the present study, however, alteration of the oxygen content in the inspired air before diving, produced strong and significant effects on the observed heart rate response during shallow and deep diving. It might be argued that as dive duration in the duck studies was short, the full chemoreceptor response could not develop. However, the differences in heart rate response of cormorants to alteration of breathing gases were established early during shallow diving (Fig. 2.7). In the case of the hypoxic exposure before deep diving, the difference was established even before submersion (Fig. 2.8).

Butler and Stephenson (1988) found that the development of the bradycardia during 'extended' dives of tufted ducks was significantly slowed following carotid body denervation. Hence, they concluded that under these circumstances carotid body chemoreceptors might become more important. It should be stressed again that the experimental situation for cormorants performing horizontal dives in the shallow dive tank of the present study was very similar to tufted ducks making 'extended' dives. Unfortunately, Butler and Stephenson (1988) did not test the effect of altering breathing gas mixtures in tufted ducks making 'extended' dives. It is conceivable that the heart rate

response of tufted ducks under these circumstances might have been qualitatively very similar to the response of double-crested cormorants.

Results from altering the gas breathed before deep diving are not easily compared with other studies. In fact, none of the studies investigating cardiac control mechanisms of voluntary diving animals incorporated the factor of depth. In a study by de Leeuw *et al.* (1998), investigating the energetic implications of body cooling for diving in tufted ducks, heart rate during dives to different depths was measured. There was no relationship between heart rate at different phases of the dive cycle and dive depth. Heart rate during diving was equally increased (when compared with resting) whether diving to 1.5 m (lasting 12.7 s) or to 5.5 m (lasting 26.5 s). Post-dive heart rate, however, increased with depth, as did the time spent at the surface between dives. Since tufted ducks in that study were diving in a similar fashion to the cormorants in the deep dive tank (*i.e.* vertical dives and not 'extended' horizontal dives) the observed heart rate patterns are in agreement with the hypothesis that horizontal dives are energetically expensive, depleting Pa_{O_2} at a fast rate, which in turn will stimulate carotid body chemoreceptors and decrease heart rate. In this context it is interesting to think about the implications for all the laboratory studies where animals perform horizontal dives in shallow, covered tanks. We might not be able to simply extrapolate from data collected under these circumstances to cardiac responses that diving animals might employ in the wild.

Butler and Stephenson (1988) found that exposure to hypoxia (9.27 ± 0.20 % O_2) significantly elevated pre- and post-dive heart rates in both control and carotid body denervated tufted ducks. Pre- and post-dive heart rates of double-crested cormorants,

however, were reduced after exposure to hypoxia. This effect was significant only for the deep dive situation, where hypoxic exposure was more severe (8 - 9 % O_2). This finding is consistent with other reports in the literature. It seems that although breathing gases of lowered oxygen content causes a consistent increase in pulmonary ventilation, the cardiovascular effects are variable depending on species and the degree of arterial hypoxaemia and hypocapnia (de Burgh Daly, 1997). While mild hypoxia most commonly causes a tachycardia, more severe hypoxia tends to produce a bradycardia.

Pa_{O_2} was not measured in this study. However, by administering 50 % O_2 for 5 min prior to forced submergence, Mangalam and Jones (1984) elevated Pa_{O_2} of double-crested cormorants almost threefold, from 80 mm Hg to 220 mm Hg. Breathing 12.8 % O_2 (combined with 3 % CO_2) reduced Pa_{O_2} to 70 mm Hg. It is most likely that the administration of the different gas mixtures used in this study for at least 10 min before diving produced the desired changes in Pa_{O_2}. Furthermore, it was the explicit purpose of this study to interfere with the birds as little as possible, to stress the voluntary and 'realistic' nature of the performed dive experiments. Interference with the birds might provoke 'chase dives', during which cardiac responses to submergence are magnified (pers. observation). Monitoring Pa_{O_2} during the dive experiments would have required handling of the birds and might have drastically altered the nature of the dives.

Increased levels of CO_2 in the breathing gas had no significant effect on diving heart rate of double-crested cormorants (Fig. 2.6 and 2.10). This finding is in agreement with results from forcibly submerged Pekin ducks (Jones *et al.* 1982), where CO_2 contributed little (~ 20 %) to the observed bradycardia, when compared with O_2 (~ 65 %). Similarly,

Butler and Stephenson (1988) found that hypercapnia had no effect on diving heart rate of voluntary diving tufted ducks. Pre- and post-dive heart rates of these ducks, however, were significantly reduced after hypercapnic exposure (5.22 % CO_2). Double-crested cormorants on the other hand showed no significant reduction in pre- and post-dive heart rates (Fig. 2.10), which could be explained by a different degree of hypercapnic exposure (3 % CO_2).

In conclusion, the results of this study clearly suggest that the cardiac responses to voluntary diving in double-crested cormorants (apart from the initial response to submersion) are strongly influenced by changes in Pa_{O_2}. Carotid body chemoreceptors, sensing arterial oxygen tensions, are the most likely facilitators of the observed cardiac responses.

Dive behaviour

The dive patterns observed during voluntary shallow diving in the present study are very similar to dive patterns reported for double-crested cormorants foraging in the wild. Ross (1974) observed double-crested cormorants diving in water 1.5 - 7.9 m deep. Dive and surface interval duration were 25.1 s and 10.3 s, respectively, resulting in a dive/pause ratio of 2.43. Dive durations during shallow and deep diving in the present study are longer than dive durations reported for double-crested cormorants foraging in shallow (1.4 m) catfish ponds (mean: 11.9 s; range: 2 - 45 s) with high prey densities (King *et al.* 1995). They are, however, certainly much shorter than the maximum dive duration (70 s) reported for double-crested cormorants (Munro, 1927). Cooper (1986) in

his review of diving patterns of 19 *Phalacrocorax* species reported dive/pause ratios of foraging birds between 1.95 and 4.36. It seems that dive duration during foraging in the majority of *Phalacrocorax* species typically exceeds the subsequent surface interval by a factor of 2 to 3, *i.e.* a dive/pause ratio of 2 - 3 (Ross, 1974; Williams and Cooper, 1983; Cooper, 1985 and 1986; Trayler *et al.* 1989; Ainley *et al.* 1990; Lea *et al.* 1996). Birds in these studies were foraging in fairly shallow water, with most dives less than 10 m deep (range: < 1 m to around 20 m). Some *Phalacrocorax* species, however, forage in deep water (*e.g.* blue-eyed shags, South Georgian shags). Since most cormorant species are benthic foragers, commuting between the surface and bottom, it is not surprising to find a positive relationship between dive time and water depth. Foraging in deep water will require long transit times and will increase dive duration and subsequent recovery periods at the surface. Accordingly, Croxall *et al.* (1991) reported very low dive/pause ratios for blue-eyed shags (0.3 - 0.4), diving to great depth (max. 116 m).

Dive duration in the present study was not different during shallow and deep diving (Fig. 2.11). Why birds increased the subsequent surface interval in the deep dive situation compared with the shallow dive situation is not readily understood. Since birds were foraging on the same prey items (herring pieces) it is not likely that increased surface times in the deep dive tank were associated with longer prey handling times. Considering the less dramatic cardiac changes associated with the deep diving situation one could speculate about the functional significance of a dive bradycardia in facilitating efficient dive patterns. The rapid decline in heart rate observed during shallow diving presumably reflects the conservation of oxygen. If birds use less oxygen during the dive, they will be able to replenish their oxygen stores faster, once ventilation resumes at the surface. A

shorter post-dive surface interval would increase the proportion of the dive cycle spent underwater, and hence, dive efficiency.

Alteration of breathing gases

The absence of an effect on dive duration due to the altered breathing gas (and hence blood gas tensions) might be accounted for by limitations of the experimental setup. Birds were feeding on herring pieces in both tanks. No attempts were made to alter 'foraging success', so that every dive was potentially a successful dive. Once cormorants reached the feeding spot, they would take a single herring piece and return to the surface, where prey was ingested. Birds did not have to make any decisions at the 'bottom' whether to continue searching or to end the dive. They did not have to chase their prey underwater, hence, actual 'bottom time' was relatively short and dive duration was dictated by the transit time. Since only dives with an obvious foraging intention were included in the analysis (*i.e.* a bird picked up a fish piece or at least checked it), it is not surprising to find that dive duration stayed constant, regardless of the breathing gas administered.

With the dive duration being dictated by the experimental setup, birds were left only with the possibility of adjusting the duration of the subsequent surface interval. The adjustment of surface interval duration in accordance to the administered gas mixture shown in Fig. 2.11 clearly illustrates the importance of blood gases (O_2 and CO_2) in controlling dive behaviour in double-crested cormorants. These findings are similar to results reported for redhead and tufted ducks (Furilla and Jones, 1986; Butler and Stephenson, 1988). In both of these studies the ducks, being stationary feeders, were able to adjust dive duration and in fact, made use of this possibility. In voluntary diving

redhead ducks, dive duration increased as O_2 in the inspired air increased (range: 10 - 50 % O_2; Furilla and Jones, 1986). Unfortunately, surface interval duration or any measures of diving efficiency were not reported. In tufted ducks dive duration was decreased after hypoxic and after hypercapnic exposure, while hypercapnia increased the surface interval duration as well (Butler and Stephenson, 1988). The reported dive efficiencies follow the same general trend as the ones reported in the present study for double-crested cormorants: hypoxia and hypercapnia both decrease dive efficiency, while hyperoxia increases efficiency. Butler and Stephenson (1988) suggested that surface interval duration is primarily controlled by CO_2 and that O_2 primarily determines dive duration. Such a clear distinction is not possible in the present study, because, given the experimental setup, birds did not adjust dive duration.

Both aforementioned studies (Furilla and Jones, 1986; Butler and Stephenson, 1988) found that diving activity of ducks ceased beyond a 'critical' O_2 or CO_2 concentration in the inspired air. Similarly, double-crested cormorants exposed to O_2 concentrations between 8 and 9 % in the deep dive tank drastically altered their dive behaviour, suggesting close proximity to such a threshold. In fact, when the O_2 concentration in the inspired air fell below 8 %, birds would not dive. The change in dive behaviour after hypoxic exposure in the deep dive situation was impressive. Surface interval duration increased by factor 4, resulting in a dive/pause ratio comparable to blue-eyed shags performing much longer and deeper dives.

Besides altering the duration of ventilation periods, it is conceivable that birds could have changed ventilation frequency in response to changes of O_2 and CO_2 in the inspired

air. Hyperventilation in hypoxia for example will result in a relatively low Pa_{CO_2} (Bouverot *et al.* 1979), which during the dive will delay any stimulatory effect of an increasing Pa_{CO_2} on ventilation, counteracting the effects of a low Pa_{O_2}. Hypoventilation during hyperoxia, on the other hand, will elevate Pa_{CO_2} (Bouverot and Sébert, 1979) which might counteract effects of an increased O_2 storage. Although ventilation was not formally measured in the present study, casual observation during the voluntary dive trials (when diving without the PVC cage) suggested that birds hyperventilated before submersion. During the altered breathing gas trials (when diving inside the PVC cage) observation was impeded. It is interesting in this context to note that hyperventilation seemed more pronounced in the deep dive situation. Hyperventilation before voluntary diving has been reported for many diving animals, and Kooyman *et al.* (1973) suggested that this could account for a lower Pa_{CO_2} and a higher Pa_{O_2} before and after diving in Weddell seals than when the animals were resting.

In conclusion, the present study strongly suggests that double-crested cormorants employ both physiological and behavioural mechanisms to maximise underwater time and, hence, potential foraging time.

Table 2.1 Heart rates (beats min⁻¹) of double-crested cormorants during resting and voluntary diving in the shallow and deep dive tank

Bird	Resting	Pre-dive	Dive	Minimum	Post-dive
Shallow diving					
1LR		375.8 (± 10.0)	152.1 (± 9.3)	71.3 (± 6.5)	392.3 (± 13.4)
LW		369.0 (± 13.9)	141.5 (± 7.4)	74.1 (± 5.1)	369.8 (± 10.1)
2G		366.1 (± 40.0)	162.0 (± 4.4)	82.2 (± 8.5)	435.4 (± 14.5)
1G	155.2 (± 20.8)	376.6 (± 29.6)	168.5 (± 20.8)	92.8 (± 13.4)	377.9 (± 12.0)
OG	142.3 (± 38.3)	393.2 (± 4.6)	155.8 (± 6.0)	89.1 (± 13.5)	398.0 (± 7.6)
OW	144.8 (± 16.3)	382.2 (± 36.6)	164.8 (± 8.3)	84.9 (± 14.5)	396.7 (± 5.5)
2P	108.7 (± 14.9)	404.2 (± 5.5)	187.0 (± 9.4)	123.7 (± 28.2)	405.6 (± 6.4)
RP	138.4 (± 15.3)	377.6 (± 38.2)	174.1 (± 19.8)	89.1 (± 33.0)	402.1 (± 8.0)
Grand mean	137.9 (± 17.5)	380.6 (± 12.6)*	163.2 (± 14.0)*,†	88.4 (± 16.1)*,†	397.2 (± 19.6)*
Deep diving					
1G		379.2 (± 17.8)	211.5 (± 8.8)	159.3 (± 10.9)	365.1 (± 24.6)
OG		377.7 (± 5.3)	213.4 (± 10.7)	175.6 (± 15.6)	377.3 (± 5.2)
OW		404.5 (± 14.8)	225.6 (± 12.1)	150.0 (± 31.6)	410.5 (± 10.0)
2P		395.5 (± 7.9)	223.7 (± 14.4)	166.7 (± 7.5)	401.5 (± 6.1)
RP		395.7 (± 14.0)	208.1 (± 20.4)	148.1 (± 16.2)	383.1 (± 21.1)
Grand mean		390.5 (± 11.6)*	216.4 (± 7.7)*,†	159.9 (± 11.5)*,†	387.5 (± 18.4)*

Values are presented as mean (± S.D.).

A grand mean is the mean of the individual cormorant means.

Resting heart rates are the mean values taken over a 20 min. session per bird.

Pre-dive and post-dive heart rates are mean values taken from 3-sec.-intervals before and after diving, respectively.

Dive heart rates are mean values averaged over the entire dive duration.

Minimum heart rates are the single minimum heart rates during each dive.

All heart rates related to diving are the mean values taken from dives lasting between 18 and 22 sec, n = 6 dives per cormorant.

*Significantly different from the resting heart rate values.

†Significant difference between 'shallow diving' and 'deep diving' value.
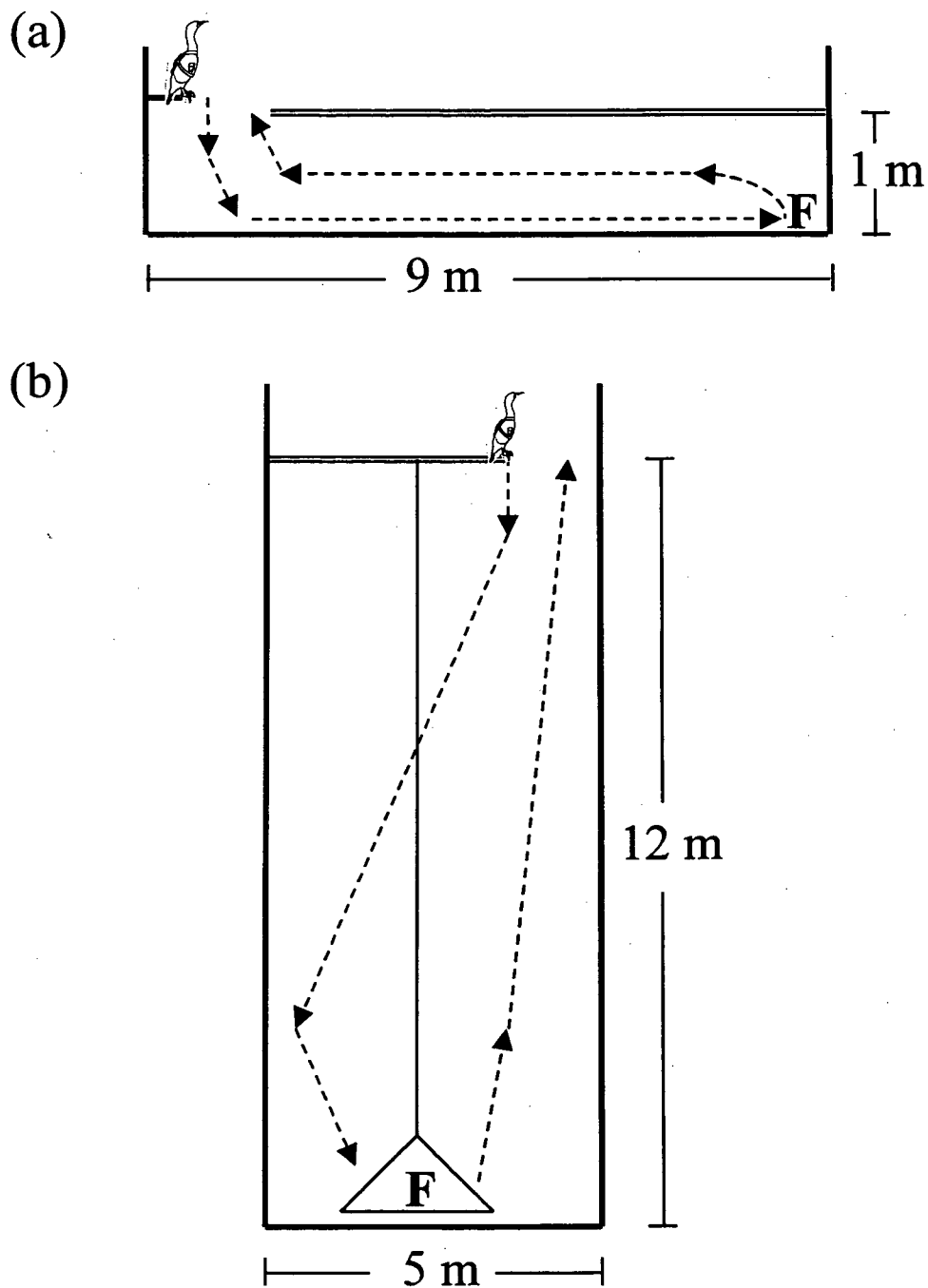
Fig. 2.1 Side view and dimensions of shallow (a) and deep (b) dive tank. 'F' indicates the feeding spot, where birds picked up chopped herring pieces. The approximate routes taken by birds are indicated by the dashed lines, with the arrowheads indicating the direction of locomotion.
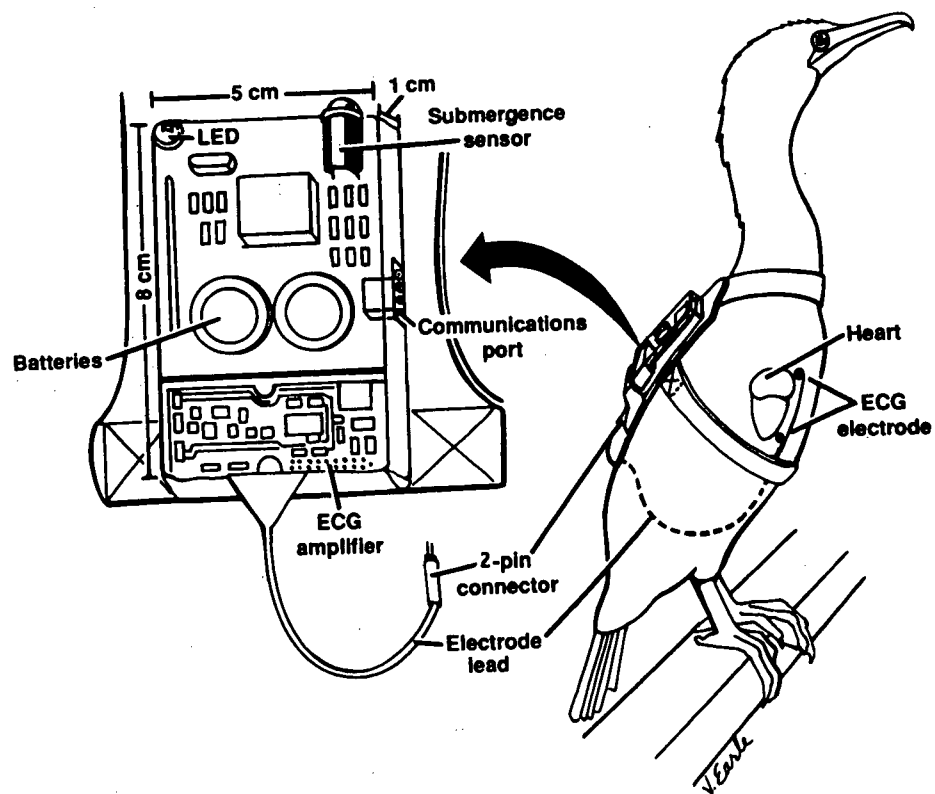
Fig. 2.2 *Left*: expanded view of data logger. *Right*: position of data logger system on double-crested cormorant during deployment. LED (light-emitting diode).
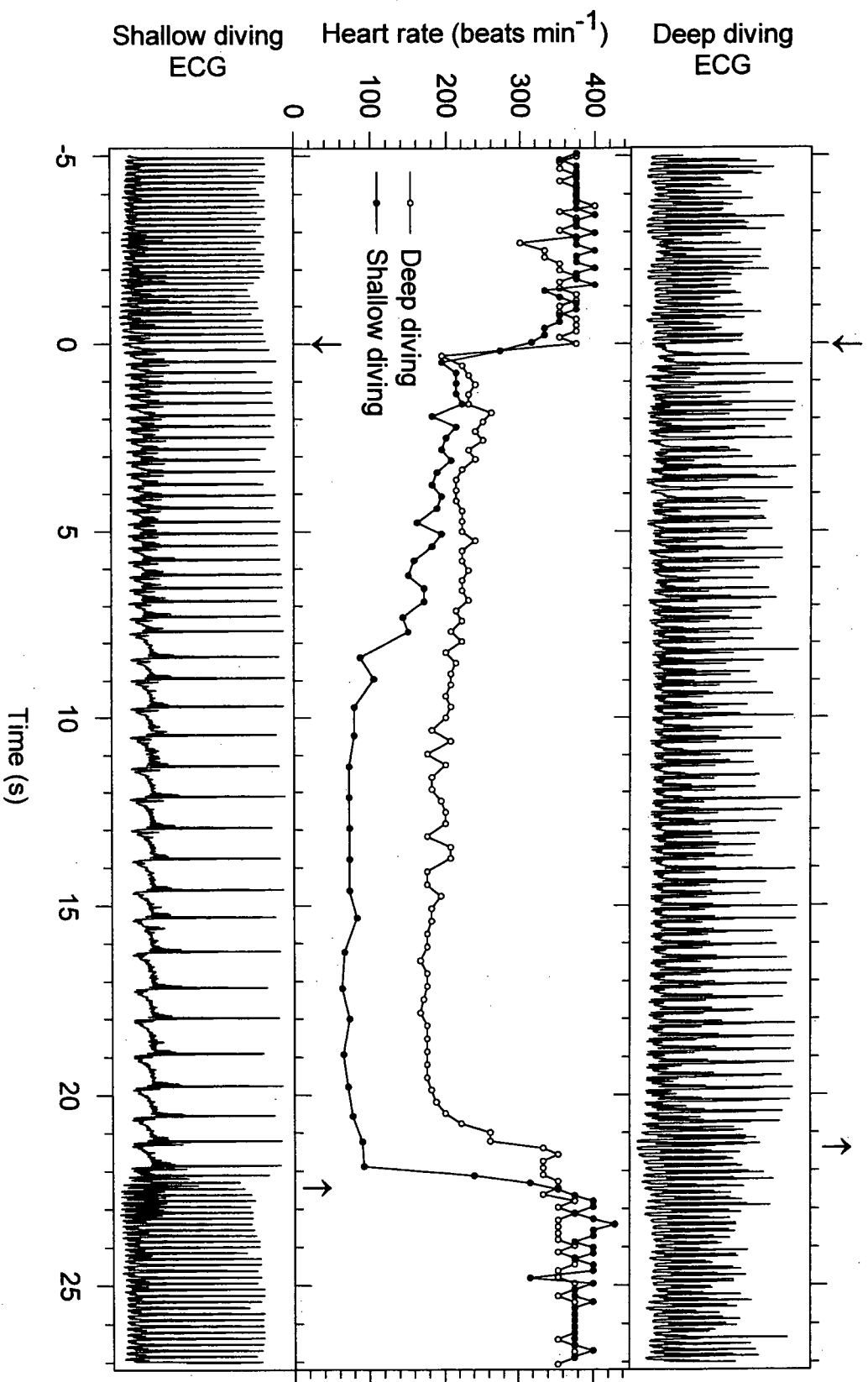
Fig. 2.3 ECG and instantaneous heart rate during individual deep and shallow dives. Top and bottom show the ECG during a deep and shallow dive, respectively. Corresponding heart rates (beats·min⁻¹) are shown in the centre. Submersion and emersion are indicated by the descending and ascending arrows, respectively.
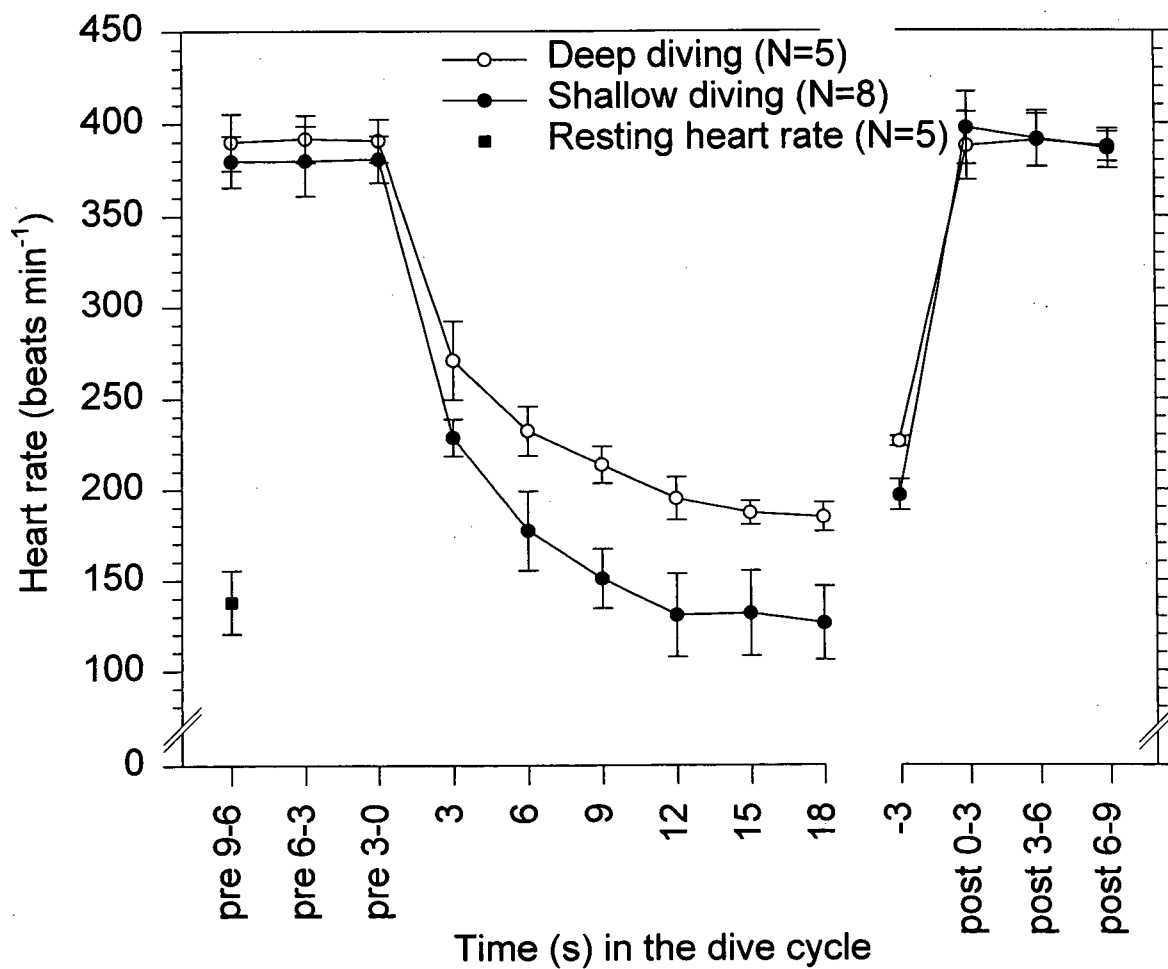
Fig. 2.4 Heart rate before, during, and after deep and shallow diving. Values are means (±
S.D.) averaged over 3-s-intervals from 6 dives per bird (all dives 18 - 22 s); N
depicts the number of individuals used. For comparison resting heart rate is
indicated.
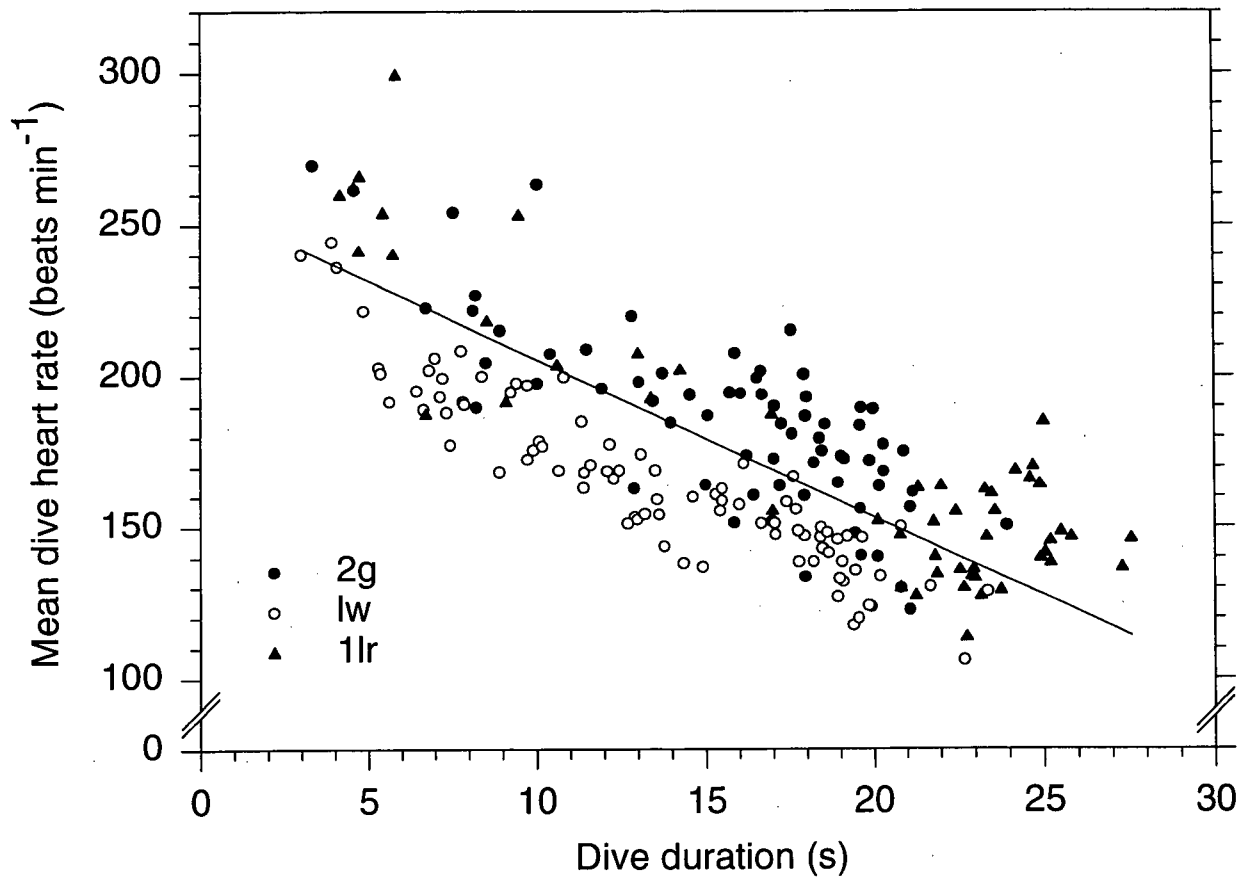
Fig. 2.5 Mean dive heart rate vs. dive duration for 3 double-crested cormorants
(cormorant 2g, 1w, and 1lr) during shallow diving (n = 208). Values for each
cormorant demonstrated a significant negative relationship, with r^2 ranging
from 0.60 to 0.84. The plotted regression line is the average relationship for all
cormorants and is described by y = 257.48 - 5.21x, where y is mean dive heart
rate and x is dive duration (r^2 = 0.761).
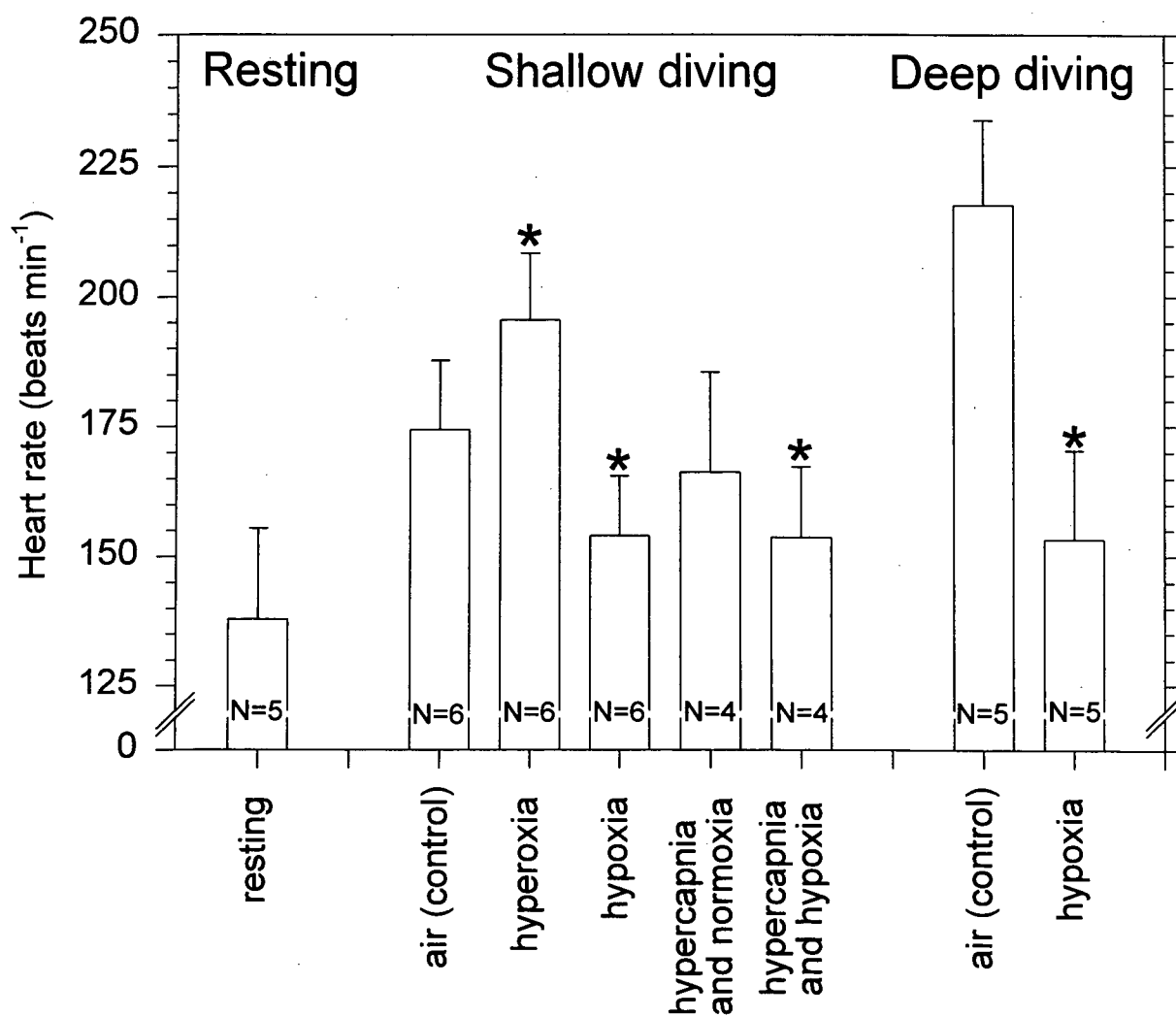
Fig. 2.6 Heart rate during resting, shallow and deep diving, and after exposure to different levels of O_2 and CO_2. Values are means ($\pm$ S.D.) from 6 dives per bird (all dives 18 - 22 s); resting heart rate was calculated from a 20 min section per bird; N depicts the number of individuals used. For gas mixtures used, see *Materials and methods*. *Significantly different from the respective control values (air).
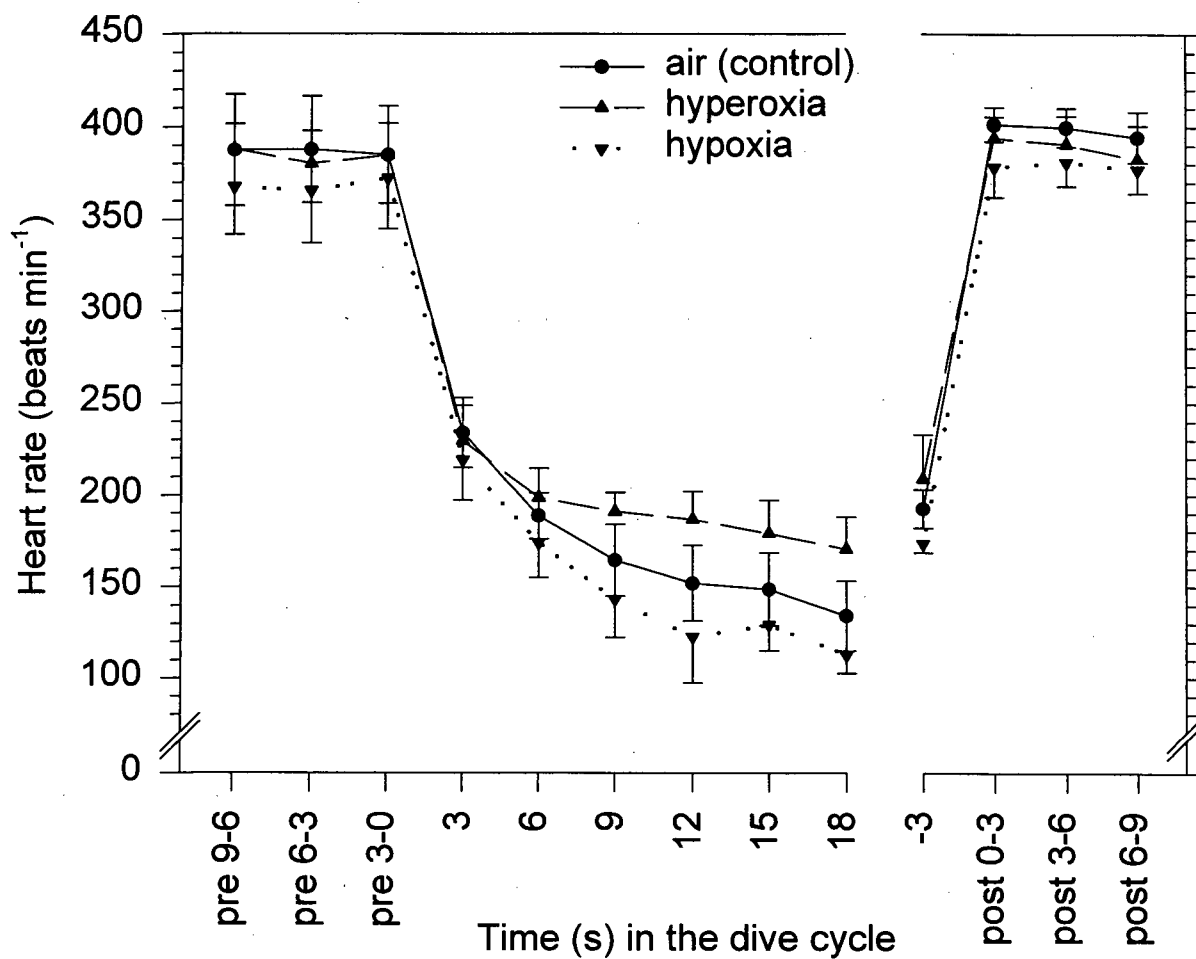
Fig. 2.7 Heart rate before, during, and after shallow diving, following exposure to different ambient oxygen levels. Values are means (± S.D.) averaged over 3-s-intervals from 6 dives per bird (all dives 18 - 22 s; N = 6 birds). For gas mixtures used, see *Materials and methods*.
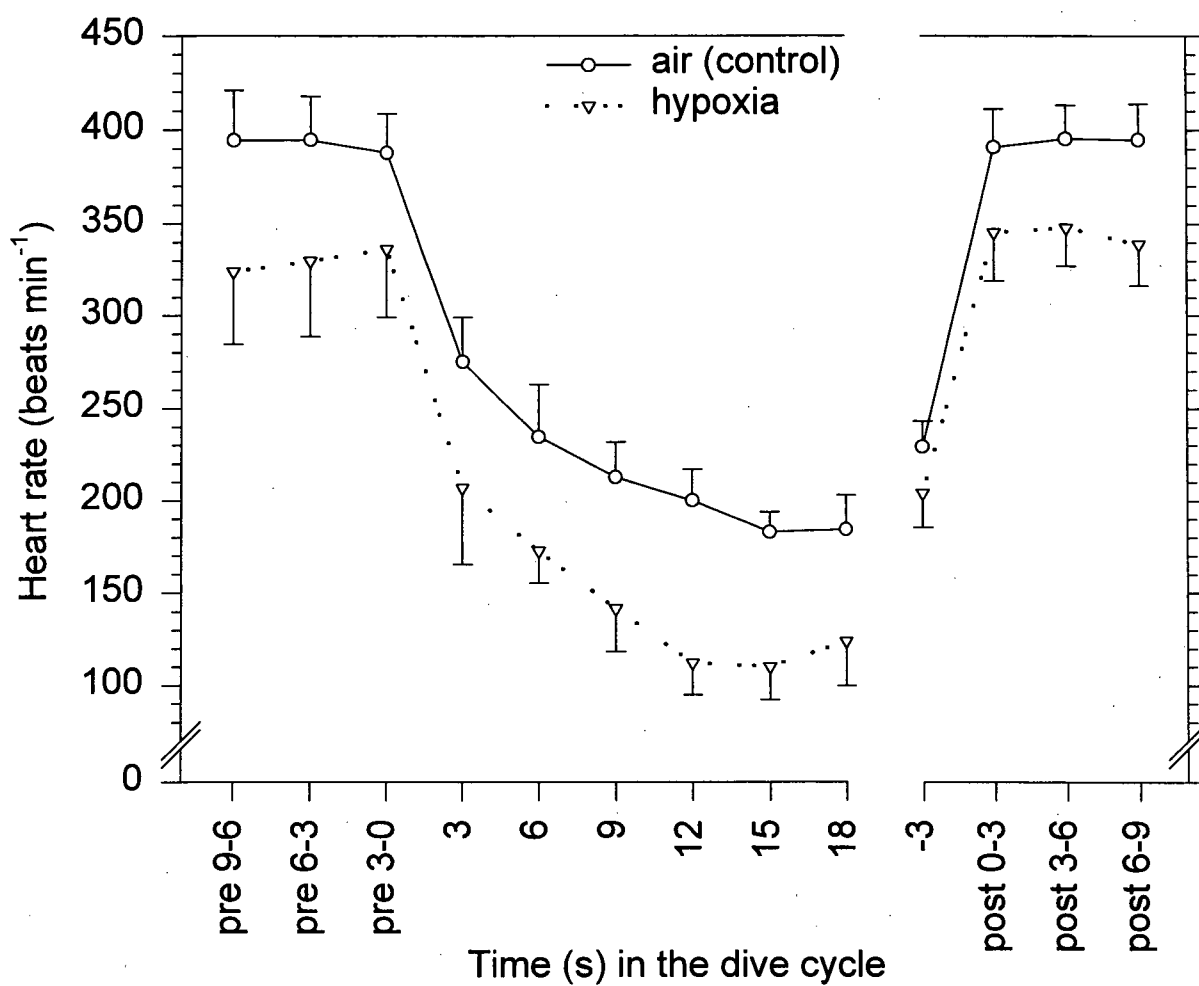
Fig. 2.8 Heart rate before, during, and after deep diving, following exposure to different ambient oxygen levels. Values are means (± S.D.) averaged over 3-s-intervals from 6 dives per bird (all dives 18 - 22 s; N = 5 birds). For gas mixtures used, see *Materials and methods*.
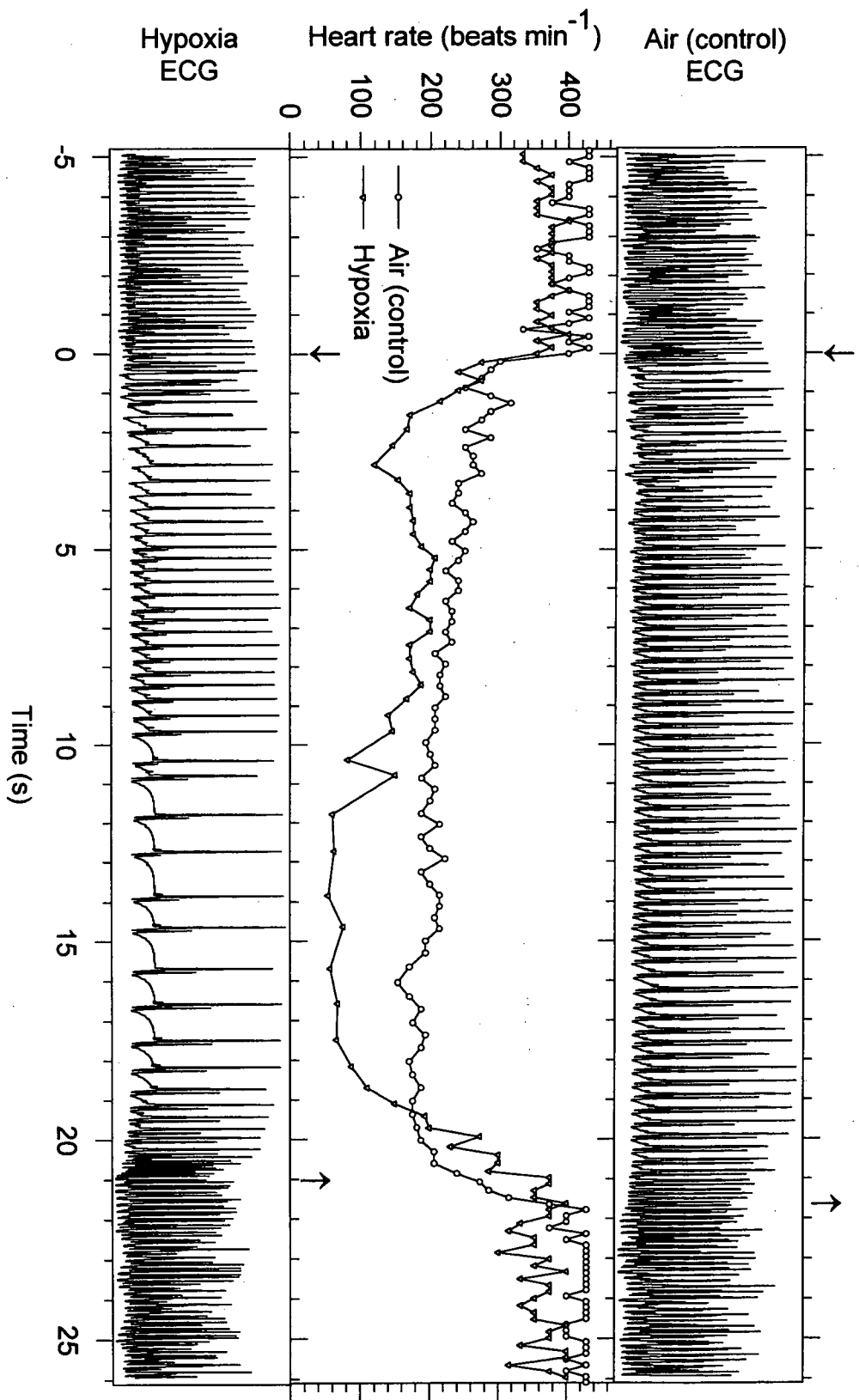
Fig. 2.9 ECG and instantaneous heart rate during 2 individual deep dives after exposure to air (control) and hypoxia. Top and bottom show the ECG during a deep dive after exposure to air and hypoxia, respectively. Corresponding heart rates are shown in the centre. Submersion and emersion are indicated by the descending and ascending arrows, respectively.
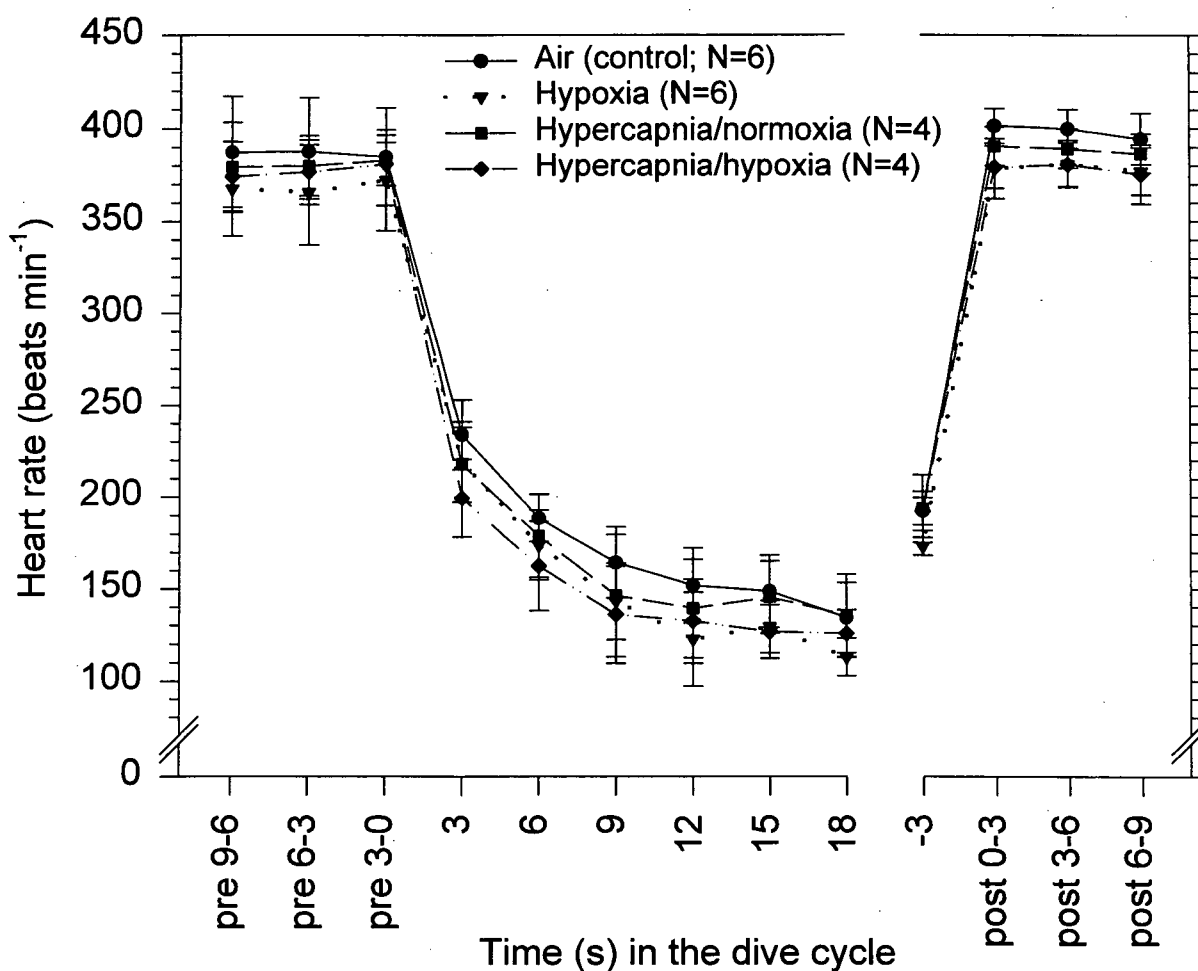
Fig. 2.10 Heart rate before, during, and after shallow diving, following exposure to different levels of O_2 and CO_2. Values are means ($\pm$ S.D.) averaged over 3-s-intervals from 6 dives per bird (all dives 18 - 22 s); N depicts the number of individuals used. For gas mixtures used, see *Materials and methods*.
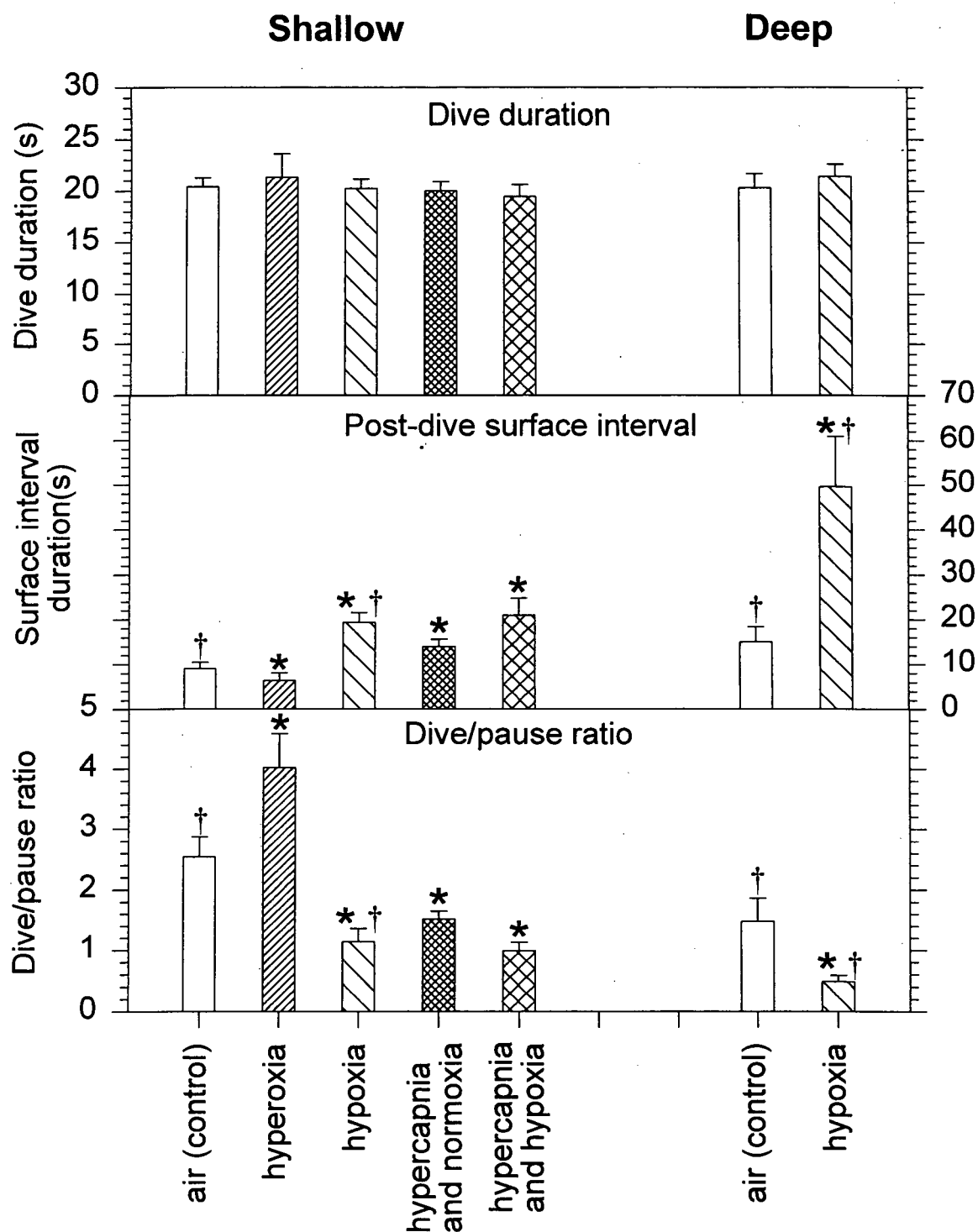
Fig. 2.11 Dive behaviour associated with shallow and deep diving and after exposure to different levels of O_2 (N = 5 birds) and CO_2 (N = 4 birds). Values are means ($\pm$ S.D.) from 10 dive cycles per bird. *Significantly different from respective control (air) values. †Significant difference between 'shallow diving' and 'deep diving' value.

Chapter 3: Ontogeny of the cardiac response to voluntary diving in double-crested cormorants

Introduction

Double-crested cormorants produce altricial chicks that hatch with naked bodies, closed eyes, and without the abilities of locomotion and thermoregulation. Hence, these chicks are completely dependent on their parents that feed them for up to 6 or 7 weeks, before chicks gain independence from their parents (fledge) and venture onto the water to take up their diving habit (Lewis, 1929).

Surprisingly little is found in the literature about the exact course of events at fledging in cormorants. Drent *et al.* (1964) report that double-crested cormorant chicks on Mandarte Island start to swim after about 6 - 7 weeks by their own choice and move away from the colony gradually to take up an independent life. Pelagic cormorant chicks (*P. pelagicus*) on Mandarte Island start nest departure when 6 to 7 weeks old (Drent *et al.* 1964). The authors did not determine, however, at what age the young finally become independent. In a tree-nesting colony of great cormorants (*P. carbo*), chicks start to fly out of the colony when 7 weeks old and become independent at 12 - 13 weeks of age (Kortlandt, 1942, cited in: Drent *et al.* 1964). Similarly, Boekelheide *et al.* (1990) report that chicks in a breeding colony of Brandt's cormorants (*P. penicillatus*) in the Farallon Islands gradually wander from the nestsite as they grow larger. After joining fledgling creches, they enter the water below the colony daily for bathing and diving, although they are still reliant on their parents, which continue to feed them for another 3 - 6 weeks.

How then do chicks acquire all the necessary skills to become successful pursuit divers? Do parents 'teach' their chicks how and where to dive, how to recognise prey and how to catch and handle it? Alternatively, are chicks left on their own to acquire all these skills? Whatever the answer might be, it is conceivable that cormorant chicks will gain many skills important for successful foraging (*e.g.* development of a prey search image or refining capture techniques) through experience.

This might be illustrated by the following observations on a group of captive double-crested cormorant chicks that were, however, not systematically investigated in this study: Individual chicks (~ 11 weeks old) responded differently when live fish (~ 10 cm long coho salmon fingerlings, *Oncorhynchus kisutch*) were offered inside a small tank (1.5 m in diameter, 0.2 m deep) in addition to the usual chopped herring pieces. While the moving fish aroused the curiosity of some chicks, others ignored them. The few chicks that seemed to 'recognise' the fish as potential prey successfully struck at the fish, killing them, but were unable to orient the fish in the position necessary for ingestion (head first). After a period of unsuccessful trials they abandoned their prey. With repeated exposures to live prey, chicks learned how to handle prey over the course of a few days. Similar observations were reported by Lewis (1929). He found that one immature double-crested cormorant which was caught after it had gained independence from its parents immediately took to catching live fish, although dead herring was also offered. In contrast, another chick (age not specified) continued to take dead herring and it took a few weeks before it learned to catch live fish.

Although there have been many studies investigating the differences in foraging behaviour of juvenile and adult birds (for review see Marchetti and Price, 1989), to my

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knowledge there is only one study that investigated age-related foraging abilities in cormorants. Morrison *et al.* (1978) compared relative foraging efficiencies of adult and immature olivaceous cormorants (*P. olivaceus*) foraging in a shallow estuarine area and on a manmade pond. They found that foraging success was significantly higher in adults than in immatures for both study sites. Besides capturing prey at a lower rate, immatures were losing captured prey more often and needed longer to handle prey (*i.e.* manipulating prey before swallowing). Immatures compensated the lower foraging success by increasing their overall foraging effort, *i.e.* immatures increased the number of foraging bouts per day.

Besides the development of behavioural patterns, anatomical and physiological maturation are indispensable components in the successful transition from a chick fed by its parents to an actively foraging immature bird. A few examples might clarify this point: Lewis (1929) found that the occipital style - a slender bone, unique to the Phalacrocoracidae and Anhingidae, which articulates with the back of the skull and serves as an additional attachment point for adductur muscles, thereby increasing the biting force (Owre, 1967; Burger, 1978) - was completely absent in double-crested cormorant fledglings that did not catch their own food. In foraging young immatures, the style was mostly cartilaginous and weak, however, ossification took place within a few weeks. Haggblom *et al.* (1988) investigated the changes in blood and muscle tissue that occurred during the development of pigeon guillemots (*Cepphus columba*) from altricial chick to fledgling to adult. Pigeon guillemots are also active pursuit divers, however, they differ from cormorants in that they employ their wings for underwater propulsion. Haggblom and co-workers (1988) found that changes in myoglobin concentration and lactate

dehydrogenase activity in heart and pectoralis muscle, as well as changes in haematocrit and haemoglobin concentration, correlated with the bird's maturation from a sedentary nest sitter to an active diver and flyer. Based on the differences in myoglobin concentrations the authors suggested that diving ability of the fledglings might be compromised and that the high concentrations found in the adult pectoralis muscle might be accomplished through the vigorous, repetitive use of these muscles.

In adult double-crested cormorants, voluntary diving is associated with abrupt changes in cardiac rhythm, *i.e.* heart rate increases before the first dive in a series followed by a steep decline upon submersion (*see Chapter 2*). In *Chapter 2,* I argued that these cardiac changes are an important component in the overall attempt to utilise oxygen economically during a dive, maximising underwater foraging time. Given that, the cardiac responses to submersion should be functional when the young take up their natural diving habit or, alternatively, should be acquired rapidly. This view is supported by the study of Morrison *et al.* (1978) on the age-related foraging abilities of olivaceous cormorants. Since the observed dive variables (dive duration, surface duration between dives, dive/pause ratio) were nearly identical between immature and adult birds, it was suggested that immatures acquire the diving abilities of adults at, or soon after, fledging. The exact age of the immatures observed was not known, however, so the precise course of events at fledging - with respect to acquisition of diving abilities - remains unclear. The observation that fledglings of double-crested cormorants and Cape cormorants *(P. capensis)* enter the swimming stage before they can fly very well (Palmer, 1962; Berry, 1976) and hence rely on diving to escape from danger, further emphasises the importance of a rapid development of the cardiorespiratory response to diving.

Few studies have investigated the developmental aspects of cardio-respiratory changes associated with diving. Castellini *et al.* (1994) in a study on northern elephant seal pups (*Mirounga angustirostris*) found evidence that pups are not born with the cardiac control associated with voluntary long duration apnoea (while sleeping on the beach), but that apnoea tolerance increases with refined cardiorespiratory control. They concluded that these pups would not be good divers before ~ 90 - 100 days old, which corresponds with the period pups spend at the beach before going to sea. Similarly, Thorson and Le Boeuf (1994) found that the 10-week period following weaning, during which juvenile northern elephant seals swim and dive near the natal rookery, is of critical importance for their survival at sea. During this developmental period the oxygen storage capacity of seals increases, while the ability to decrease their metabolic rate during diving improves, enabling seals to maximise underwater time.

Keijer *et al.* (1988) monitored heart rate in tufted ducklings during their first-ever and subsequent voluntary whole-body submergences. They found no evidence for a 'sensitive phase' during which cardiac control associated with diving is acquired or refined. Instead, cardiac control seemed to be fully developed at the time of first voluntary submergence, as the average cardiac response during these submergences closely resembled the response seen in adult tufted ducks. Rey (1971), on the other hand, reported that the bradycardia during forced submergence of domestic ducks differed qualitatively for different ages. Bradycardia was most rapid and pronounced in the youngest ducklings, declining over the next 5 months, until no further changes in the cardiac response to forced submergence were observed. Similarly, West (1981) found that newly hatched mallard ducklings (*Anas platyrhynchos*) display a profound bradycardia during forced

head submersion. Hence, the central neural pathways associated with the heart rate response to forced submergence are functional upon hatching. Although the youngest ducklings again showed the most profound bradycardia, this was clearly related to the pre-forced submergence heart rate. Since pre-forced submergence heart rates of ducklings declined over time (from 1 to 12 weeks after hatching), the initial heart rate drop upon submersion was strongest and most rapid in the youngest ducklings, while the lowest absolute heart rate values were accomplished by the oldest ducklings.

The purpose of this study was to investigate the ontogeny of the cardiac response to voluntary diving in double-crested cormorants, *i.e.* to establish whether the cardiac response during voluntary diving of double-crested cormorant chicks differs from that seen in adults. As pointed out in *Chapter 1*, by recording heart rates of chicks during their first ever and subsequent voluntary submergences it should be possible to determine whether the cardiac response during voluntary diving is in fact 'only' a modified 'forced submergence response'. It should be furthermore possible to establish whether habituation might be involved as a means to 'learn' the appropriate cardiac response to voluntary submergence. Finally, the cardiac responses during first ever and subsequent voluntary and forced submergences were compared.

Materials and methods

Birds

Nine double-crested cormorant chicks were collected from a breeding colony on Mandarte Island, B.C., in September 1995, when chicks were approximately 5 weeks of

age. Birds were housed in a sheltered outdoor pen with no access to water at the Animal Care Facilities of the University of British Columbia, Vancouver. Chicks were initially fed about 400 g of a mixed diet daily, which was reduced to about 10 percent of their body weight after a few weeks. Diet consisted of Pacific herring (*Clupea harengus*) and rainbow smelt (*Osmerus mordax*), supplemented with vitamin B1 tablets (Thiamine hydrochloride, Stanley Pharmaceuticals Ltd., North Vancouver, B.C.). After about 4 weeks in captivity the chicks 'fledged' (accompanied by marked behavioural changes) and the experimental trials started. At 'fledging', mean body mass of the chicks (2.33 ± 0.14 kg; range: 2.14 - 2.60 kg) had reached that of the adult birds (2.36 ± 0.17 kg, N = 9; see *Chapter 2*).

Instrumentation

A purpose built data logging system was used to record heart rate from the cormorant chicks (see *Chapter 2, Material and methods* and Andrews, 1998 for technical details and its application to the cormorants). The ECG electrodes (stripped sections of insulated stainless steel biomedical wire [Cooner Wire Co., Chatsworth, CA, USA]) were inserted subcutaneously, one placed 2 cm lateral to the posterior cervical region and one 4 cm lateral to the caudal end of the synsacrum, on opposite sides of the midline from one another. Adjacent to each insertion site a 2 cm section of the ECG lead was sealed and glued to the feathers with 5 minute epoxy. Before every experimental trial chicks were caught and fitted with the harness. The data logger's ECG electrode leads were connected to the implanted ECG leads and the sampling mode of the data logger was triggered (the

ECG was sampled at 100 Hz and the submergence sensor at 2 Hz). Every effort was made to minimise handling time before trials.

Experimental trials

All double-crested cormorant chicks used in this study had never been exposed to water before the 'first ever voluntary submergence' and the 'first ever forced submergence' trials. Water temperature during the trials ranged from 4 to 9 $^\circ$C.

Voluntary submergences

Six chicks (~ 10 weeks old) were used to investigate the cardiac responses displayed during their first ever voluntary head submergence and during their early dives. At the beginning of a trial the instrumented chick was kept inside an enclosure for about 10 min, before opening a door, which allowed access to the dive tank (9 m long, 3 m wide, 1 m deep). Most chicks would not dive during the first trials but only submerged their heads. Since these head submergences were not detected by the data logger's submergence sensor, cormorants were filmed by a video camera equipped with an internal clock (0.1 s resolution). Data logger and VCR time was synchronised before every trial. At the end of a trial, which generally lasted around 30 - 60 min, birds were recaptured to disconnect the ECG leads and remove the harness. To avoid the initiation of 'escape dives' when catching chicks (especially in the early trials), chicks were gently encouraged into the enclosure and the door was closed. After each chick had completed 5 trials, ECG electrodes were removed and all chicks were housed inside the fenced dive tank. Chicks

had to dive for their food daily and as they got accustomed to the tank, the surface of the tank was gradually covered, until only a small section (1m x 1.5m) at one end of the tank remained open. Birds would submerge here, swim underwater to the opposite end of the tank to pick up fish pieces and return to the opening to swallow their prey. After about two weeks, five of the chicks were reinstrumented to monitor heart rate during the longer dives which the chicks were then performing. Dive duration during these dives (chicks were ~16 - 17 weeks old) was typically between 10 and 20 s.

Forced submergences

To compare the cardiac response to voluntary diving with the response to forced submergence, a separate group of three chicks (~11 weeks old) was used in forced submergence experiments only. Chicks were held by an investigator and submerged to a depth of about 30 cm inside the diving tank. Submergence times of 3 and 10 s were alternated for a total of 12 submergences, followed by a final forced submergence of 30 s. Between submergences chicks were given a one minute period to recover.

Resting heart rate

Resting heart rate was recorded from all of the chicks at ~12 weeks of age. Birds were equipped with ECG leads and the data logger 'backpack' as described above, but then they were returned to their holding pens. Birds would perch immediately after release and return to their routine shortly after the investigator left. Heart rate was recorded during these trials and the birds were either observed from a blind or filmed by a videocamera.

Trials lasted for approximately one hour. All trials were done during daylight hours with postabsorptive birds that were awake and perched in the upright position.

Data analysis and statistics

Submergence and emergence times were determined from the record of the data logger's submergence sensor. For the short head submergences, submergence periods were determined by visual analysis of the recorded video tapes. Cardiac interbeat intervals were derived from the ECG trace after identifying the QRS peaks by eye. The mean interbeat interval for each period of interest (*e.g.* an individual dive or forced submergence) was then converted to heart rate in beats per minute (beats·min^{-1}).

Data were grouped into the following categories: (1) 'first' voluntary head submergence, (2) the 'six' following voluntary head submergences, (3) the 'first' voluntary dive, (4) the 'six' following voluntary dives, (5) the 'later' (longer) voluntary dives, (6) the 'first' forced submergence, and (7) the 'six' following forced submergences. The 'first' forced submergence lasted 3 s, while the 'six' following forced submergences were matched pairs of 3 and 10 s submergences.

As chicks in their early voluntary submergence trials apparently had to 'learn' how to submerge, a dive was defined as a complete head submergence, accompanied by forward propulsion. To allow comparison between the different submergence categories, heart rates before submergence, during submergence, and after surfacing were averaged over 1-, 2- or 3-s-intervals. Mean values were calculated for all chicks for the different submergence categories. For each submergence category a grand mean was calculated

from the means of the individual birds. Heart rates during 'later' (longer) dives were compared with heart rates obtained from eight adult double-crested cormorants performing dives in the same dive tank (6 dives were analysed for each individual; see *Chapter 2*), lasting between 18 and 22 s (mean: 20.1 ± 0.8 s).

To calculate resting heart rates, instantaneous heart rate over the entire trial period was plotted against time. Heart rate was elevated due to handling at the beginning of the trial but fell to a baseline value within 10 min in all birds. After heart rate had stabilised, a section of 20 min was chosen for the calculation of a single value, representing the resting heart rate. A mean value was calculated from all interbeat intervals during that period and converted into beats·min^{-1}.

Heart rates during the different submergence categories were compared using one-way repeated-measures analysis of variance (ANOVA) with Student-Newman-Keuls pairwise multiple comparisons. When single comparisons were made, as in comparing values obtained from the chicks with values from the adult birds, Student's t-test was used. A significant difference was accepted at the level of $P < 0.05$. All mean values are presented with standard deviation (± S.D.).

Results

Behaviour during first dive trials

When first placed on water, none of the chicks started to dive spontaneously. Instead they floated or swam on the surface, often engaged in hygienic activities ('bathing'). During 'bathing' chicks submerged their heads briefly while vigorously flapping their

wings. Brief head submergences and 'dives' in a more exploratory context and not accompanied by hygienic activities occurred gradually during the first two sessions in all chicks. However, during these initial 'dives' chicks submerged their heads while swimming towards a submersed feeding platform without gaining depth. Chicks appeared to be unable to overcome buoyancy during these initial 'dives'. Gradually they seemed to adjust their buoyancy so they could reach the herring pieces at 0.5 - 1 m depth. By the end of the initial experimental phase (5 trials per chick) the dive behaviour of the chicks was indistinguishable from that of the adults.

Resting heart rates, voluntary head submergences and dives

The grand mean for resting heart rate (143.2 ± 24.6 beats·min^{-1}; N = 9 chicks) was not different from the resting heart rate of adult cormorants (137.9 ± 17.5 beats·min^{-1}; N = 5 adults). Resting heart rate was less than half the rate recorded from chicks just before the start of voluntary head submergence or diving (Table 3.1). As can be seen from Table 3.1, for all five categories of voluntary head submergences and dives, heart rates during submergence were significantly different from heart rates before submergence and after surfacing. Heart rates (before submergence, during submergence and after surfacing), however, were not significantly different between the different voluntary submergence categories. In all categories of voluntary head submergence/dive, heart rate fell immediately on submergence by 60 - 100 beats·min^{-1}. The similarity in the immediate (first second) heart rate response in the first ever dive (mean dive duration: 2.3 ± 1.6 s)

compared with that in the much longer (mean dive duration: 14.0 ± 3.0 s), later dives was particularly striking (Table 3.1).

When making their first head submergence, 3 of the 5 chicks showed a noticeable decline in heart rate (Fig. 3.1a), while in the other 2, this was not the case (Fig. 3.1b). The major difference between these two types of response was pre-submersion heart rate. When pre-submersion heart rate was high, the decline on submersion was accentuated. A similar picture holds for first ever dives (Fig. 3.1c and 3.1d). In 2 of the 6 chicks pre-dive heart rate was in the range of 200 - 250 beats·min^{-1} and the decline associated with submersion, although present, was not particularly obvious (Fig. 3.1d). In the next six dives, the variability in pre-dive heart rate was reduced (Table 3.1) and all animals showed a pronounced immediate decline in heart rate on submersion.

When tested later, after having learnt to dive for food, heart rate was high in the pre-dive period and declined immediately on submersion to about 220 - 240 beats·min^{-1} (Fig. 3.1e and 3.1f). Heart rate levelled off and remained stable at around 200 beats·min^{-1} throughout these longer dives, before increasing in the period just before surfacing. Voluntary dives of 4 chicks (later, longer dives) were compared with dives of 8 adult cormorants (Fig. 3.2). Pre-dive heart rate was significantly lower (333.9 ± 31.2 beats·min^{-1}) in chicks than adults (380.6 ± 12.6 beats·min^{-1}). Over the first 3 s of submergence, mean heart rate in chicks and adults was nearly identical (Fig. 3.2). Heart rate declined very slowly in chicks but much more rapidly in adults and, after 9 s into the dive, heart rate in adults was 50 beats·min^{-1} below that in chicks. This difference was significant as was the difference in mean dive heart rate (chicks: 212.4 ± 16.9 beats·min^{-1};

adults: 163.2 ± 14.0 beats·min^{-1}) and post-dive heart rate (chicks: 354.9 ± 27.3 beats·min^{-1}; adults: 397.2 ± 19.6 beats·min^{-1}).

<u>Forced submergence</u>

Heart rate was extremely low (134.9 ± 53.7 beats·min^{-1}) before the first ever forced submergence and an immediate decline in heart rate was not obvious (Table 3.1, Fig. 3.3a and 3.3b). Mean heart rate during 3 s of forced submergence (112.5 ± 66.2 beats·min^{-1}) was not significantly different from the pre-submergence value. Pre-forced submergence heart rate increased with repetitive submergences (172.5 ± 79.5 beats·min^{-1}) and a significant fall in heart rate (to 134.5 ± 47.7 beats·min^{-1}) occurred upon submersion (Table 3.1, Fig. 3.3c). Chicks displayed sinus arrhythmia which was especially prominent in one chick, and the forced submergence heart rate profile was established often before the actual act of submersion (Fig. 3.3d). The last forced submergence in each bird was extended to 30 s and in all chicks, heart rate was between 50 - 100 beats·min^{-1} during the last few seconds of submergence (Fig. 3.3e).

Discussion

<u>Voluntary submergence</u>

In adult double-crested cormorants, diving in shallow water, heart rate increased prior to submersion to about three times the resting heart rate (137.8 ± 17.4 beats·min^{-1}; see *Chapter 2*) and halved on submersion. After this initial decline, heart rate fell much more slowly, reaching 150 beats·min^{-1} after 9 s of submergence. Although the cardiac responses

to voluntary submergence were variable, in the majority of chicks initial heart rates during first ever head submergence and first ever voluntary, shallow dives were similar to the adult birds. For the cormorant chicks, which were ~10 weeks old, this was their first exposure to water. Hence, these results do not support the hypothesis that the cardiac response to voluntary diving is 'only' a modified 'forced submergence response'. The present data do not suggest that cormorants 'learn' the appropriate cardiac response to voluntary diving via habituation or conditioning of the 'classical dive response'. In both cormorant chicks and adults the initial cardiac response to submersion was moderate and heart rate established early in the dive (first 3 s) was around 225 beats·min^{-1} (Table 3.1 and Fig. 3.2). Much of the variability in the cardiac responses of cormorant chicks was due to the heart rate immediately before diving. If pre-dive heart rate was low, then the initial cardiac response to diving was not obvious. In fact, similar arguments can be advanced to explain much of the variability in initial cardiac responses to diving in tufted ducklings (Keijer et al. 1988).

Similar to the findings of Keijer et al. (1988), the observed response in double-crested cormorant chicks does not support the hypothesis that habituation is involved in the development of the initial cardiac adjustments to voluntary diving. The present results suggest that these adjustments are reflex in nature and this reflex is fully developed by the first submergence event. This does not, however, deny the potential for modification of the cardiac response during the dive by components of the CNS above the reflex level (e.g. anticipatory tachycardia before surfacing, see Elsner et al. 1989). Furthermore, as pointed out in Chapter 1, conditioning could not be investigated with the methodology

used in the present study. Hence, the hypothesis that the initial cardiac response to voluntary diving may be conditioned is still valid.

The nature of the reflex pathway initiating the decline in heart rate in cormorants during the first few seconds of the dive is unknown. This decline is present from the first head submergence or dive, although only markedly when pre-dive heart rate is at least double the resting rate. An initial rapid decline in heart rate is seen in many birds, in both voluntary dives and forced submergence, when the pre-submergence heart rate is markedly elevated above resting (Jones and Butler, 1982; Jones *et al.* 1982; Kanwisher *et al.* 1981; Woakes and Butler, 1983). Although nasal or other upper respiratory tract receptors are believed to initiate the cardiac responses in diving birds and mammals, Furilla and Jones (1986) showed that anaesthetisation of the narial region has only minor effects on the initial cardiac retardation during escape dives of redhead ducks. Since cormorants lack external nares (nares are open slits in hatching double-crested cormorants but close abruptly after 29 - 30 days, due to the ingrowth of a horny sheath; Palmer, 1962; Berry, 1976), stimulation of nasal receptors with water is not likely the cause of the initial heart rate decline.

The importance of cessation of respiration (apnoea) to the initiation of cardiac events in diving has been seen in Humboldt penguins (Butler and Woakes, 1984) as well as in some cormorant chicks of the present study. In Humboldt penguins there is often a prominent sinus arrhythmia when the bird is on the surface, which terminates at the heart rate during expiration on head submersion (it is not known however, if Humboldt penguins dive on expiration; Butler and Woakes (1984) suggest diving on inspiration). A similar picture was seen in some of the whole body forced submergences of the

cormorant chicks. In animals displaying prominent sinus arrhythmia (Fig. 3.3d), the submergence heart rate was established before submersion at the pre-submergence exhalation. Volitional control of breathing in birds is undisputed. Claims of volitional control of heart rate *per se*, on the other hand, are a red herring. The close linkage between breathing and heart rate provides a plausible mechanism for the initial cardiac adjustments to diving. Whether double-crested cormorants, diving voluntarily, dive on inspiration or expiration remains to be established however.

In adult cormorants, after the initial cardiac adjustment to submergence, heart rate continued to decline throughout shallow dives of up to 22 s in duration (see *Chapter 2*). In contrast, in adult diving ducks the initial cardiac response is typically the lowest heart rate of the dive. Heart rate then increases to a more or less stable level before rising markedly prior to surfacing (Butler and Woakes, 1979). The cardiac response observed during the later (longer) dives of double-crested cormorant chicks more closely resembled the response of adult diving ducks than adult cormorants. In these dives, heart rate was maintained more or less at the initial level before rising in anticipation of surfacing. In *Chapter 2* I argued that the secondary decline in heart rate, observed during shallow diving in adult cormorants, is facilitated by intravascular chemoreceptors. Chemoreceptor driven declines in heart rate can be habituated in forcibly submerged diving and dabbling ducks (Gabbott and Jones, 1985; Gabbott and Jones, 1987). This is, however, unlikely to be the explanation for the stable diving heart rate during the later (longer) dives of cormorant chicks, since habituation was not detectable in adult birds with a much longer 'dive history'. Furthermore, the possibility that chemoreceptors are not fully developed in the chicks seems improbable, given the strong decline in heart rate during the longer

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forced submergence trials (30 s, Fig. 3.3e), which most likely is facilitated by chemoreceptors (Jones and Larigakis, 1988). On the other hand, adult cormorants making deep dives show a similar cardiac response as chicks in their later (longer) shallow dives (see *Chapter 2*). As the depth of submergence increases, so will the arterial oxygen tension (at least initially), unloading the arterial chemoreceptors. Consequently, in the absence of habituation, one could speculate that stable heart rates in diving chicks are a consequence of higher arterial oxygen tensions throughout the dive. Given the present evidence, it is difficult to conceive how this might be accomplished. Another possibility that cannot be ruled out is that animals 'learn' to further decrease their heart rate during the dive by conditioning (*e.g.* animals might learn that a further decline in heart rate makes the breath-hold easier or shortens the recovery period). In this context it would have been interesting to record heart rates from the chicks during deep diving and, at a later stage (as immatures or young adults), again during shallow diving.

As pointed out in *Chapter 1*, precocial tufted ducklings and altricial double-crested cormorant chicks follow distinctly different developmental patterns. The results of the present study suggest that despite these marked differences, the cardiorespiratory system of both species is ready to support their diving existence, once they venture onto water. Although this might seem surprising at first, it fits into the picture, if in fact the cardiorespiratory adjustments of birds during diving are under reflexogenic control (Butler and Jones, 1997). In this case, cormorants do not need environmental cues to complete their cardiac response but can rely on a reflexogenic system that matured during the prolonged period on land. However, recalling the observed physical difficulties cormorant chicks faced when they first tried to dive, one can imagine that chicks in the

wild will pass through a phase of intense learning (adjusting buoyancy, locomotion, etc.) when they first enter the water. Morrison *et al.* (1978) suggested that immature olivaceous cormorants acquire the diving abilities of adults at, or soon after, fledging. This suggestion was based on their finding that dive patterns (dive duration, recovery period, dive/pause ratio) were nearly identical between immature and adult birds. It is unquestionable that overall dive patterns of birds observed in the wild and in a semi-laboratory setting will differ. However, in support of Morrison *et al.* (1978), double-crested cormorant chicks in this study, after free access to the dive tank for a few days, displayed dive patterns very similar to the adults. Acquisition and perfection of techniques related to prey recognition, capture and handling, on the other hand, might require more time.

Forced submergence

The low heart rates before the first ever forced submergence are largely accounted for by the strong sinus arrhythmia displayed by the chicks. Over the following submergences sinus arrhythmia gradually disappeared and heart rate was kept at an elevated level before submergence. The cardiac response of chicks during early forced submergence, lasting up to 10 s, was comparable to the forced submergence response of immature double-crested cormorants (~5 months old) which had breathed 100 % oxygen before forced submergence (Jones and Larigakis, 1988). Heart rate decline upon forced submergence was less dramatic in these immature cormorants after breathing oxygen before submergence, when compared to breathing air beforehand. Heart rate decreased from

~190 beats·min^{-1} to ~130 beats·min^{-1} within the first 5 s of submergence. Again, it is tempting to argue that chemoreceptor reflex sensitivity may have not been fully matured in the chicks. On the other hand, forcibly submerging chicks for 30 s (Fig. 3.3e) caused heart rate to fall within the same range (~50 beats·min^{-1}) as in forcibly submerged adults (Mangalam and Jones, 1988), suggesting a well developed chemoreceptor mechanism. Sinus arrhythmia, as observed during the forced submergence trials, was less obvious in voluntarily submerging chicks. Heart rate before voluntary submergence was double the rate before forced submergence and never fell below ~190 beats·min^{-1} during submergence (including the later, longer dives). Hence, cardiac responses of double-crested cormorants to forced submergence are more extreme than responses during voluntary submergence, as shown previously (Kanwisher *et al.* 1981). The present results show that this difference is apparent in chicks from their very first exposure to water, although much of the difference might be dependent on pre-submergence heart rate, as has been shown for diving ducks (Furilla and Jones, 1987).

Table 3.1 Dive duration and heart rates of double-crested cormorant chicks associated with initial and later voluntary and forced submergence

		Voluntary submergence					Forced submergence	
		First ever head submergence	Next 6 head submergences	First ever dive	Next 6 dives	6 later (longer) dives	First ever forced submergence	Next 6 forced submergences
Duration (s)		0.7 ± 0.2	0.8 ± 0.1	2.3 ± 1.6	2.5 ± 2.4	14.0 ± 3.0	3.5 ± 0.5	7.5 ± 0.2
Heart rate (beats·min⁻¹)								
Before submergence	-3 to 0 s	327.2 ± 56.5	346.5 ± 43.0	338.7 ± 85.0	340.9 ± 26.4	333.9 ± 31.2	134.9 ± 53.7†	172.5 ± 79.5†
	-1 to 0 s	328.6 ± 56.1	348.7 ± 39.0	341.1 ± 79.5	350.3 ± 26.8	345.3 ± 27.5	150.4 ± 39.3†	171.1 ± 63.4†
	0 to 1 s	232.5 ± 42.9*	285.9 ± 41.8*	249.2 ± 44.9*	240.1 ± 18.4*	243.3 ± 17.2*	116.6 ± 4.8†	134.5 ± 47.7*,†
	0 to 2 s			209.0 ± 34.0*	247.3 ± 27.1*	234.3 ± 12.8*	107.9 ± 44.9	119.7 ± 66.7*
During submergence	0 to 3 s				224.2 ± 0.6*	222.6 ± 8.3*	143.8 ± 51.2	120.3 ± 64.4*
	3 to 6 s					205.4 ± 14.4*		
	6 to 9 s					203.4 ± 13.7*		
	mean	233.7 ± 40.4*	273.8 ± 39.5*	247.3 ± 45.6*	241.0 ± 20.4*	212.4 ± 16.9*	112.5 ± 66.2†	115.0 ± 60.1*,†
After surfacing	0 to 1 s	318.8 ± 77.8	354.4 ± 31.4	366.9 ± 46.5	349.8 ± 16.5	350.6 ± 24.0	175.1 ± 32.4†	178.3 ± 52.9†
	0 to 3 s	346.9 ± 54.2	358.8 ± 26.0	355.8 ± 50.4	350.2 ± 19.2	354.9 ± 27.3	198.7 ± 25.9†	210.3 ± 56.6†

Values are presented as mean ± S.D.
'Mean' refers to the mean submergence heart rate, which was derived from the mean interbeat interval during submergence.
The 'first ever forced submergence' was 3 s in duration, while the 'next 6 forced submergences' were matched pairs of 3 and 10 s submergences. Heart rates during the first 3 s only were included in analysis.
Number of double-crested cormorant chicks contributing to the mean values is 6 for the 'first ever dive', 4 for the 'later (longer) dives', and 5 for all other voluntary submergence categories. 3 animals contributed to the forced submergence categories.
*Significantly different from 'before submergence (-3 to 0 s)' and 'after surfacing (0 to 3 s)' values.
†Significant difference between forced submergence and voluntary submergence values.
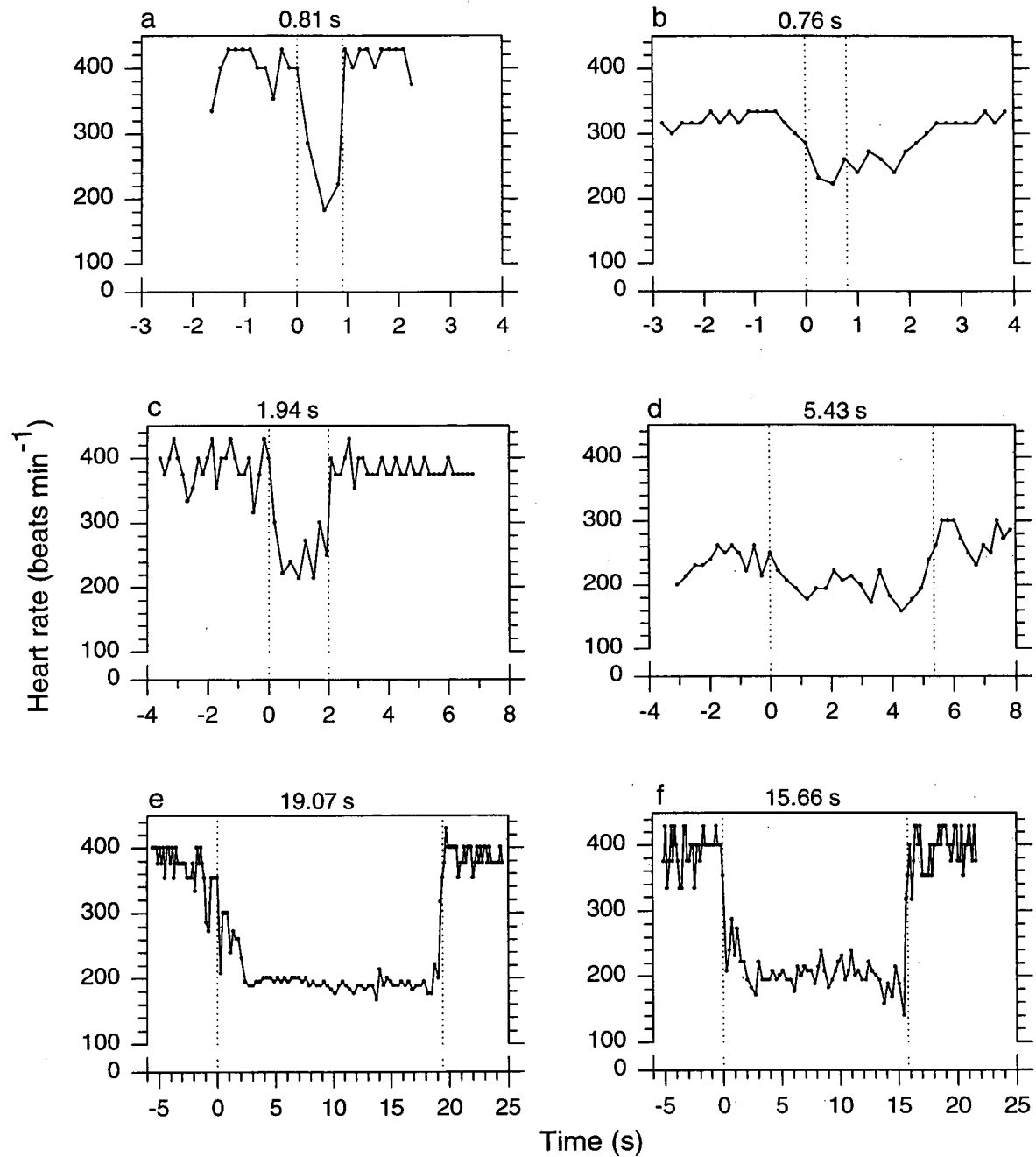
Fig. 3.1 Cardiac responses of double-crested cormorant chicks to first ever voluntary head submergence (3.1a and 3.1b), first ever voluntary dive (3.1c and 3.1d), and later (longer) voluntary dives (3.1e and 3.1f). Figures are instantaneous heart rate records of individual chicks. Submergence periods are indicated by the dashed lines, with the negative time values referring to the pre-submergence period and zero indicating submersion. Submergence duration is shown on top of figures a-f.
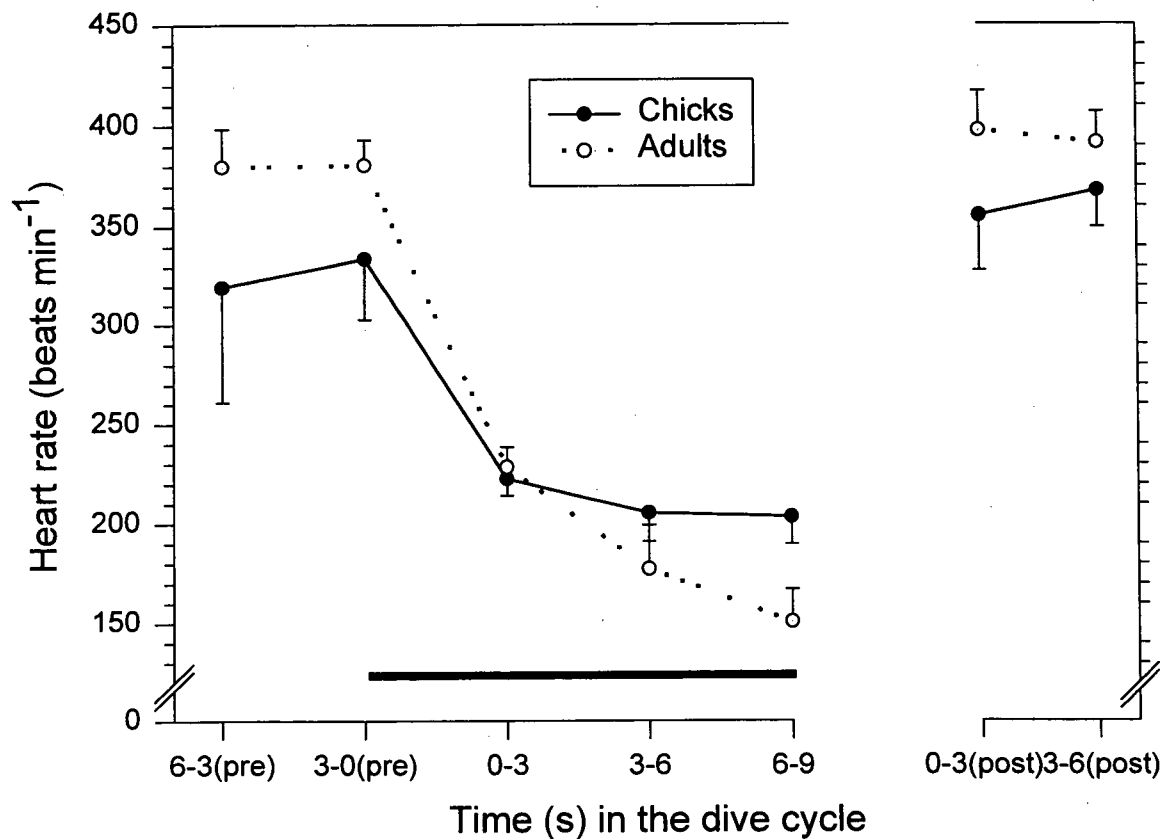
Fig. 3.2 Heart rates (beats·min^{-1}) associated with the first 9 s of voluntary shallow diving in double-crested cormorant chicks (N = 4) and adults (N = 8). Values are means ± S.D., averaged over 3-s-intervals (6 dives were analysed per individual). Mean dive duration for chicks and adults was 14.0 ± 3.0 and 20.1 ± 0.8 s, respectively. Dive period is indicated by the solid bottom line. 'Pre' and 'post' refer to the pre- and post-dive period, respectively.
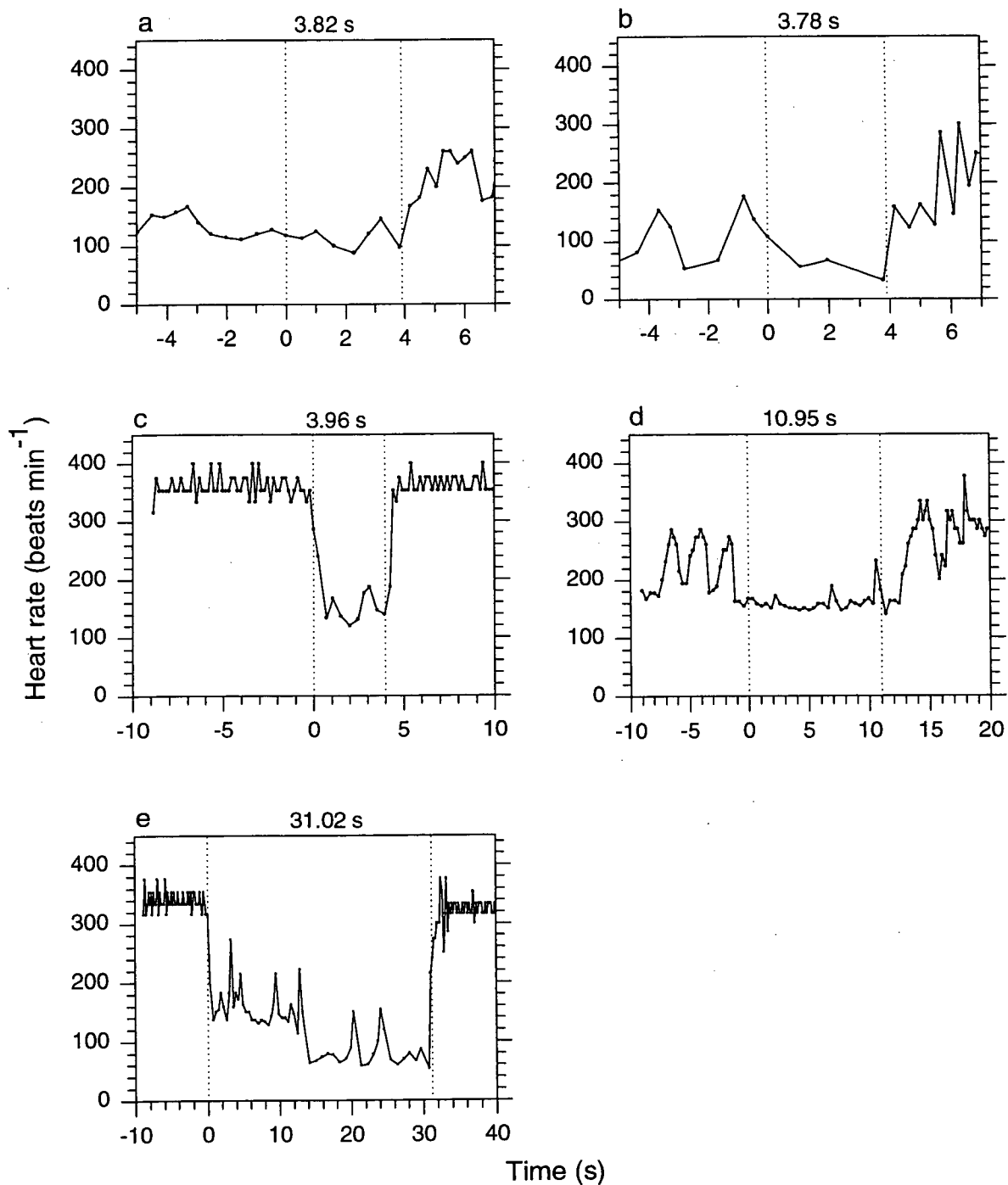
Fig. 3.3 Cardiac responses of double-crested cormorant chicks to forced submergence. Figures are instantaneous heart rate records of individual chicks during their first ever forced submergence (3.3a and 3.3b), during forced submergence after being subjected to repetitive forced submergences (3.3c and 3.3d) and during one 'extended' forced submergence (3.3e). Submergence periods are indicated by the dashed lines, with the negative time values referring to the pre-submergence period and zero indicating submersion. Submergence duration is shown on top of figures a-e.

Chapter 4: Conclusion

This thesis shows that double-crested cormorants, like many other diving vertebrates, undergo marked cardiac changes during voluntary diving. In preparation for a dive and in response to a terminated dive, heart rates are elevated to about three times the resting value, presumably facilitating the quick loading of O_2 and unloading of CO_2. At the onset of dives lasting between 18 and 22 s, heart rate drops immediately, followed by a secondary decline after about 5 - 10 s (shallow diving) or a relatively stable (or much slower declining) heart rate during the rest of the dive (deep diving). Heart rate increases towards the end of the dive, reaching pre-dive levels upon surfacing. Experiments in which birds were exposed to different breathing gases before diving (to manipulate Pa_{O_2} and Pa_{CO_2}) suggest that intravascular chemoreceptors (most importantly sensing Pa_{O_2}) play an important role in the development of the cardiac response during voluntary diving. Hence the difference in the cardiac responses to voluntary diving in the shallow and deep dive situation can be explained by the different degree of chemoreceptor stimulation in both situations. Birds diving to depth will experience a compression hyperoxia during descent and the elevation in Pa_{O_2} will delay any chemoreceptor mediated reinforcement of the initial heart rate drop, so that the reduction in heart rate during these deep dives will be less pronounced. Shallow diving birds, in contrast, will not experience a compression hyperoxia, hence Pa_{O_2} will decrease early in the dive, accelerating the chemoreceptor mediated cardiac inhibition. Beside the effects on cardiac

performance, blood gases play an important role in controlling the dive behaviour of double-crested cormorants. While dive duration did not vary with the different breathing gases administered (most likely due to the limitations of the experimental setup), post-dive surface interval duration did, resulting in the most efficient dive pattern (highest dive/pause ratio) after hyperoxic exposure. In conclusion, my results suggest that blood gas levels (O_2 and CO_2) are important in the overall cardiac and behavioural responses of double-crested cormorants associated with voluntary diving.

The cause of the initial heart rate drop upon submersion in double-crested cormorants remains unclear. Since cormorants lack external nares, stimulation of nasal receptors with water is not likely to be the cause. The finding of this thesis that initial heart rates during first ever head submergence and first ever voluntary, shallow dives of double-crested cormorant chicks were similar to the adult birds does not support the hypothesis that cormorants 'learn' the appropriate cardiac response to voluntary diving via habituation or conditioning of the 'classical dive response'. Hence, the cardiac response to voluntary diving is not just a modified forced submergence response. It was furthermore shown that habituation in general is not involved in the development of the initial cardiac adjustments to voluntary diving, while conditioning (apart from conditioning of the 'classical dive response') could not be investigated with the methodology used in the study. Hence, I suggest that these adjustments are reflex in nature and this reflex is fully developed by the first submergence event. Although the nature of this reflex pathway is obscure, cessation of breathing before submersion and the close linkage between breathing and heart rate might provide a plausible mechanism.

Relevance to freely diving double-crested cormorants

The significance of chemoreceptors to the cardiac responses of double-crested cormorants diving in the wild remains unknown. Dive depths and durations observed in the wild suggest that the birds might employ a mixture of the two dive modes investigated in this study (shallow and deep diving). Many dives in the wild might be to less than 12 m. It is unlikely, however, that birds in the wild will perform extended horizontal dives in close proximity to the surface, as they did in the shallow dive tank. Dive durations of birds in this study were similar to durations reported from double-crested cormorants foraging in the wild. Although the crucial role of chemoreceptors as modulators of the cardiovascular responses of cormorants might be masked during relatively short deep dives (20 - 30 s), it will become obvious during extended dives. The decrease in heart rate during diving observed in this study should reflect a redistribution of blood flow. Blood is preferentially sent to tissues that cannot survive without oxygen (*e.g.* heart and brain). The reduced perfusion of other tissues and organs (*e.g.* splanchnic and peripheral vascular beds) will lead to a suppression of the aerobic metabolism and will facilitate the economic utilisation of limited oxygen stores during diving, hence prolonging underwater foraging time. There are many situations when extending dives might be crucial to cormorants. Ydenberg (1988) suggested that predators feeding on mobile prey items might 'work hard' once they encounter prey (*e.g.* a fish school). Under these circumstances it might be sensible to prolong dive duration and minimise the post-dive recovery period, so as to catch as many prey items as possible, before prey contact is lost. Double-crested cormorants are opportunistic foragers (Robertson, 1974) and will take pelagic prey if encountered. They furthermore swallow prey items under water, if

small enough (pers. observation). Hence, the scenario for pursuit divers, suggested by Ydenberg (1988), might be valid for double-crested cormorants. If, on the other hand, double-crested cormorants forage predominantly on benthic fish, then the same time constraints on prey availability that apply for pelagic foragers may not operate, since the fish are less mobile. Monaghan (1996) found that European shags responded to a decrease in food availability by increasing the number of dives performed per foraging trip, increasing dive duration and dive depth. The strongest response observed in these bottom foragers, however, was a decrease in dive/pause ratio, due to an increase of the post-dive recovery period. It was suggested that shags worked much harder during these dives to search for prey at the bottom, again stressing the importance of the economic utilisation of the limited oxygen stores via cardiovascular mechanisms. Walton *et al.* (1998) suggested that it would be more important for bottom foragers to maximise the portion of the dive cycle (dive time plus surface time) spent at the site of resource gain (*i.e.* the bottom) than to maximise the overall dive/pause ratio. One possible strategy would be to keep travel time (to and from the bottom) and recovery time (at the surface) low, hence dive shallow and within the aerobic limits. Burger (1991), on the other hand, suggested that even prolonged, and hence anaerobic dives may increase overall foraging efficiency for seabirds foraging on prey at considerable depth, by reducing the proportion of time spent travelling underwater. The ability to make long and/or repetitive dives will also be advantageous when trying to escape predators.

Double-crested cormorants are easily disturbed when approached (see Kanwisher *et al.* 1981; Grémillet and Wilson, 1998). Hence, reports on dive duration and dive depth were achieved by opportunistic observation of birds diving close to shore or in fishponds and

might therefore be biased. Double-crested cormorants typically forage alone (although some social foraging has been described; Bartholomew, 1942) and individual birds might have different foraging preferences. Grémillet et al. (1998) found that individual great cormorants and European shags had very flexible feeding habits that allowed them to use both pelagic and benthic resources. Until instruments that allow some direct insight into the foraging patterns of double-crested cormorants in the wild are deployed, the overall significance of cardiac responses during submergence to their daily foraging behaviour remains elusive.

However, the present study clearly suggests that these cardiac responses are one important physiological strategy that allows double-crested cormorants to maximise their underwater foraging time. Other strategies that would prolong underwater foraging time have been suggested and are related to a decrease in overall metabolism. Wilson and Wilson (1995) suggested that cormorants might have developed a strategy that leads them to conduct deeper, and thus longer, dives at the beginning of a foraging trip, when upthrust is still high, and gradually reduce dive depth and duration over time, as buoyancy decreases (due to water penetration into the plumage and ingested prey). In doing so, birds might minimise the energetic costs of swimming caused by positive buoyancy. Grémillet et al. (1998), however, found no evidence for such a strategy when investigating foraging techniques of Great cormorants and European shags. Bevan et al. (1997) reported a progressive reduction in abdominal temperature of South-Georgian shags within dive bouts. Similarly, variability in core temperature (derived from thoracic and abdominal temperatures) and abdominal cooling has been reported from freely diving king penguins by Culik et al. (1996) and Handrich et al. (1997), respectively. This

temperature decrease in certain tissues is another consequence of the preferential redistribution of blood flow during diving. If the reported temperature declines reflect temperature changes in other tissues as well, then overall metabolic rate might be reduced during diving. Hypometabolism during diving is certainly one possible mechanism that would facilitate the maximisation of underwater foraging time. In this context, I think it would be most rewarding to employ instruments on freely diving double-crested cormorants in order to investigate their natural foraging patterns and the facilitating physiological strategies.

My thesis shows that double-crested cormorant chicks, like tufted ducklings, seem to have a fully developed cardiac response to diving once they venture onto water. Given the distinctly different developmental patterns of both species, this is somewhat surprising. It is unlike the situation in northern elephant seal pups, which go through a period of cardiorespiratory maturation that lasts as much as 3 months after weaning. During this time the seals spend more and more time in the water near the natal rookery, eventually performing long dives and catching prey once they are able to tolerate long duration apnoea. However, while elephant seal pups can 'afford' such a prolonged phase of maturation while fasting because of their large blubber stores, cormorant chicks cannot rely on such a strategy but must quickly start foraging successfully upon fledging. In addition, the fact that double-crested cormorant fledglings enter the swimming stage before they can fly very well, and hence rely on diving to escape from danger, emphasises the importance of a mature cardiorespiratory response to diving at the start of their diving 'career'.

In conclusion, the present thesis suggests that cardiac and behavioural adjustments are important components of a strategy that allows double-crested cormorants to maximise the time spent underwater and hence, potential foraging time. While it is unclear what causes the initial cardiac response to diving, it is present from the very first submersion event (chicks). The initial bradycardic response appears to be maintained or even augmented by intravascular chemoreceptors, which permits cormorants to perform extended dives.

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