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Corticosterone responses to captivity and sampling  
stress in the mallard (*Anas platyrhynchos*) and  
grey duck (*Anas superciliosa*)

A thesis presented in partial fulfilment of the requirements for the  
degree of

Masters of Science  
in Physiology  
at Massey University

Mark Forman  
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Abstract

1. The aim of this study was to investigate the influence of capture and captivity stress on plasma corticosterone levels and breeding success in mallard and grey ducks. Measurements of plasma corticosterone levels were used to identify factors that cause stress and to identify individual birds with low stress responses and a greater likelihood of a successful breeding in captivity than their peers. The effect on corticosterone levels of the stress associated with the collection of blood samples was investigated and different sampling regimes for measuring corticosterone responses to stress were examined. The use of exogenous glucose administration and the placing of ducks into darkened boxes to lower corticosterone levels was also studied.
2. Corticosterone levels in wild mallards after capture were higher than levels in ducks held captive for 3 or 5 months. Corticosterone levels decreased in captive ducks in relation to the time spent in captivity and amount of contact spent with people.
3. Corticosterone levels repeatedly measured over 8 months varied between individual captive grey duck. The only 2 female ducks to rear ducklings, and their mates, all had lower corticosterone levels before the breeding season than the remaining 4 female and 3 male ducks.
4. The variation in corticosterone levels and responses between individual grey duck and the negative relationship between corticosterone levels and body weight may have been due, in part, to the existence of a dominance hierarchy amongst the grey ducks.
5. Corticosterone levels in winter may indicate potential breeding success and, can be used to identify stressful factors in the captive environment.
6. The observation of an increase in corticosterone levels with the sampling and handling of ducks depends on the magnitude of levels in the first sample obtained and on the frequency with which samples are obtained thereafter.
7. High corticosterone levels will decrease if a duck is placed in a darkened individual box or given an oral dose of 10 ml of 0.84M or 1.38M

glucose.

8. It is concluded that the measurement of corticosterone levels can be used to indicate factors that may affect the breeding performance of birds. Methods for minimising the stress associated with the capture and captivity of wild birds can also be identified from the measurement of corticosterone levels.

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## Chapter 1:

### General introduction

## 1. General introduction

Captive breeding of some native New Zealand birds occurs for conservation purposes. The removal of birds from predation should help to increase breeding success while guarantee a readily obtainable food supply. However despite the placement of birds in a captive environment which, as based on habitat studies, mimic natural habitats the birds may not breed at all or even die. In such situations the birds are unable to adapt to captivity and are said to be under stress.

In this study the stress associated with capture and captivity was measured in wild mallard ducks, with plasma levels of the adrenal gland hormone corticosterone used as a measure of stress. Ways to reduce capture and captivity stress were examined. The variation between individual mallards in their corticosterone responses to capture and captivity was noted and further investigated amongst a group of native New Zealand Grey duck over 8 months and discussed in conjunction with their breeding performance during this time.

Grey ducks were used in this study as they provided a species of waterfowl known to be reluctant breeders in captivity (Marchant and Higgins, 1990) and hence more likely to be influenced by any stress associated with captivity than their Mallard counterparts. Mallards were used for studies of, and ways to relieve, stress associated with capture and captivity as they provided a species of waterfowl abundant around the area of study and hence readily obtainable by trapping.

### 1.1 Stimuli, stressors, stress and distress

Before discussing stress associated with the capture and captivity of wild birds it is important to define the context in which the terms stress, distress and stimuli that are stressful (stressors) are used. Firstly, stimuli that elicit physiological responses that may include a rise in corticosterone levels are discussed. Next the variation in the range of physiological responses that various authors use to indicate stress, and hence stressors and distress, is

discussed. Finally the context in which stress is used in this study is explained.

Numerous stimuli, both artificial and natural, such as temperature extremes, starvation, disease organisms, electric shock, handling, repeated sampling, transportation, chasing and loud noises have been found to increase corticosterone levels in birds (reviewed by Mench, in press). Such stimuli have also been found to induce increases in the catecholamines, adrenaline and noradrenaline, released from the adrenal gland (reviewed by Harvey *et. al.*, 1984). Other responses exhibited by birds towards the above stimuli may be stimulus specific such as neurally mediated skin vasodilation in hot temperatures and withdrawal behaviour from the source of an electric shock (Siegel, 1971). Corticosterone, catecholamine and stimulus-specific responses are said to occur when the bird has interpreted the stimuli as threatening, or perceiving to threaten, its homeostasis or well-being.

In order to encompass the corticosterone, catecholamine and stimulus-specific responses of an animal exposed to stimuli affecting (or perceived to affect) its homeostasis Selye (1936) used the term General Adaptation Syndrome (G.A.S.). The G.A.S. consists of three distinct sequential stages or reactions; the alarm reaction, the stage of resistance and finally the stage of exhaustion. Siegel (1980), cited Selye (1963) in further stating that the stimuli inducing such responses as outlined in the G.A.S. are stressors and stress is the general term used to describe the animal's defensive response to the stressor.

The alarm reaction involves responses such as catecholamine mediated vasodilation and stimulus specific behaviour such as ruffling of feathers to increase cooling in response to hot temperatures. The stage of resistance involves the release of corticosteroids while the exhaustion stage occurs when stimuli such as disease organisms cannot be either avoided or adapted to by corticosterone, catecholamine or stimulus-specific responses.

The problem with defining stress in terms of the G.A.S. is the presence of situations where only the alarm reaction occurs (Freeman, 1985). For example, the ruffling of feathers to increase cooling during hot temperatures

could be thought of as an alarm reaction and hence interpreted as indicative of stress. As stress is generally perceived as having a negative influence upon an animal's well-being the use of stress in describing an animal's defensive response to such environmental stimuli is confusing (Harvey *et. al.*, 1984; Freeman, 1985). However, Williams (1984) stated that the use of the term stress is generally positive and adaptive in that it describes reactions combating or accommodating stimuli.

Although still describing alarm reactions as indicative of stress, Henry (1993) cited Seyle (1974), in using the term "distress" to describe a response to a stressor that involves corticosterone release while stress without distress occurs in predominantly alarm reactions to stressors. Other authors further defined distress in describing corticosteroid responses to stressors only where the magnitude and duration of the response is above that exhibited by the animal in response to innocuous stimuli such a midday rise in ambient temperature (Ewbank, 1973; Harvey *et. al.*, 1984; Freeman, 1985). Hence distress is used to describe situations which are unpleasant, incur some cost or damage to the animal and possibly causes suffering (Ewbank, 1992).

In this study, the use of the word stress is limited to describing the presence of stimuli believed to have induced a rise in corticosterone levels in the birds studied. For example, "capture stress" is used to describe the stimuli associated with the capture of birds which elevated their corticosterone levels. The individual stimuli which may have contributed to the overall capture stress, such as the presence of humans or being contained within a trap, are labelled stressors. The use of the term distress is avoided in this study as it is not known whether any of the observed corticosterone responses of the mallards or grey ducks are any different from responses that could result from innocuous stimuli.

## 1.2 Physiological measurements of stress

The use of corticosterone levels as one physiological parameter in describing whether certain stimuli are stressful is based upon experiments

demonstrating elevation of corticosteroid levels in animals when they are exposed to stimuli labelled stressful e.g. restraint (Harvey *et. al.*, 1984). Corticosterone levels can be used to measure and compare the severity of stress in birds (Brown, 1961) as the magnitude and duration of any corticosterone response depends on the stressor applied (Harvey *et. al.*, 1984).

However, the use of parameters such as plasma corticosterone levels in quantifying the disturbance caused by certain stimuli means all stimuli which increase corticosterone levels, including egg laying in chickens, are termed stressors (Freeman, 1985). Ewbank (1992) avoids describing a relatively innocuous increase in corticosterone levels as being indicative of stress by comparing any increase in levels with previous corticosterone responses induced by stimuli that caused some damage or disadvantage to the animal, such as debeaking of chickens, that is presumably noxious.

Duncan (1981) suggests using more than one physiological parameter when deciding whether a stimulus is stressful to a bird. Situations could exist where the absence of a corticosterone response could be interpreted to indicate the absence of any disturbance created by certain stimuli, but the catecholamine response could be as marked as responses to other stimuli said to be stressful.

Despite the limitations of using only plasma corticosterone levels to detect stress, measurements of corticosterone levels could help to detect stimuli in the environment of birds which are stressful and that could be removed to improve the bird's welfare. For example, comparisons of the corticosterone responses of birds placed in solitary as opposed to communal confinement for transportation may reveal which type of confinement is the least stressful (Freeman, 1971). The maintenance of low levels of environmental stress is especially important in captive breeding programmes where greatest success will be achieved from captive environments in which the birds are exposed to as few stressors as possible.

### 1.3 Plasma corticosterone levels and the hypothalamo-pituitary-adrenal axis

In understanding why stressful stimuli inducing an increase in plasma corticosterone levels should be avoided among captive birds if possible, an appreciation of how corticosterone acts to resist or adjust to such stimuli within the bird is useful. Increased circulating levels of corticosterone can induce the mobilization of energy stores (gluconeogenesis), suppression of reproduction, immunosuppression and suppress inflammation (Siegel 1971, 1980; Harvey *et. al.*, 1984; Axelrod and Reisine 1984). The suppression of reproduction is of greatest concern in captive breeding programmes of wild birds.

For stressful stimuli to induce an increase in plasma corticosterone levels, a series of hormonal events must occur within the bird before the presence of such stimuli in the birds environment leads to elevated corticosterone levels. The hypothalamo-pituitary-adrenal (HPA) axis modulates the corticosterone response to stress in birds. The release of corticotrophic releasing factor (CRF) and arginine vasotocin (AVT) from the hypothalamus triggers the release of adrenocorticotrophic hormone (ACTH) from the pituitary gland which in turn releases corticosterone from the adrenal gland (Harvey and Hall, 1990).

CRF and AVT are released from paraventricular nuclei (PVN) neurons in the hypothalamus when they are stimulated by the catecholamine neurotransmitters adrenaline and noradrenaline and other  $\beta$ -adrenergic agonists (Rees *et. al.*, 1985a). Serotonin and gamma-amino-butyric acid (GABA) are another two neurotransmitters that are released from neurons synapsing on PVN neurons and they may stimulate CRF and AVT release (Harvey and Hall, 1990).

Certain stimuli are implicated in increasing the turnover of the neurotransmitters that induce CRF and AVT release. For example, adrenaline and noradrenaline content and turnover in hypothalamic neurons is increased when birds are starved while serotonin turnover is increased during water deprivation but not starvation (Harvey and Hall,

1990). Hence it is the presence of stressful stimuli that triggers hypothalamic neural activity, HPA activation and finally corticosterone release.

CRF is secreted into the hypothalamic portal vasculature from CRF containing neurons while AVT containing neurons terminate on the posterior pituitary. CRF and AVT act synergistically to evoke the release of ACTH at the pituitary with CRF also inducing the transcription of pro-opiomelanocortin (POMC) mRNA, the precursor for ACTH synthesis (Harbuz and Lightman, 1992).

Although the release of ACTH from the pituitary is primarily controlled by levels of CRF and AVT, its release may be inhibited by hypothalamic somatostatin (SRIF) and by corticosterone negative feedback (Harvey and Hall, 1990). Corticosterone feedback regulation of ACTH is directed towards inhibiting a surge in ACTH release that occurs in response to stressors (Harbuz and Lightman, 1992). CRF levels are also controlled by a corticosterone feedback inhibition which regulates basal CRF levels rather than inhibiting any surge in CRF as seen in response to stressors (Harbuz and Lightman, 1992).

Corticosterone secretion is primarily stimulated by ACTH, though ACTH-like factors originating from the pineal or hypothalamic sites possibly also have a stimulatory effect (Holmes and Phillips, 1976; Harvey *et. al.*, 1984). ACTH causes the proliferation of adrenocortical tissue and induces concentration-dependent increases in the release and biosynthesis of corticosterone. Corticosterone release and biosynthesis may also occur autonomously from adrenal gland cells (Holmes and Phillips, 1976).

Other hormones that can stimulate corticosterone release include pituitary growth hormone (GH) and prolactin (PRL) from the pituitary gland (Carsia *et. al.*, 1984, 1985; Cheung *et. al.*, 1988), while parathyroid hormone (PTH) from the parathyroid gland can induce corticosterone biosynthesis in the same manner as ACTH (Rosenberg *et. al.*, 1988). Triiodothyronine ( $T_3$ ) may reduce corticosterone release as hypothyroidism (low  $T_3$ ) potentiates corticosterone synthesis (Carsia *et. al.*, 1985) while prolonging the half-life of corticosterone in plasma (Kovacs and Peczely, 1983). The catecholamines, adrenaline and noradrenaline, released from

adrenomedullary cells in the adrenal may potentiate ACTH induced corticosterone secretion (Rees *et. al.*, 1985).

The effect of gonadal steroids on plasma corticosterone levels is uncertain. Assenmacher *et. al.* (1975) state that testosterone increases plasma levels of both corticosterone and corticosterone binding proteins while Carsia *et. al.*, (1987) found that testosterone inhibits corticosterone biosynthesis. These variable effects of testosterone on corticosterone biosynthesis and release could be due to seasonal variation in the responsiveness of the adrenocortical tissue to gonadal steroids (Silverin, 1979).

Other factors that may cause increased plasma levels of corticosterone include alterations in adrenal blood flow (Harvey *et. al.*, 1984) and changes in the characteristics of corticosterone binding proteins (Gould and Siegel, 1978; Wingfield *et. al.*, 1984). Increased plasma levels of corticosterone decrease the sensitivity of adrenocortical cells to ACTH, demonstrating a negative feedback relationship of corticosterone on its own biosynthesis and release (Carsia *et. al.*, 1983).

In conclusion, while an increase in plasma corticosterone levels can be due to the presence of stress and subsequent activation of the HPA axis, other factors such as the plasma levels of gonadal or thyroid hormones may effect the magnitude and duration of any corticosterone response.

## Chapter 2:

Influence of capture, captivity and sampling stress  
on plasma corticosterone levels in the mallard  
(*Anas platyrhynchos*)

## Abstract

1. The aim of this study was to measure corticosterone responses to capture and captivity in mallards and to examine variations in responses with duration and situation of captivity. The use of different sampling regimes in measuring corticosterone responses was also examined.

2. Corticosterone levels in wild mallards after capture were higher than levels in ducks held captive for 3 or 5 months, with levels decreasing among captive ducks in relation to the time spent in captivity. Corticosterone levels were lowest in ducks either reared indoors or held in an aviary for one month.

3. High corticosterone levels will decrease if the duck is placed in either a darkened communal or individual box.

4. If blood samples are collected at 20 min intervals a corticosterone response to sampling will apparently only occur if initial corticosterone levels are basal.

5. Sampling at 5 min intervals increases corticosterone levels whereas sampling at 20, 30 or 60 min intervals does not.

6. In conclusion, placement of wild ducks into darkened boxes may reduce stress associated with capture. The duration and possibly environment of captivity may affect corticosterone levels. An allowance of an acclimatisation period to captivity should be made before anticipation of breeding, while the captive environment may determine the duration of any acclimatisation period.

## 2.1 Introduction

The captive breeding of birds for conservation purposes is based on the belief that placing birds in captivity may enhance breeding success. The environment in which they are held should contain a guaranteed supply of food, ample nesting sites and be free of predators. Initially the birds have to undergo the stress of capture and transportation before being released into their captive environment. Novel and potentially stressful situations which may be present in a captive environment include confinement in an aviary, presence of humans and the use of feeders and pelleted feed. Some birds adapt to captivity and breed, some do not and some may even die.

By using physiological and/or behavioural parameters it is possible to quantify the degree of stress involved in capture and captivity. Ways to minimise or neutralise any stress involved in capture and captivity could be discovered thereby accelerating the acclimatisation of the bird to captivity and improving reproductive performance.

One physiological parameter used to quantify the stress associated with capture is plasma corticosterone levels. Increased corticosterone levels after capture have been found in the starling (*Sturnus vulgaris*, Dawson and Howe, 1983), White-throated sparrow (*Zonotrichia albicollis*, Schwabl *et. al.*, 1988), Garden warbler (*Sylvia borin*, Schwabl *et. al.*, 1991) and Harris Hawk (*Parabuteo unicinctus*, Mays *et. al.*, 1991). The magnitude of any such increase in corticosterone levels however, may vary with reproductive state, as demonstrated in the female White-crowned sparrow (*Zonotrichia leucophrys*, Wingfield *et. al.*, 1982).

Holding birds in communal boxes for transportation after capture maintains raised corticosterone levels resulting from capture (Wingfield *et. al.*, 1982). Birds placed in individual boxes may also demonstrate raised corticosterone levels. Chickens (*Gallus domesticus*) placed in a box for several hours either as a group (Beuving and Vonder, 1978) or individually (Craig and Craig, 1985) responded with increased corticosterone levels.

Birds placed in captive environments can also maintain elevated corticosterone levels. For at least 33 days corticosterone levels in White-

crowned sparrows held in outdoor aviaries were just as high as levels measured after capture from the wild (Wingfield *et. al.*, 1982).

Measurements of corticosterone levels in birds could indicate ways to accelerate acclimatisation to captivity. The identification of factors that increase corticosterone levels could allow aviculturalists to then remove these factors from the bird's environment. In chickens factors that have been shown to affect corticosterone levels include cage design (Compton *et. al.*, 1981, Gibson *et. al.*, 1986), group size (Jones and Harvey, 1987) and heat stress (Edens and Siegel, 1975). Similarly, factors that affect corticosterone levels in birds other than chickens include heat stress in turkeys (El-Halawani *et. al.*, 1973), cold stress in the pigeon (*Columbia livia*, Jeronen *et. al.*, 1976) and duck (Landsberg and Weiss, 1975) and intrusion by foreign birds on Japanese Quail (Satterlee *et. al.*, 1983).

To investigate the degree of stress imposed by capture and captivity as measured by corticosterone levels it is important to know the basal corticosterone levels in birds prior to capture. There is a lag of 60 sec in the pekin duck (Harvey *et. al.*, 1980) and starling (Dawson and Howe, 1983) between the capture or removal of the bird from its normal environment and an increase in corticosterone levels. Corticosterone levels in samples collected in less than 60 sec will therefore reflect true basal levels without sampling artefacts.

Characterisation of the corticosterone response to different factors such as cage design requires the collection of several blood samples, usually within one hour. Repeated handling and blood sampling of the birds may cause a corticosterone response in its own right (Wingfield *et. al.*, 1992) thereby disguising effects on corticosterone levels of the factor being studied. This potential problem can be overcome if samples are collected far enough apart to allow any sampling induced increase in corticosterone levels to abate.

When investigating corticosterone responses one must recognise that variation between individual birds may occur due to differences in age (Holmes *et. al.*, 1989, 1990a & 1990b), sex (Wingfield *et. al.*, 1992) and stock (Edens and Siegel, 1975). The environment from which the animal is

obtained has also been shown to affect corticosterone responses in mice (Guila, 1991) and may also do so for birds.

The magnitude of a corticosterone response may depend upon the time of day at which samples have been collected. Daily rhythms in corticosterone levels have been found in ducks (Wilson *et. al.*, 1982), turkeys (El-Halawani *et. al.*, 1973 and Proudman, 1991), pigeons (Joseph and Meier, 1973) and quail (Boissin and Assenmacher, 1970; Kovacs and Peczely, 1983). In hens, a daily rhythm in corticosterone levels occurs with a peak that coincides with egg laying (Beuving and Vonder, 1977; Wilson and Cunningham, 1981). However, Freeman and Flack (1980) found no daily rhythm in corticosterone levels in 5 different strains of non-laying chicks.

There is apparently only one study in which corticosterone levels were measured in wild birds from capture through to captivity (Wingfield *et. al.*, 1982). However suggestions of ways to reduce corticosterone responses to capture and accelerate adjustment to captivity were not reported in this study nor was any individual variation that may have occurred.

The corticosterone responses to various factors have been investigated as previously stated, but any variation in these responses between birds from different environments has not. Discovering such environmental variability could be important as information could be gained that may help in decisions about which environments most suit particular birds. Furthermore, individual variability in the corticosterone responses to a novel stressor could indicate which birds would or would not habituate well to other novel stressors such as captivity.

The study described here therefore had three main objectives. (1) Corticosterone levels were measured in mallards after capture and after various periods in several captive environments in order to allow ways of lowering corticosterone levels after capture and during captivity to be evaluated. (2) Repeated sampling was used to investigate if the corticosterone responses of ducks to the novel stressor of sampling vary between groups of ducks from different environments. Individual variability in this corticosterone response was considered, together with ways of identifying individuals with a predisposition to acclimatise and

breed in captivity. (3) Corticosterone responses to sampling were also investigated to discover the best sampling regime to use for observing and inducing corticosterone responses.

## 2.2 Materials and methods

Blood samples were collected from mallards (Table 2.1) obtained from the wild (Groups A to D) or held captive in the Avian Physiology Unit, Massey University for various lengths of time after capture from the wild (Groups E to J). Blood samples were also collected from a group of ducks reared in captivity at the Avian Physiology Unit (Group K).

All results were obtained over a 6 month period from January in mid summer to June in mid winter, with sampling starting at 9.30am and finishing by 11.00am to minimise any diurnal rhythm effect. All ducks housed at the Avian Physiology Unit had access to food (duck pellets, J.F. Cockrem, Massey University) and water *ad libitum*.

### 2.2.1 Sample collection

Blood samples (0.5 - 2.0 ml) from the brachial vein were taken into heparinised syringes, centrifuged and the plasma frozen for later analysis of corticosterone levels.

For ducks in groups A to H and K initial blood samples were obtained in less than 90 sec from the duck being handled. If corticosterone levels do not increase in response to handling within 90 sec then levels in these samples reflect levels in the duck prior to handling and can be designated to occur at 0 min (Table 2.1). The collection of further samples allowed the measurement of any change in corticosterone levels in response to sampling. If no sample had been obtained by 90 sec sampling was abandoned to ensure that the duration of sampling experienced by the duck was the same at each sampling time.

Blood samples from group I and J ducks were collected 5 and 30 min after initial handling respectively (Table 2.1). A maximum of four samples was collected from each duck during an experiment, so the omission of a sample at 0 min allowed later sampling to coincide with samples collected from some other groups. Again, sampling was abandoned if no sample had been obtained by 90 sec from the duck being handled.

Table 2.1: Experimental groups

Group	Sex	n	Initially sampled at	Subsequent samples collected at
A	M	5	0 min	20, 40 & 60 min
B	F	10	0 min	20, 40 & 60 min
C	M	12	0 min	20, 40 & 60 min
D	F	5	0 min	20, 40 & 60 min
E	F	5	0 min	60, 120 & 180 min
F	F	5	0 min	20, 40 & 100 min
G	F	10	0 min	20, 40 & 60 min
H	M	4	0 min	20, 40 & 60 min
I	F	5	5 min	10, 15 & 20 min
J	F	5	30 min	60, 90 & 120 min
K	F	5	0 min	20, 40 & 60 min

## 2.2.2 Comparisons of plasma corticosterone levels in different groups of mallards

Ducks were obtained from wild or captive situations, so a comparison of corticosterone levels in mallards in response to capture and after various periods of captivity could be made using initial samples from group A to H ducks. Samples collected at 20 min intervals were used to compare corticosterone responses to sampling stress between groups of ducks obtained from wild or captive situations. Along with samples collected at 20 min intervals, samples collected at various other frequencies were used to describe corticosterone responses of ducks to sampling stress.

### 2.2.2.1 Plasma corticosterone levels in wild and captive mallards

Wild ducks were caught in traps placed on the edge of ponds 2 km from the Avian Physiology Unit. The traps consisted of two compartments (2.0 x 1.0 x 1.0m and 2.0 x 2.0 x 1.0m; length x width x height) with funnel entrances from the outside into one compartment and between compartments. The traps were baited with wheat after several days of pre-baiting and checked the following morning. After the trap had been darkened with a large cover ducks were removed and sampled individually. After initial blood sample collection ducks were placed into individual darkened boxes (0.40 x 0.25 x 0.30m; length x width x height) and further samples collected every 20 min for 1 h. Ducks sampled in this way are included in groups A and B (Table 2.1).

Some ducks (groups C and D) were taken from the trap and placed into a darkened communal box (0.94m x 0.56m x 0.34m; length x width x height), transported to the Avian Physiology Unit (a journey which lasted 15 to 30 min), left for 1 h and then sampled. In comparison with groups A and B ducks, group C and D ducks allow effects of communal boxing on corticosterone levels in wild ducks to be discussed. Again, further samples

were collected every 20 min for 1 hr with the ducks being placed in individual boxes between sampling.

Captive ducks were held at the Avian Physiology Unit in either outdoor pens or aviaries. On experimental days ducks in outdoor pens were herded into an aviary and confined within a darkened space approximately 1 m in diameter. Ducks were individually removed from the group and taken to another room where they were restrained by a handler and the initial blood sample taken. Ducks sampled in this way are included in groups E, F and G with the time spent in captivity prior to sampling being of 1, 6 or 26 weeks duration respectively. After the initial blood sampling, ducks were placed in individual darkened boxes and further samples collected at various frequencies.

Ducks housed in aviaries were also confined within a darkened space approximately 1 m in diameter within their aviary before being individually removed and taken to another room for sampling. Again, samples were collected every 20 min for 1 h with these ducks being included in group H.

An additional group of ducks were reared indoors from the day of hatching as part of a separate experiment performed by Dr. Cockrem and were sampled for comparison (group K). The ducks reared indoors were sampled in the same way as those in the aviary with samples also being collected at 20 min intervals for 1 h.

Ambient temperatures at a site 1 km from the Avian Physiology Unit were obtained from the Grasslands Division, Department of Scientific and Industrial Research, Palmerston North to determine whether temperature differences may have been responsible for the variation in corticosterone levels between groups E to H ducks who were sampled on different days.

#### 2.2.2.2 Plasma corticosterone responses of wild and captive mallards to repeated sampling at 20 min intervals

As shown in Table 2.1, groups A to D, G, H and K were sampled every 20 min for 1 h. This standard sampling regime allowed comparisons of the corticosterone responses to a consistent stressor between groups of wild and

captive ducks .

### 2.2.2.3 Effects on plasma corticosterone levels of the time taken to obtain a blood sample, the order of sampling and various frequencies of repeated sampling

Only captive female mallards held in an outdoor pen were used for this experiment (groups E to G, I and J). Ducks were sampled as described earlier for ducks from the outdoor pen. After the first sample (or, in groups I and J, handling but not sampling) ducks were placed in individual darkened boxes until and after the collection of subsequent samples as outlined in Table 2.1.

### 2.2.3 Measurement of plasma corticosterone

Corticosterone levels were measured by radioimmunoassay in extracted plasma. Briefly, 100 $\mu$ l plasma samples were extracted into 1 ml dichloromethane. After mixing and centrifugation a 500 $\mu$ l aliquot of the dichloromethane were removed and placed in another test tube and dried under a stream of air at 37 $^{\circ}$ c. Dried extracts were reconstituted in 200 $\mu$ l of phosphate-buffered saline with gelatin and left overnight at 4 $^{\circ}$ c. Aliquots of buffer (20  $\mu$ l) were removed, made up to 100 $\mu$ l and frozen at -20 $^{\circ}$ C until assayed. The extraction efficiency measured using a spike of tritiated corticosterone was  $93.8 \pm 2.7\%$ .

The antiserum (batch No. B3-163, Endocrine Sciences, Tarzana, CA) cross-reacted with other steroids at < 1.0%: progesterone (0.6%), desoxycorticosterone (0.2%), testosterone (0.1%), estradiol (0.03%) and aldosterone (0.02%). Reconstituted extracts were incubated with 100 $\mu$ l of antiserum and 100 $\mu$ l of tritiated corticosterone (approximately 5,000 cpm; Amersham) overnight at 4 $^{\circ}$ C. Separation of bound and free hormone was achieved by the addition of 0.5 ml of dextran coated charcoal. Tubes were allowed to stand for 18 min from the last charcoal addition and were then

promptly centrifuged at 3000 rpm in a Heraeus Christ 5000S refrigerated centrifuge. A 500 $\mu$ l aliquot of the supernatant (containing bound labelled corticosterone) was removed and placed in a disposable scintillation vial. Scintillant mixture (0.005g dimethylPOPOP, 0.05g POP, 2 l toluene) was added (3 ml), and the tubes shaken for 1 h in an orbital shaker. After 1 h vials were counted in a Beckmann LS7500 scintillation counter for 10 min.

Displacement curves for increasing amounts of duck plasma and of the corticosterone standard were compared following logit-log transformation and found to be parallel. Corticosterone (0.62 to 10.00 ng/ml) was added to duck plasma and was quantitatively recovered ( $102.2 \pm 5.6\%$ ,  $n=4$ ). The limit of sensitivity of the assay was 0.40 ng/ml. The intra- and inter-assay coefficients of variation were 6.5% and 9.3% respectively.

#### 2.2.4 Statistical analyses

Corticosterone levels in the first sample obtained from each duck were compared between groups using one-way ANOVA with Bonferroni adjusted probability levels used for subsequent post-hoc tests. Where the Bartlett's test indicated heterogeneity of variance comparisons of corticosterone levels between each group was performed using Kruskal-Wallis nonparametric ANOVA.

The effect of time, or sampling stress, on corticosterone levels within each group of ducks was also investigated by one-way repeated measures ANOVA with Bonferroni adjusted probability levels used for subsequent post-hoc tests. Where the Bartlett's test indicated heterogeneity of variance the effect of sampling stress on corticosterone levels within each group was investigated by using Friedman nonparametric ANOVA.

Relationships between corticosterone levels and either ambient temperature or the time taken to obtain a blood sample were examined using regression analysis. Effects of sampling order on corticosterone levels were examined using the Kruskal-Wallis nonparametric ANOVA (Wilkinson, 1990).

Log transformed data were used for statistical analysis. Summary data are shown as mean  $\pm$  standard error of the mean (SEM).

## 2.3 Results

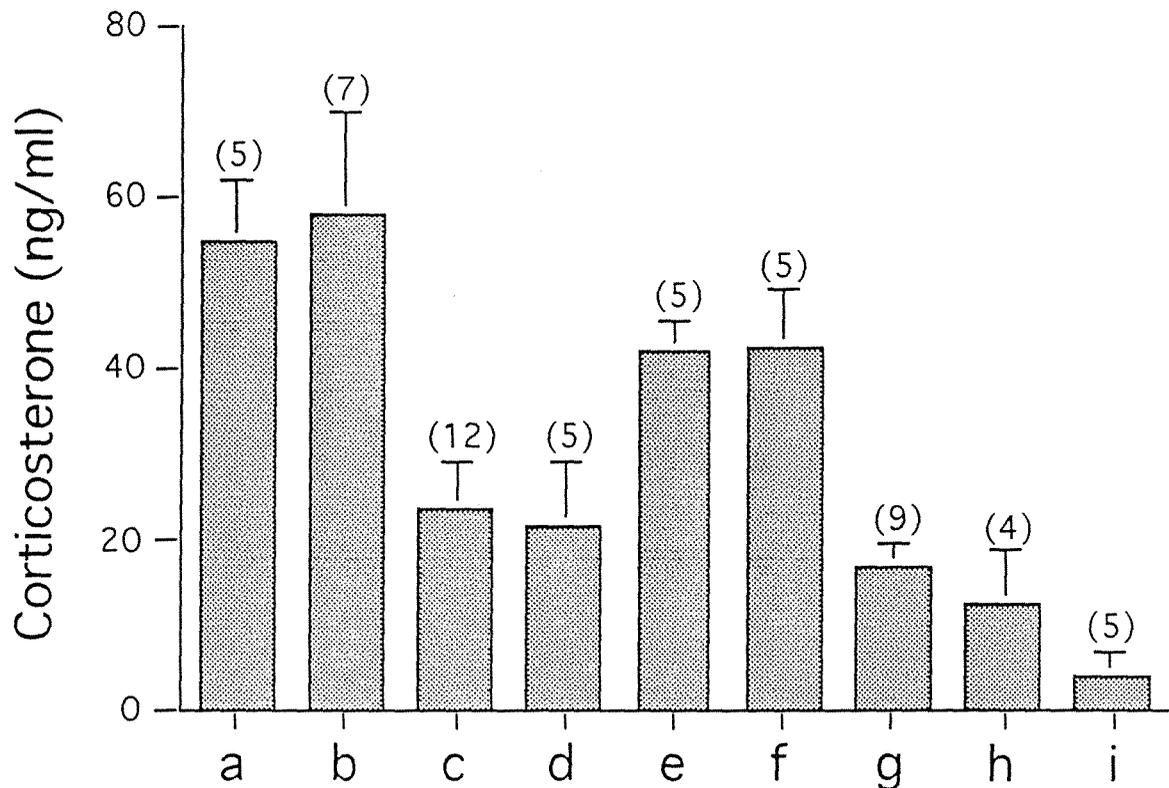
### 2.3.1 Plasma corticosterone levels in wild and captive mallards

There was large variation in corticosterone levels between groups of mallards obtained from various environments (Fig 2.01). Corticosterone levels in both male (group A) and female ducks (group B) were significantly higher when they were sampled directly from the trap than if they were obtained from a darkened communal box 1 h after transportation from the trap ( $p < 0.05$ , group C and D).

For the ducks kept in captivity, corticosterone levels were significantly higher in ducks held in an outdoor pen for 1 (group E) or 6 weeks (group F) than levels in ducks held for 5 months (group G,  $p < 0.01$ ). The ducks held in an outdoor pen for either 1 or 6 weeks also had significantly higher corticosterone levels than ducks held in aviaries (group H,  $p < 0.05$ ) or reared in an indoor room (group K,  $p < 0.01$ ). The ducks held for 5 months in an outdoor pen (group G) had similar corticosterone levels to ducks held in aviaries (group H,  $p = 0.719$ ), but significantly higher levels than in ducks reared in an indoor room (group K,  $p < 0.05$ ). The corticosterone levels in ducks reared in an indoor room were not different from levels in ducks held in aviaries (group H,  $p = 0.395$ ).

Some experimental groups had a larger range of corticosterone values than other groups, as shown in Figure 2.02. All the male ducks from the trap (group A) had higher corticosterone levels than male ducks sampled from a communal box (group C). For the female ducks, only 3 of 7 ducks sampled from the trap (group B) had corticosterone levels higher than the females from a communal box (group D).

The effect of time in captivity on corticosterone levels can also be seen by comparing individual corticosterone values. Of the ducks held for 1 (group E) or 6 weeks (group F) in an outdoor pen only 1 of the 10 ducks had corticosterone level as low as the levels in any of the ducks that had spent 5 months (group G) in captivity. All of the 9 ducks held for 5 months in an outdoor pen had corticosterone levels within the range observed in ducks



- a & b - Male (a) and female (b) ducks from trap (groups A and B respectively)
- c & d - Male (c) and female (d) ducks trapped, transported and left for 1 h in a communal box (groups C and D respectively)
- e to g - Female ducks after 1 wk (e), 6 wks (f) and 5 months (g) in outdoor pen (groups E, F and G respectively)
- h - Male ducks after 2 months in an outdoor pen followed by 1 month in an aviary (group H)
- i - Female ducks 3 months old, reared from hatch in an indoor room (group K)

Figure 2.01: Mean plasma corticosterone levels in wild and captive mallards. Numbers of ducks in each group are in brackets above columns.

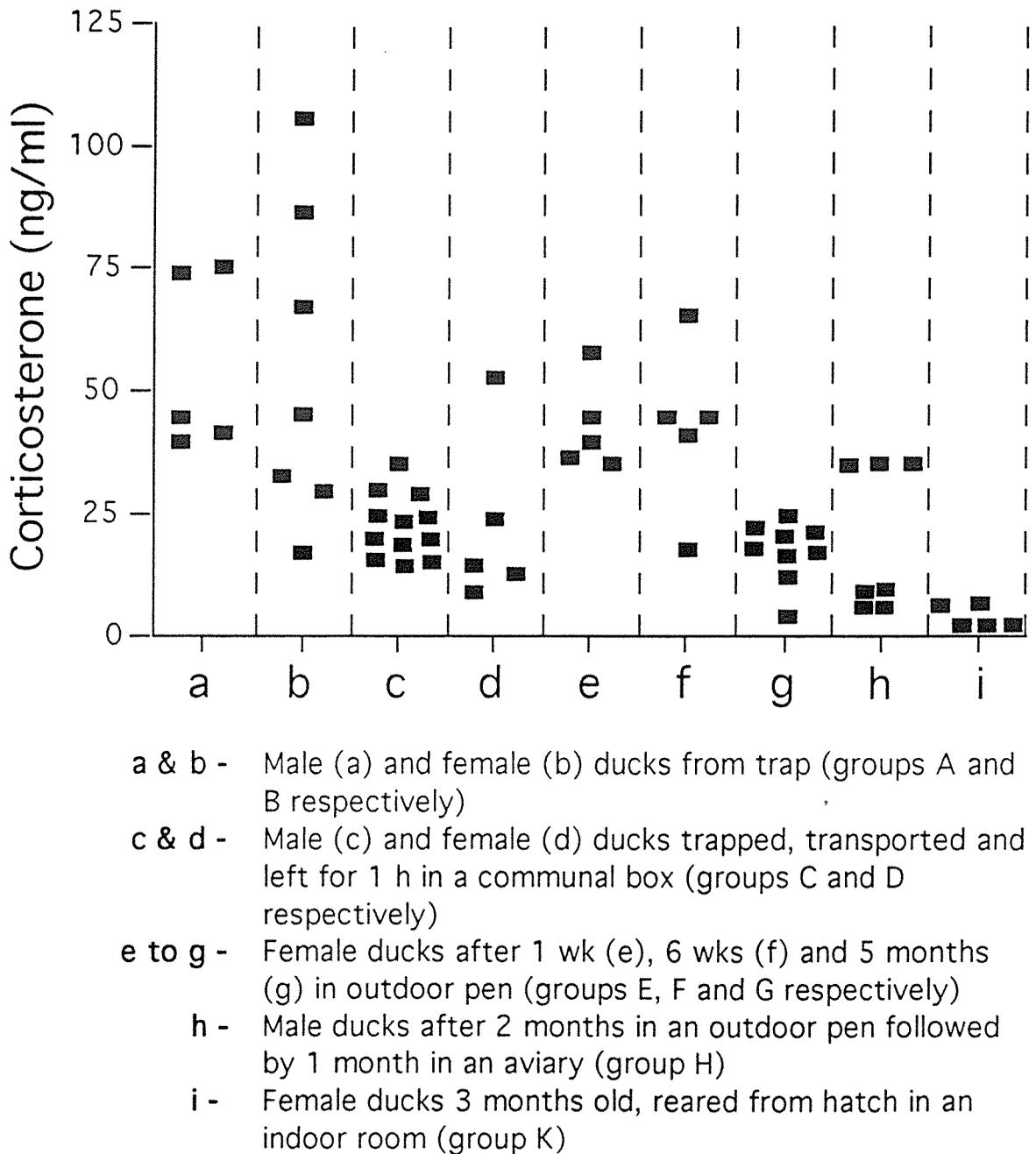


Figure 2.02: Plasma corticosterone levels in wild and captive mallards

held in aviaries (group H), but only 1 of the 9 ducks had levels as low as levels in ducks reared in an indoor room (group K).

The variation in corticosterone levels between groups of ducks held for varying lengths of time in captivity was not related to the ambient temperature on the day they were sampled ( $p=0.617$ , Fig 2.03).

### 2.3.2 Plasma corticosterone responses of wild and captive mallards to repeated sampling at 20 min intervals

Mean corticosterone levels in both male (group A) and female ducks (group B) obtained from the trap were significantly lower at 20 min than levels at 0 min, with this decrease sustained until 60 min ( $p<0.05$ , Fig 2.04). Corticosterone levels in all 5 male (group A) and 7 female ducks (group B) were lower at 20 min than levels at 0 min with this decrease sustained in all 5 male ducks and in all 5 of the female ducks from which complete sets of blood samples were obtained (Fig 2.05).

No change in mean corticosterone levels was seen in response to repeated blood sampling in either male (group C,  $p=0.438$ ) or female ducks (group D,  $p=0.714$ ) obtained from a communal box (Fig 2.04). Mean corticosterone levels at 20, 40 and 60 min in both male and female ducks obtained from the communal box were not significantly different from levels measured at 20, 40 and 60 min in ducks obtained directly from the trap. Corticosterone levels generally remained relatively constant during repeated sampling of individual male ducks obtained from the communal box (group C, Fig 2.05). In the female ducks from the communal box (group D) corticosterone levels in 2 of the 5 ducks remained below 12 ng/ml whereas the other 3 female ducks had high levels (Fig 2.05).

Mean corticosterone levels in female ducks from the outdoor pen (group G) decreased in relation to levels measured at 0 min ( $p<0.05$ , Fig 2.04). A decrease in corticosterone levels was seen in 7 of these 9 ducks (Fig 2.05). No change in mean corticosterone levels ( $p=0.272$ ) was seen for the male ducks from an aviary (group H, Fig 2.04) with levels remaining relatively constant in individual ducks (Fig 2.05).

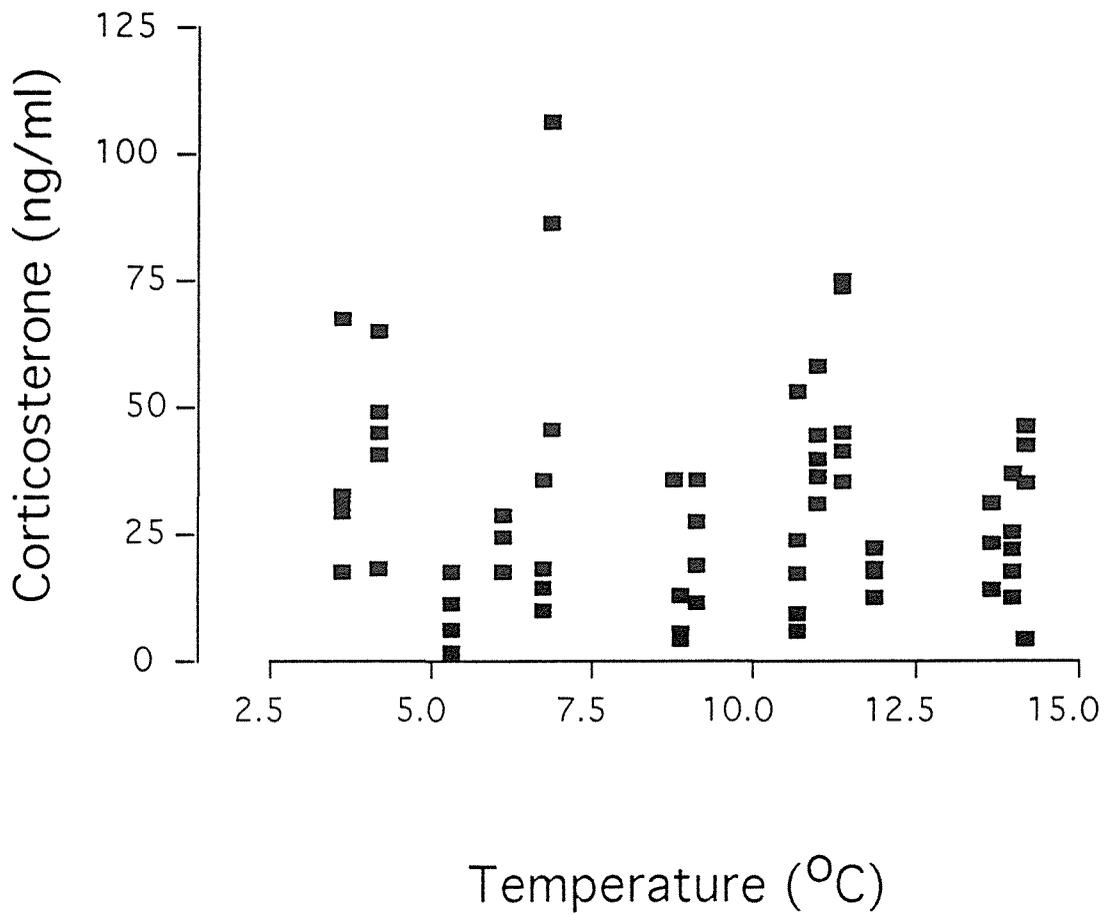
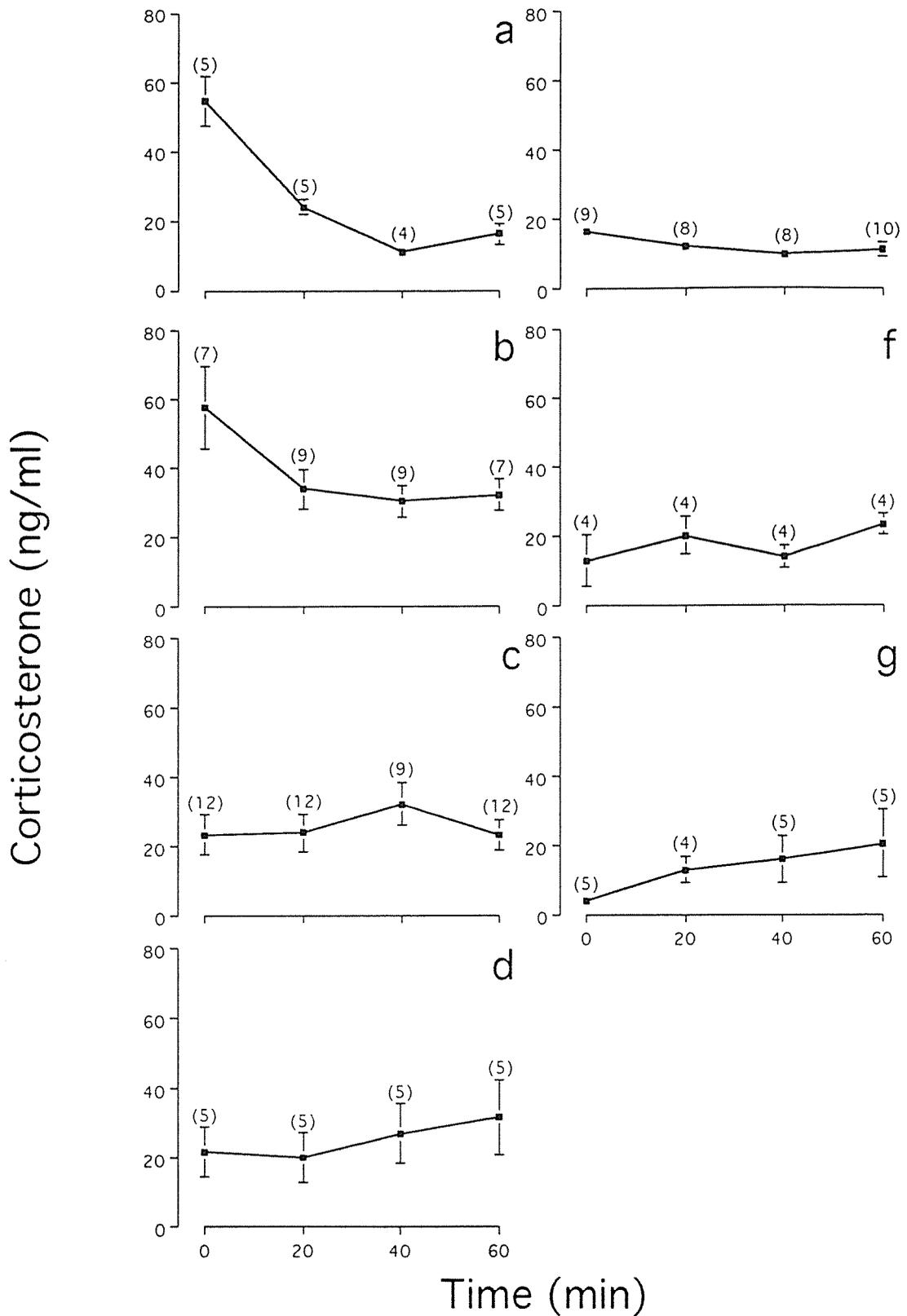
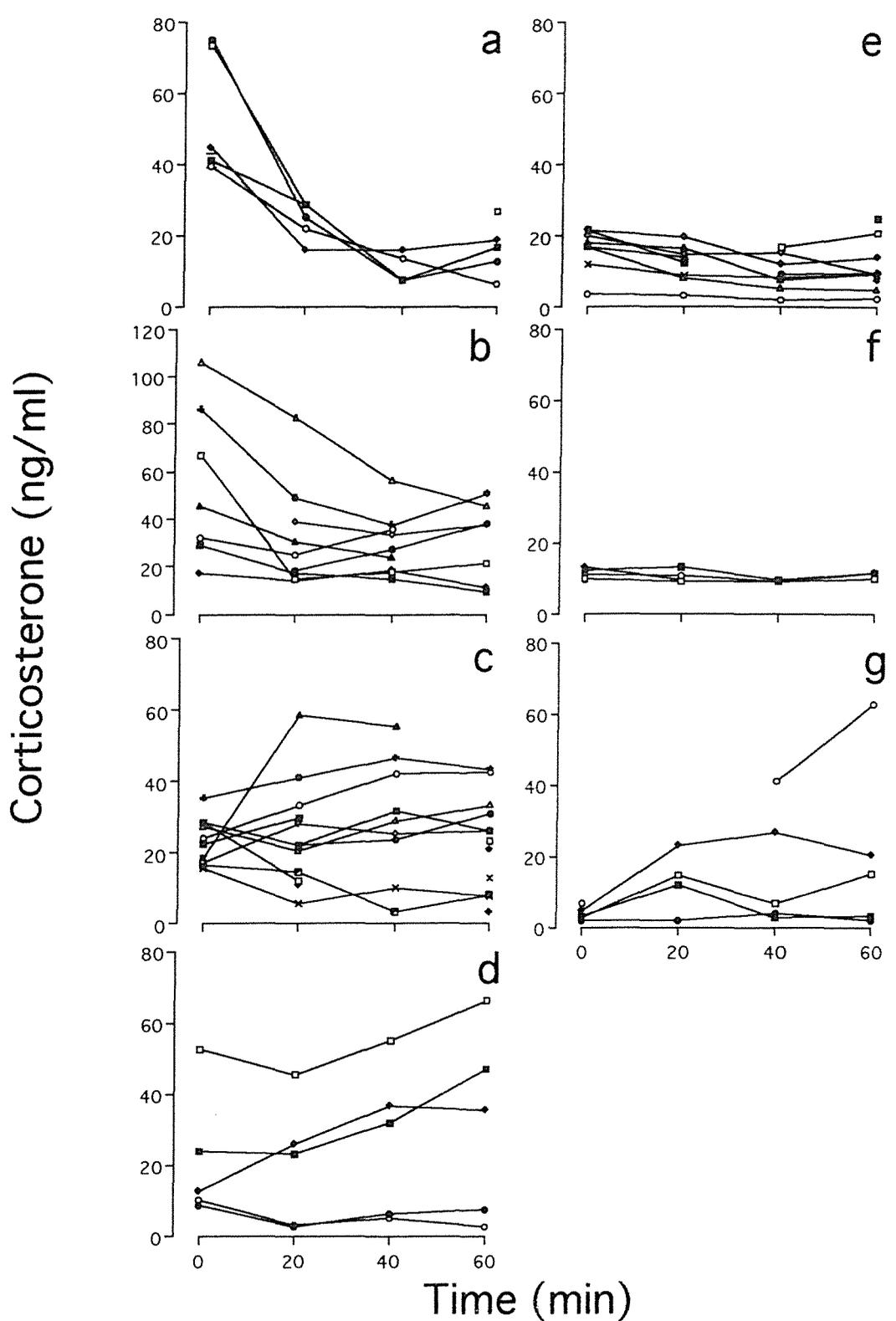


Figure 2.03: Relationship between plasma corticosterone levels and ambient temperature on the morning of sampling. The temperature was measured at the Grasslands Division, Department of Scientific and Industrial Research, Palmerston North, New Zealand.



- a & b - Male (a) and female (b) ducks from trap (groups A and B respectively)
- c & d - Male (c) and female (d) ducks after transportation and 1 h in communal box (groups C and D respectively)
- e - Female ducks after 5 months in an outdoor pen (group G)
- f - Male ducks after 2 months in an outdoor pen followed by 1 month in an aviary (group H)
- g - Female ducks 3 months old, reared from hatch in an indoor room (group K)

Figure 2.04: Mean plasma corticosterone levels in wild and captive mallards. Numbers of ducks sampled are in brackets above columns.



- a & b - Male (a) and female (b) ducks from trap (groups A and B respectively)
- c & d - Male (c) and female (d) ducks after transportation and 1 h in communal box (groups C and D respectively)
- e - Female ducks after 5 months in an outdoor pen (group G)
- f - Male ducks after 2 months in an outdoor pen followed by 1 month in an aviary (group H)
- g - Female ducks 3 months old, reared from hatch in an indoor room (group K)

Figure 2.05: Plasma corticosterone levels in wild and captive mallards.

No significant change in mean corticosterone levels ( $p=0.168$ ) occurred with sampling in female ducks obtained from an indoor room (group K, Fig 2.04). However, in 4 of the 5 ducks corticosterone levels at either 20 or 40 min had reached 12 ng/ml or more and were higher than levels measured at 0 min. Corticosterone levels in one group K duck never rose above 4 ng/ml (Fig 2.05).

### 2.3.3 Effects on plasma corticosterone levels of the time taken to obtain a blood sample, the order of sampling and various frequencies of repeated sampling

#### 2.3.3.1 Effects on plasma corticosterone levels of the time taken to obtain a blood sample and of the order of sampling

Corticosterone levels in female ducks from groups E, F and G did not exhibit any significant trend with time when samples were obtained within 90 sec of the duck being handled ( $p=0.567$ , Fig 2.06). The removal of a duck from its group did not have any significant effect on the corticosterone levels in the remaining ducks of that group, because there was no relationship between the order in which a duck was picked up for sampling and its corticosterone level ( $p=0.132$ , Fig 2.07).

#### 2.3.3.2 Plasma corticosterone responses of mallards to various frequencies of repeated sampling

Mean corticosterone levels in female ducks sampled every 20 min for 1 h (group G) decreased in relation to levels measured at 0 min ( $p<0.05$ , Fig 2.08). A decrease in corticosterone levels was seen in 7 of these 9 ducks (Fig 2.09).

Sampling every 20 min for 40 min and again at 100 min (group F), did not significantly change mean corticosterone levels from levels at 0 min ( $p=0.682$ , Fig 2.08). However, there was large variation between ducks in

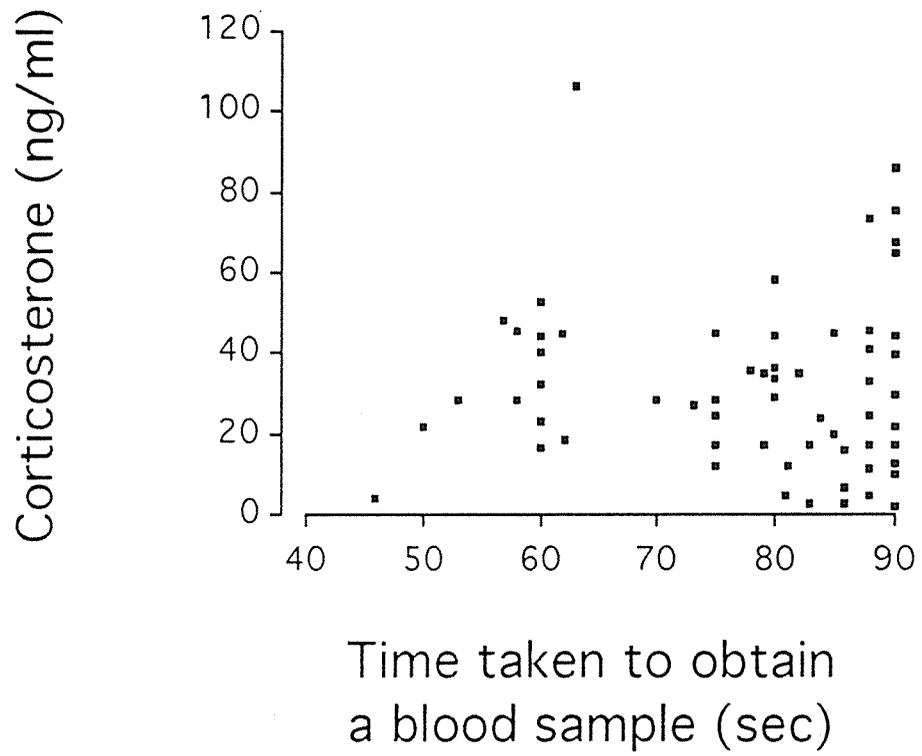


Figure 2.06: Relationship between plasma corticosterone levels and the time taken to obtain the first blood sample in captive female mallards.

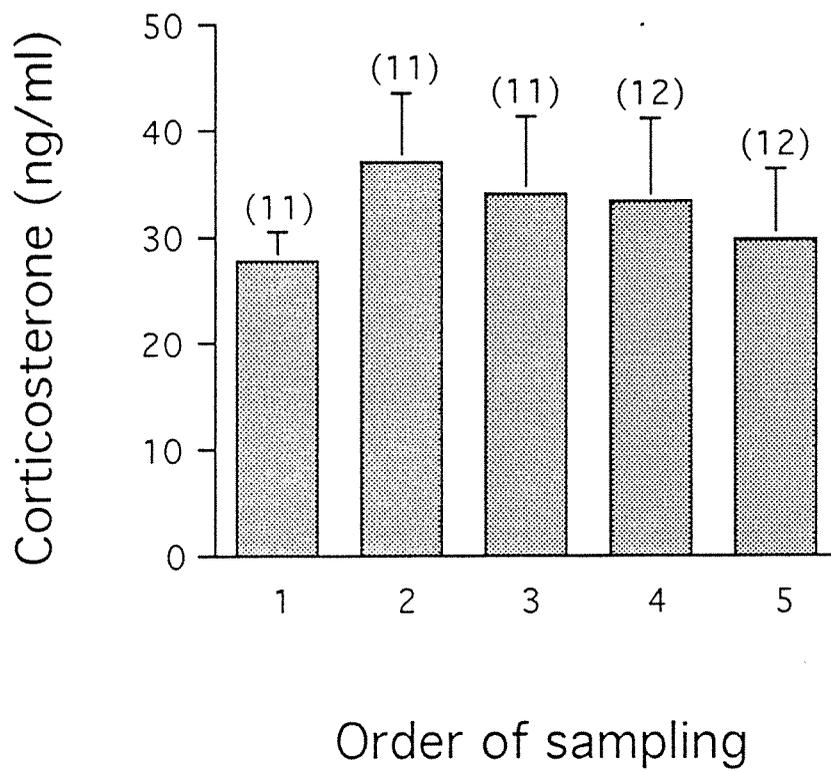


Figure 2.07: Relationship between plasma corticosterone levels in the first sample and the order of sampling in captive female mallards.

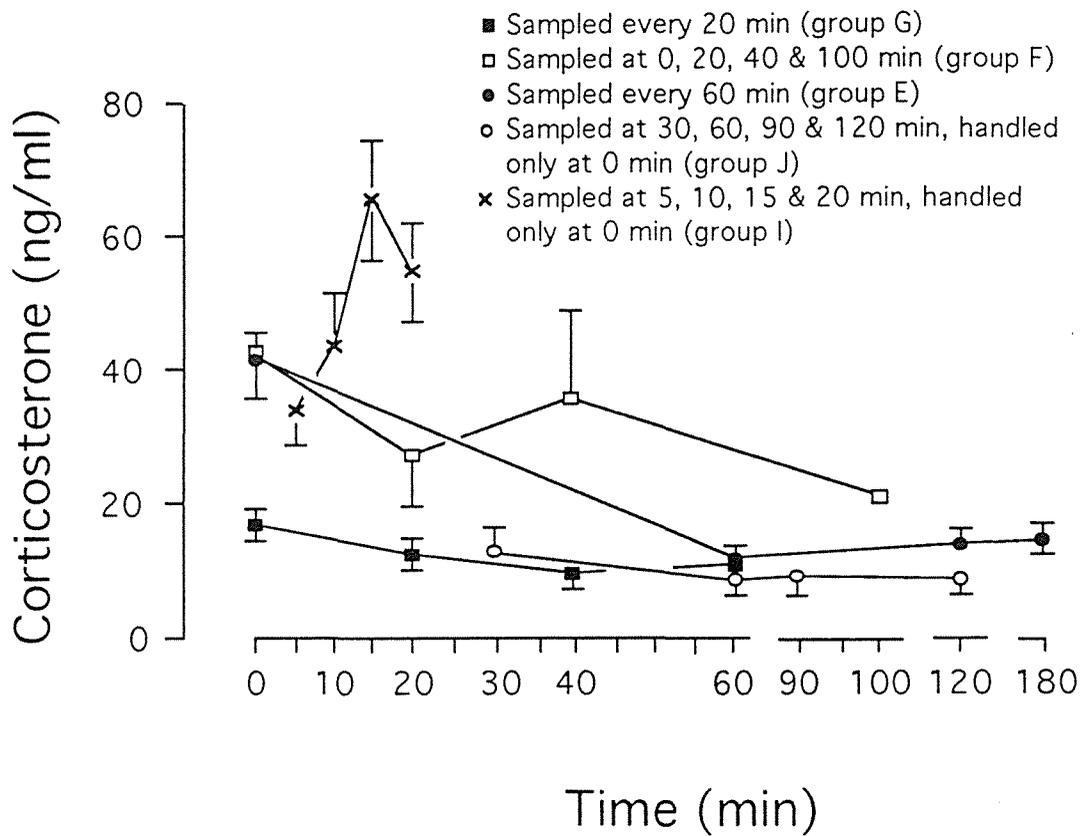
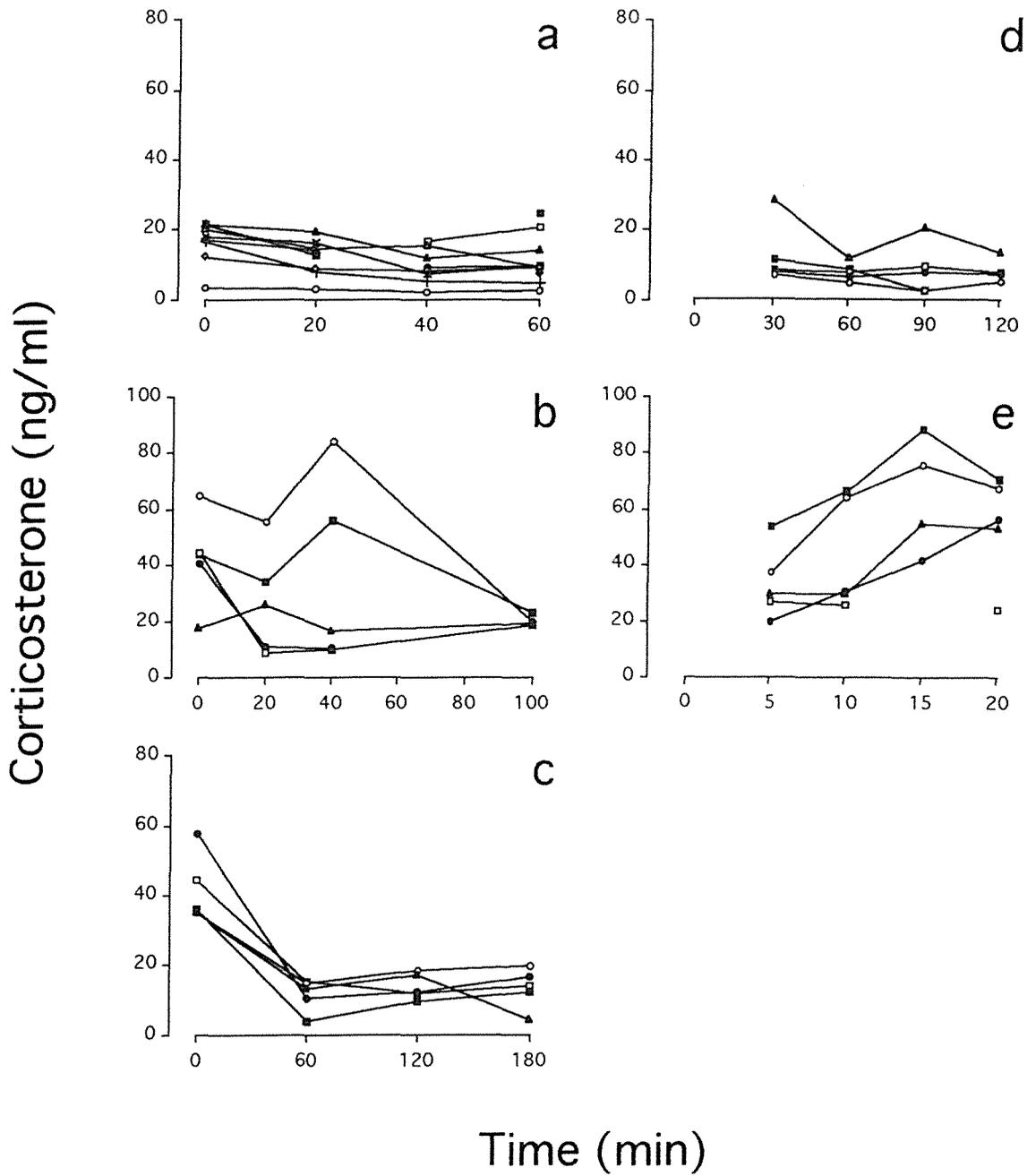


Figure 2.08: Mean plasma corticosterone levels in groups of female mallards sampled at various time intervals.



- a Sampled every 20 min (group G)
- b Sampled at 0, 20, 40 & 100 min (group F)
- c Sampled every 60 min (group E)
- d Sampled at 30, 60, 90 & 120 min, handled only at 0 min (group J)
- e Sampled at 5, 10, 15 & 20 min, handled only at 0 min (group I)

Figure 2.09: Plasma corticosterone levels in female mallards sampled at various intervals.

their corticosterone response in this group. Despite a range of corticosterone levels at 40 min of 10.0 to 84.0 ng/ml, levels were between 18.9 to 23.2 ng/ml at 100 min (Fig 2.09).

Mean corticosterone levels at 60, 120 and 180 min (group E) were consistently lower than levels at 0 min ( $p < 0.01$ , Fig 2.08). This pattern was seen in all 5 ducks (Fig 2.09).

Sampling every 30 min for 120 min (group J) did not significantly change mean corticosterone levels from levels at 30 min ( $p = 0.357$ , Fig 2.08). No sample was taken when the ducks were first handled, so corticosterone levels at 0 min are unknown. Corticosterone levels did not exceed 11.3 ng/ml in 4 of the 5 ducks between 30 and 120 min (Fig 2.09).

Corticosterone levels increased between 5 and 20 min in group I ducks sampled every 5 min ( $p < 0.01$ , Fig 2.08). Again no sample was taken when the ducks were first handled at 0 min, so corticosterone levels at that time are unknown. An increase in corticosterone levels was seen in 4 of the 5 ducks between 5 and 20 min (Fig 2.09).

No duck left undisturbed in an individual box for a period of 60 min had corticosterone levels higher than 23.2 ng/ml (i.e. group F from 40 min and group E between each sample), despite previously having levels as high as 84.0 ng/ml (Fig 2.09). Some ducks did exhibit corticosterone increases. The highest such increase in corticosterone levels in any duck left for 60 min in a box were 10 to 18.9 ng/ml.

The corticosterone levels at 20 min were significantly higher in ducks sampled three times by 20 min (group I) than in the ducks of group G who had been sampled only once by 20 min ( $p < 0.01$ , Fig 2.08). However, corticosterone levels in another group of ducks also sampled once by 20 min (group F) were no different to levels in group I ducks at 20 min ( $p = 0.062$ , Fig 2.08), perhaps due to the high variation in levels in group F ducks at 20 min (Fig 2.09).

## 2.4 Discussion

In summary, high corticosterone levels found in ducks after capture can be reduced by placing each duck in either an individual or a communal box. Once in captivity wild ducks react more to the presence of humans, exercise and/or confinement than other ducks who have been captive for 3 months or more. Whether the environment in which ducks are held captive affects corticosterone levels or responses to stress is uncertain. Large individual variation exists in corticosterone responses to blood sampling and could be indicative of the birds likely response to other stressors such as captivity. The observation and inducement of a corticosterone response to blood sampling depends on the frequency of sampling.

### 2.4.1 Plasma corticosterone levels in wild and captive mallards

In this study, corticosterone levels from ducks obtained from a trap were higher than levels in any other group of ducks (Fig 2.01). High corticosterone levels in response to capture were expected, as the capture of other wild birds has been shown to increase corticosterone levels (e.g. Dawson and Howe, 1983; Schawbl *et. al.*, 1988; Schwabl *et. al.*, 1991; Mays *et. al.*, 1991). Although corticosterone levels have apparently not been measured in wild ducks before, in domestic ducks levels below 12 ng/ml are said to be basal (Harvey *et. al.*, 1980; Harvey and Phillips, 1982; Rees *et. al.*, 1983 & 1985a; Klingbeil, 1985). If a similar corticosterone level were basal for wild ducks, that would indicate that capture elevates levels and is therefore stressful.

Besides the effects of capture in elevating corticosterone levels the confinement of the ducks with other birds and the absence of water in the trap could also contribute to a elevation in levels. Pukekos (*Porphyrio porphyrio*) were found in the trap on all occasions in which ducks were sampled. The presence of other birds in close proximity to an established group of quail has been shown to raise corticosterone levels (Satterlee *et. al.*, 1983) and pukekos might have had a similar effect on the ducks confined in

the trap. It is not known whether the trapped ducks were part of an established flock, nor is it known whether birds need to be part of an established group before confinement with other birds will elevate corticosterone levels.

Another possible contributor to the high corticosterone levels in trapped ducks could be a response to an absence of water in the trap. Ducks could have been in traps for up to 18 h prior to sampling as the traps were baited and set during the late afternoon on the previous day. Lack of access to water for 18 h has been shown to increase corticosterone levels in ducks (Harvey *et. al.*, 1982). However, the decline in corticosterone levels in ducks after they had been placed in individual darkened boxes (Fig 2.04) indicates that the absence of water is not entirely responsible for the high levels measured after trapping.

Ducks taken from the trap and sampled after transportation and a 1 h wait in a darkened communal box had corticosterone levels similar to levels in ducks held for 20 min in an individual box. If it is assumed that corticosterone levels were high when the ducks were taken from the trap, these observations suggest that placement of ducks in a communal box lowers corticosterone levels. The reduction of corticosterone levels in ducks in a communal or individual box might occur because darkness apparently reduces the arousal levels of many animals including chickens (Kilgour and Dalton, 1984).

Other environmental stimuli could have caused the corticosterone levels to be higher in ducks taken from the trap than levels in ducks kept in a communal box for 1 h. For example, a wild cat or stoat may have been trying to get at the ducks in the trap on the mornings ducks were sampled directly from the trap but not on the mornings when the ducks were placed in a communal box. However, placing the ducks from the traps into individual boxes for 20 min did lower corticosterone to levels similar to those in ducks from the communal box, indicating that effects of stressful stimuli prior to sampling can be largely overcome by the placing of ducks into boxes.

Confinement in captivity may be stressful to birds because of the

presence of humans, inability to escape, new habitat and the imposition of a new social situation. Some birds habituate to the stress of captivity and breed, some do not breed and some even die. Corticosterone levels in wild White-crowned sparrows held in aviaries remained above basal levels for at least 33 days (Wingfield *et. al.*, 1982), indicating a period of adjustment to captivity. In this study, corticosterone levels in ducks after 1 (group E) or 6 weeks (group F) in a outdoor pen were higher than levels in ducks held for 5 months (group G, Fig 2.01). Presuming similar conditions on the days of sampling, ducks held captive in an outdoor pen therefore had lower corticosterone levels with increased time spent in captivity. This indicates that the ducks underwent a period of adjustment to captivity.

With all the groups of ducks sampled here there was no relationship between ambient temperature and corticosterone levels (Fig 2.03) so that the differences in levels between groups E, F and G ducks were not likely to be due to temperature induced changes. That is supported by the results of El-Halawani *et. al.* (1973), Jeronen *et. al.* (1976) and Landsberg and Weiss (1975), who found no relationship of corticosterone levels and temperature over ranges of ambient temperatures which were greater than the range in this study.

Ducks held captive for 3 months, the last month of which was in an aviary, (group H) had corticosterone levels similar to ducks held captive for 5 months. Two explanations are possible for the similarity in corticosterone levels. (1) After 3 months of captivity, or longer, all ducks could have adjusted to captivity and had the same corticosterone levels. (2) The placing of ducks in the aviary could have shortened the period of adjustment to captivity, possibly as ducks held in aviaries experienced the presence of humans more than ducks held in outdoor pens. In comparison, ducks reared in an indoor room (group K) who experienced the greatest contact with humans had the lowest corticosterone levels of all ducks sampled. The presence of humans as a potential stimulant to corticosterone release has been recognised in studies of diurnal corticosterone rhythms where sampling involves venous cannulation and remote blood removal to avoid birds perceiving the presence of humans (e.g. Joseph and Meier, 1973;

Proudman, 1991).

If experience of human presence is reflected in corticosterone levels, then other factors also present during the obtaining of ducks for sampling may have an influence on resulting corticosterone levels. As the duck becomes more accustomed to such factors with increasing time spent in captivity any corticosterone response may diminish. Other possible factors besides the presence of humans could include the confinement of ducks prior to sampling and herding of the ducks held in outdoor pens into aviaries prior to sampling.

(1) Confinement either as a group or individually raises corticosterone levels (Beuving and Vonder, 1978; Craig and Craig, 1985). In this study, ducks held in aviaries or indoor rooms were housed in greater densities than ducks in outdoor pens and when feeders were refilled the ducks often huddled in a group over an area not dissimilar to which they were confined prior to sampling. Such familiarity with confinement may have resulted in lower corticosterone levels in ducks held in an aviary or indoor room as opposed to outdoor pen as the corticosterone response to novel situations is reduced with repeated exposure (Harvey and Phillips, 1982; Rees *et. al.*, 1983; Harvey and Hall, 1990).

(2) The herding of ducks prior to sampling could have been another factor that may have influenced corticosterone levels and possibly contributed to the difference in levels between ducks held outdoors and those held in an aviary. Exercise on a treadmill has been shown to increase corticosterone levels in ducks (Harvey and Phillips, 1982; Rees *et. al.*, 1983), although the total distance walked in these studies was at least 11 times greater than the estimated distance the ducks covered during herding in the present study.

The magnitude of individual variations in corticosterone levels differed between the groups of ducks sampled, with the wild ducks sampled from the trap having the greatest individual variation in levels. Such variation suggests that individual ducks experiencing the same captive situation react differently to their peers in respect to corticosterone levels. Those ducks which demonstrate a lower corticosterone response to capture

may also respond less to being placed in a novel captive environment and hence have a predisposition to breed in captivity. Whether a comparatively low corticosterone response to a stressful situation such as capture by individual ducks indicates a predisposition to breed in captivity is addressed in the following chapter.

In conclusion, greater experience of human presence and confinement and the absence of herding may have contributed to the lower corticosterone levels in ducks held in an aviary or indoor room as opposed to an outdoor pen. The lower corticosterone levels in ducks held in an outdoor pen for at least 5 months as opposed to 1 or 6 weeks after capture indicate that the ducks may become accustomed to captivity between 6 weeks and 5 months. The capture of wild ducks raises corticosterone levels with the placing of ducks into darkened boxes reducing levels. Individual variation in corticosterone levels exists and, with respect to the wild ducks sampled from a trap, may provide a criterion for identifying ducks likely to breed in captivity.

#### 2.4.2 Plasma corticosterone responses of wild and captive mallards to repeated sampling at 20 min intervals

Equally large variations in the corticosterone responses to repeated sampling were seen here in the captive and wild ducks. Such variations could result from the large variation in initial corticosterone levels as discussed previously. If ducks have high initial corticosterone levels then repeated sampling may not be potent enough to induce a further increase in levels as the presence of one stressor has been shown to decrease the corticosterone response to another separate stressor (Rees *et. al.*, 1985b). Hence ducks with low initial corticosterone levels have a greater likelihood of demonstrating a corticosterone response to repeated sampling as was shown in this study.

In conclusion, future studies of corticosterone responses to repeated sampling (as distinct from initial corticosterone levels) in birds after capture may also indicate individuals that have a predisposition to captive breeding.

The repeated sampling of birds from different captive environments may indicate whether the environment in which a bird is held affects the corticosterone responses of birds to such a novel stressor. For both these types of study low initial corticosterone levels are needed before any rise in levels as a result of repeated sampling will be seen. Ideally birds should be obtained in ways that do not cause pre-sampling elevation in corticosterone levels. For small birds the use of mist nets for capture within aviaries could be considered, while for larger birds such as the duck stress-free capture for sampling may be impossible unless the birds are kept in a small enclosure and chasing can be avoided.

### 2.4.3 Effects on plasma corticosterone levels of the time taken to obtain a blood sample, the order of sampling and various frequencies of repeated sampling

#### 2.4.3.1 Effects on plasma corticosterone levels of the time taken to obtain a blood sample and of the order of sampling

Corticosterone levels in this study were measured in blood samples collected in less than 90 sec. There was no rise in corticosterone levels during this 90 sec period, so the measured levels reflected those in ducks immediately before they were sampled (Fig 2.06). In comparison, Harvey *et al.* (1980) showed no rise in corticosterone levels in samples collected in less than 60 sec. In the present study allowing 90 sec rather than 60 sec avoided rushing the procedure and thereby ensured a high success rate in obtaining each blood sample.

No increase in corticosterone levels was seen among ducks as their peers were removed from the group (Fig 2.07). However, a large proportion of the corticosterone levels were above 12 ng/ml indicating the presence of a prior stressor which may have obscured a response to sampling order (Rees *et al.*, 1985b). On the other hand, there may have been no effect of sampling order on corticosterone levels in the present ducks as reported previously

for white crowned sparrows (Dusseau and Meier, 1971), ducks (Harvey *et. al.*, 1980; Harvey and Phillips 1982; Jones and Harvey, 1987) and chickens (Lagadic *et. al.*, 1990). One study demonstrated a sampling order effect in the barheaded goose (*Anser indicus*, Dittami, 1981).

#### 2.4.3.2 Plasma corticosterone responses of mallards to various frequencies of repeated sampling

The magnitude of a rise in corticosterone levels with repeated sampling apparently depended on the frequency with which samples were collected and the magnitude of initial corticosterone levels. In the present study only sampling at 5 min intervals (group I) increased corticosterone levels (Fig 2.08), whereas sampling ducks at intervals of 20 min (group G) did not raise levels and allowing 60 min between samples (group E) resulted in a decrease in levels during the first 60 min.

If ducks have low corticosterone levels at 0 min then sampling at 20 min intervals may be frequent enough to observe any corticosterone response. All 5 of the ducks raised in an indoor room (group K) had low corticosterone levels with 4 of the ducks demonstrating a rise in levels when sampled at 20 min intervals (Fig 2.05). Furthermore, Harvey *et. al.* (1980) demonstrated a small rise in corticosterone levels of approximately 7 ng/ml 30 min after initial sampling in the duck, indicating that even 30 min intervals should be sufficient to observe any change in levels with sampling. However as the metabolic half life of corticosterone in ducks is approximately 10 min and even less when the duck is stressed (Bradley and Holmes, 1971; Harvey *et. al.*, 1980), sampling 20 min or more after initial sampling may not record peak corticosterone levels even if initial levels are low.

Placing ducks in individual darkened boxes apparently lowered previously high corticosterone levels in ducks, since the highest level 60 min after initial sampling was 23.2 ng/ml despite levels initially being as high as 84.0 ng/ml (Fig 2.09). However no control group was included and corticosterone levels may have dropped in ducks 60 min after a previous

sampling regardless of whether they were placed in an individual darkened box.

In conclusion, the observation of any corticosterone responses to repeated sampling depends on the frequency with which samples are collected and the magnitude of initial corticosterone levels. Sampling at 5 rather than 20 min intervals should allow the detection of any rise in corticosterone levels with sampling and of the maximum level induced by the sampling procedure despite possibly high initial levels. The placing of ducks in individual darkened boxes 1 h before sampling may possibly reduce individual variations in corticosterone levels between ducks and lower previously high levels.

## Chapter 3:

Plasma corticosterone levels and response to  
sampling stress in relation to breeding success in  
the grey duck (*Anas superciliosa*)

## Abstract

1. The aim of this study was to investigate in grey ducks individual and monthly variations in both initial corticosterone levels, as measured in the first blood samples obtained from the ducks, and corticosterone responses to sampling stress.
2. Both initial corticosterone levels and responses to sampling stress varied between individual grey duck sampled over the 8 months of the experiment. The only 2 female ducks to rear ducklings, and their mates, had lower initial corticosterone levels before the breeding season than the remaining 4 female and 3 male ducks.
3. There was a negative relationship between initial corticosterone levels and body weight in male and female grey duck.
4. The variation in corticosterone levels and responses between individual grey duck may have been due, in part, to the existence of a dominance hierarchy amongst the grey ducks.
5. Initial corticosterone levels and responses to sampling stress did not vary significantly across the 8 months of the experiment from late autumn to mid summer.
6. In conclusion, corticosterone levels may provide an indirect measure of potential breeding success in captivity, and could also be used to help identify stressful stimuli which could then be removed or neutralised.

### 3.1 Introduction

Studies of corticosterone levels in mallards after capture and captivity have shown that a large degree of individual variation exists (Chapter 2). Such individual variation in a corticosterone response to a constant stressor has also been found in chickens (Bradley and Holmes, 1971; Beuving and Vonder, 1978) and wild starlings (Dawson and Howe, 1983). In this study initial corticosterone levels and the responses to sampling stress were examined in a captive flock of Parera or New Zealand grey duck (*Anas superciliosa*) to see if any variation between ducks in stress responses was consistent during the year, and to determine whether there was any relationship between stress responses and breeding success. Grey duck were used as they are a native waterfowl species that is widespread throughout the wetlands of New Zealand but, more importantly, had proven difficult to breed in captivity (Marchant and Higgins, 1990). In a previous breeding season at the Avian Physiology Unit, Massey University, from a flock of approximately 20 male and 20 female grey ducks in their first year of captivity only 3 female ducks bred and produced about 30 eggs. This is substantially less than the approximately 300 eggs produced by a group of about 20 male and 20 female mallards in their first breeding season in captivity. The study of individual variation in both corticosterone levels and breeding success among grey duck in captivity may yield information on the reasons for the difference in breeding success within and between species held in captivity.

Besides monthly measurements of corticosterone levels, body weight and body condition data were collected and observations were made of aggressive, reproductive and territorial behaviours. Such information in previous studies has helped to explain why individual variation in corticosterone levels exists (e.g. Silverin and Wingfield, 1982; Schawbl *et. al.*, 1988) and may therefore indicate which factors affect corticosterone levels and breeding success. Stressors identified in this way in grey ducks could then be removed or neutralised, thereby improving breeding success in grey duck and possibly other native waterfowl captive breeding programmes.

Samples were collected monthly for 8 months that included the breeding season in order to determine whether individual variation in initial corticosterone levels and responses to sampling stress among grey ducks was consistent. Hence, besides individual variation, monthly variation in initial corticosterone levels and responses to sampling stress was investigated in the grey duck. Examples of species demonstrating seasonal variation in corticosterone levels with higher levels during the breeding than the non-breeding season include the white crowned sparrow (*Zonotrichia leucophrys gambelii*, Wingfield and Farner, 1978), pied flycatcher (*Ficedula hypoleuca*, Silverin, 1979; Silverin and Wingfield, 1982), Harris hawks (*Parabuteo unicinctus*, Mays *et. al.*, 1991), male ring dove (*Streptopelia risoria*, Lea *et. al.*, 1986), starling (Dawson and Howe, 1983), free range chicken (Szelenyi *et. al.*, 1985) and male Pekin duck (*Anas platyrhynchos*, Assenmacher *et. al.*, 1975). The white-browed sparrow (*Plocepasser mahali*) is an example of a bird in which seasonal variation in corticosterone levels was absent (Wingfield *et. al.*, 1991).

Possible reasons for any seasonal variation in corticosterone levels include a diurnal phase shift in peak corticosterone levels, and gonadal and thyroid hormone influences. If a bird is always sampled at the same time each experimental day throughout the year then a shift of peak corticosterone levels from after sunset during winter to after sunrise during the breeding season, as in the white throated sparrow (*Zonotrichia albicollis*), could be interpreted as a seasonal variation in levels (Dusseau and Meier, 1971). Examples of species in which a diurnal rhythm of corticosterone levels has been demonstrated include the quail (Boissin and Assenmacher, 1970), turkey (El-Halawani *et. al.*, 1973; Proudman, 1991); pigeon (*Columbia livia*, Joseph and Meier, 1973), chicken (Wilson and Cunningham, 1981) and Khaki Campbell duck (Wilson *et. al.*, 1982).

Seasonal changes in gonadal or thyroidal hormone levels and their effects on corticosterone synthesis, release and metabolism could account for observed seasonal variations in corticosterone levels, though exactly what effects they impose is uncertain. Testosterone has been shown to have a stimulatory effect on plasma corticosterone or corticosterone binding

globulin (CBG) levels (Assenmacher *et. al.*, 1975; Assenmacher and Jallageas 1978; Ketterson *et. al.*, 1991) while other studies have shown an inhibitory effect of testosterone on the adrenal gland, ACTH, corticosterone or CBG levels (Chan and Phillips, 1973; Peczely, 1979; Carsia *et. al.*, 1983; Kovacs and Peczely, 1991). Thyroid hormones have been shown to decrease corticosterone levels in some studies (Assenmacher *et. al.*, 1975; Assenmacher and Jallageas, 1978; Gibson *et. al.*, 1986), while others demonstrate an opposite effect (Peczely, 1979; Kovacs and Peczely, 1991).

The corticosterone response to sampling stress was also investigated as well as monthly variation in corticosterone levels in the grey duck. In some situations a seasonal variation in the corticosterone response to a consistent stressor, such as blood sampling, has been demonstrated. For example, a reduced corticosterone response to capture and sampling stress has been shown in the garden warbler (*Sylvia borin*) prior to migration (Schwabl *et. al.*, 1991). Schwabl *et. al.*, (1991) suggested that a reduction in the corticosterone response to stress might avoid utilisation of the birds migratory energy stores that corticosterone would otherwise induce. Examples of birds where a reduced corticosterone response to a stressor was shown during the breeding season include the white crowned sparrow (*Zonotrichia leucophrys gambelii*, Wingfield *et. al.*, 1982; Wingfield, 1988), female inca doves (*Scardafella inca*), female Abert's towhees (*Pipilo aberti*), black-throated sparrows (*Amphispiza bilineata*), cactus wren (*Campylorhynchus brunneicapillus*) and the curve-billed thrasher (*Toxostoma curvirostre*, Wingfield *et. al.*, 1992). Greenberg and Wingfield (1987) suggested that resistance to a stressor, as demonstrated by a reduced corticosterone response, may be advantageous to birds in environments where a rise in corticosterone levels may disrupt reproductive functions long enough to cause the bird to miss a short breeding season.

The disruption of reproductive functions by increased corticosterone levels has been suggested by studies showing a correlation between increased corticosterone levels and decreased reproductive hormones on the male Pekin duck (Assenmacher *et. al.*, 1975) and tree sparrow (*Spizella arborea*, Wilson and Follett, 1975). The effect of exogenous corticosterone

administration on gonadal function varies between different seasons in the redheaded bunting (*Emberiza bruniceps*, Chaturvedi and Suresh, 1990). In the semipalmated sandpiper (*Calidris pusilla*), elevated corticosterone levels in response to sampling stress correlated with a decrease in testosterone levels in birds with initially high testosterone levels, while birds with initially low testosterone levels demonstrated an increase (Gratto-Trevor *et. al.*, 1991). Hence the exact effects of corticosterone upon reproductive function may vary between species, time of season and reproductive state (Wingfield, 1988).

The study described here had three main objectives. (1) Initial corticosterone levels and responses to sampling stress were measured in grey ducks across 8 months to investigate the consistency of any individual variation. (2) The collection of body weight and body condition data and reproductive, territorial and aggressive behavioural observations allowed the effects of, and possible reasons for, individual variation in corticosterone levels and responses to be discussed. (3) Initial corticosterone levels and responses to sampling stress of the grey ducks were measured for 8 months to determine whether there was any seasonal changes in these parameters.

## 3.2 Materials and methods

Five male and six female New Zealand grey ducks (*Anas superciliosa*) aged from 8 to 10 months old were used for this study. They had been reared at the Avian Physiology Unit, Massey University, New Zealand (40°23'S, 175°37'E), and had access to food (duck pellets, J.F. Cockrem, Massey University) and water *ad libitum*. They were held with two other male and one other female grey duck in an outdoor pen containing ponds and 8 nestboxes (Fig 4.01). All ducks had their primary feathers trimmed so as to inhibit flight. Monthly average ambient temperatures and daylength on experimental days were measured by the Grasslands division, Department of Scientific and Industrial Research, Palmerston North approximately 1 kilometre from the Avian Physiology Unit and are shown in figure 4.02.

### 3.2.1 Sample collection

Blood samples were obtained over a 8 month period from May to December, on the second week of each month, with sampling starting at 9.30am and finishing by 11.00am to avoid any diurnal rhythm effect. Male grey ducks were always sampled on the first experimental day each month, with females the following day. The same 5 male ducks were sampled throughout the study but a total of 6 female ducks were used as one of the females was exchanged for another female in August due to uncertainty about her sex (subsequent cloacal examination during the breeding season showed she was a female).

### 3.2.2 Variation between ducks in initial plasma corticosterone levels

To obtain blood samples from the grey ducks housed in an outdoor pen, all ducks were herded from the outdoor pen into an aviary where they were rounded up into a darkened confined space approximately 1m in diameter. Ducks of the sex needed were identified visually from coloured

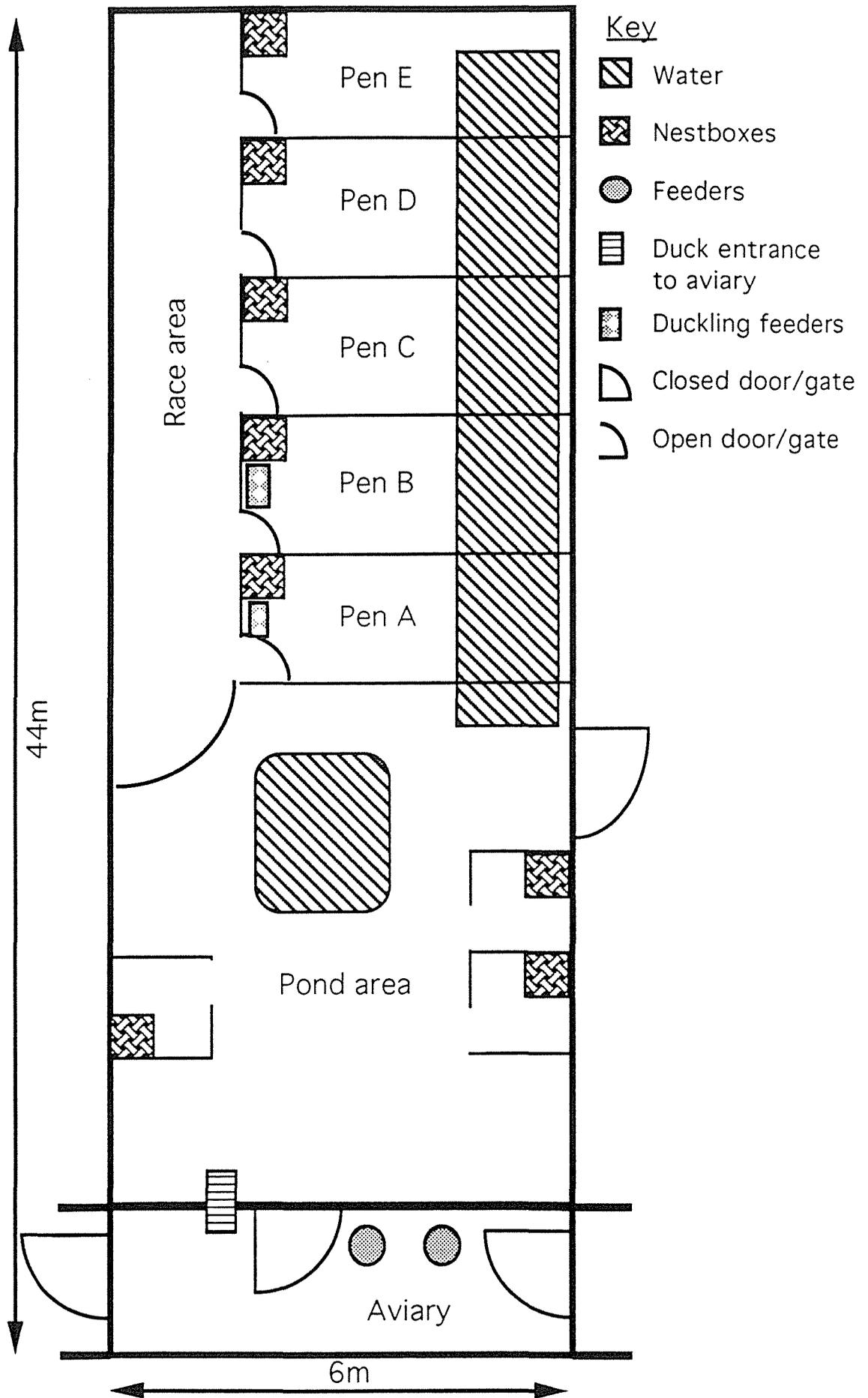


Figure 3.01: Outline of outdoor pen in which grey duck were held for duration of study

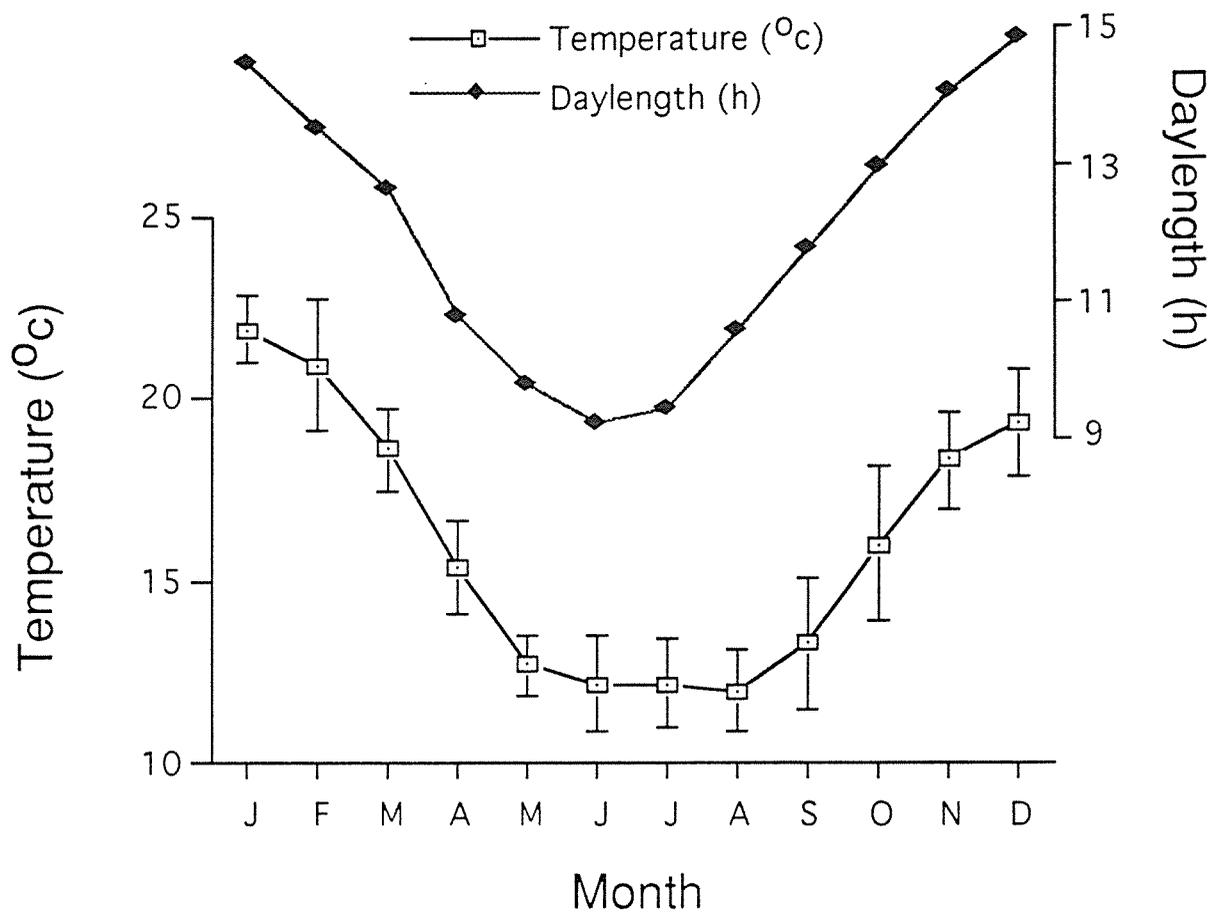


Figure 3.02: 1992 monthly average maximum temperatures and daylength at the Grasslands division of the Department of Scientific and Industrial Research, Palmerston North, New Zealand (40°23'S 175°37'E).

leg bands, individually picked up and taken to another room. A blood sample (0.5-2.0 ml) was immediately collected from the brachial vein was taken into an heparinised syringe, centrifuged and the plasma frozen for later analysis of corticosterone levels. All blood samples were obtained within 90 sec of the duck being picked up.

Female grey ducks #356 and #360 were incubating eggs, or rearing ducklings from October to December when samples were due for collection. These females could therefore not be herded from the outdoor pen to the aviary for sample collection. Blood samples were obtained from these females by removing them from their nest or the section of the outdoor pen reserved for them and their ducklings, but are excluded from the various analyses involving corticosterone levels as the method of obtaining these samples was different from that used with other ducks.

### 3.2.3 Variation between ducks in plasma corticosterone responses to sampling stress

After the first blood sample ducks were placed in individual darkened boxes (0.40 x 0.25 x 0.30m; length x width x height) and further samples collected every 20 min for 1 h. The samples collected from each duck at 0, 20, 40 and 60 min were analysed for plasma corticosterone levels for determination of the corticosterone response to sampling stress. All blood samples were obtained within 90 sec of the duck being picked up.

### 3.2.4 Measurement of social status, territorial area and body weight and condition

#### 3.2.4.1 Social status

Observations of both aggressive and reproductive behaviours were made once a week from the start of September to the second week in December. The grey duck were confined to the pond area of the outdoor pen (Fig 3.01) and aggressive and reproductive behaviours over a 1 h period

noted by a stationary observer.

Aggressive behaviour generally consisted of chasing and the occasional grabbing of feathers of one duck by another. This behaviour was taken to indicate that the chasing duck was dominant, and these observations were used to determine the relative social status of the male grey ducks. The female ducks did not interact enough amongst themselves, or with the male ducks, for any social status to be determined.

The reproductive behaviours noted were those described by Williams (1969) and were used to determine the pairing of male and female ducks. The events of the reproductive cycle of each pair were determined by both the weekly observations of reproductive behaviours and by daily checks on nestboxes.

#### 3.2.4.2 Territorial area

Three times a week from the start of September to the second week in December the position of each individual grey duck in the outdoor pen was noted by an observer in the morning. This was done with the aid of a hide some 10 m away from the pen, allowing the observation of individual sections of the pen without the ducks being aware of the observer.

#### 3.2.4.3 Body weight and condition

The grey duck were weighed monthly from July to December after sampling had been completed. The body condition of the grey ducks was subsequently calculated using an index which incorporated both body weight and winglength measurements.

$$\text{Condition Index} = \frac{\text{Body Weight (Kg)}}{\text{Winglength (m)}^3}$$

Winglength was measured from the elbow joint to the end of the last primary feather. For the relationship between corticosterone levels and both

body weight and condition index, only initial corticosterone values in the first sample from each duck were used.

Body condition indices are only available for 2 of the 6 female grey ducks because the measurement of winglength was done in January of the year following the completion of the study when only 2 of the female grey duck remained at the Avian Physiology Unit.

### 3.2.5 Measurement of plasma corticosterone

Corticosterone levels were measured in plasma samples using the procedure outlined in Chapter 2, Section 2.2.3. As stated previously, the intra- and inter-assay coefficients of variation were 6.5% and 9.3% respectively, and the minimal detectable corticosterone concentration was 0.40 ng/ml.

### 3.2.6 Statistical analyses

The relationship between corticosterone levels and the time taken to obtain a blood sample was examined using regression analysis. Effects of sampling order on corticosterone levels was examined using the Kruskal-Wallis nonparametric ANOVA.

Initial corticosterone levels in each duck were compared between individual ducks using independent one-way ANOVA. Relationships between initial corticosterone levels and either body weight or condition were examined using regression analysis.

The presence of any monthly variation in initial corticosterone levels across the 8 months of sampling was examined by an independent one-way analysis of variance (ANOVA).

The difference between male and female grey duck in initial corticosterone levels was investigated by Students T-Test.

The effect of time, or sampling stress, on corticosterone levels within each group of ducks was investigated by one-way repeated measures ANOVA with Bonferroni adjusted probability levels used for subsequent

post-hoc tests. Where the Bartlett's test indicated heterogeneity of variance the effect of sampling stress on corticosterone levels within each group was investigated by using Friedman nonparametric ANOVA (Wilkinson, 1990).

Log transformed data were used for statistical analyses. Summary data are shown as mean  $\pm$  standard error of the mean (SEM).

### 3.3 Results

#### 3.3.1 Individual variation in initial plasma corticosterone levels and responses to sampling stress

##### 3.3.1.1 Individual variation in plasma corticosterone levels

None of the male grey ducks had consistently lower initial corticosterone levels than any other duck (Fig 3.03). For female grey ducks, duck #360 consistently had the lowest initial corticosterone levels each month from July to September (Fig 3.04) with all levels below 4 ng/ml. When sampled directly from their nest or during the rearing of ducklings corticosterone levels remained below 12 ng/ml for both ducks #356 and 360 in October to December.

Mean corticosterone levels in the first sample (Fig 3.05) varied significantly between female ducks ( $p < 0.001$ ), while differences between males approached significance ( $p = 0.068$ ).

##### 3.3.1.2 Individual variation in the plasma corticosterone response to sampling stress

The corticosterone response to sampling stress is defined by the corticosterone levels in blood samples collected at 20 min intervals for 1 h from each grey duck. Duck #355 and duck #360 had the smallest and most consistent responses of the male and female grey ducks respectively (Fig 3.06 and 3.07).

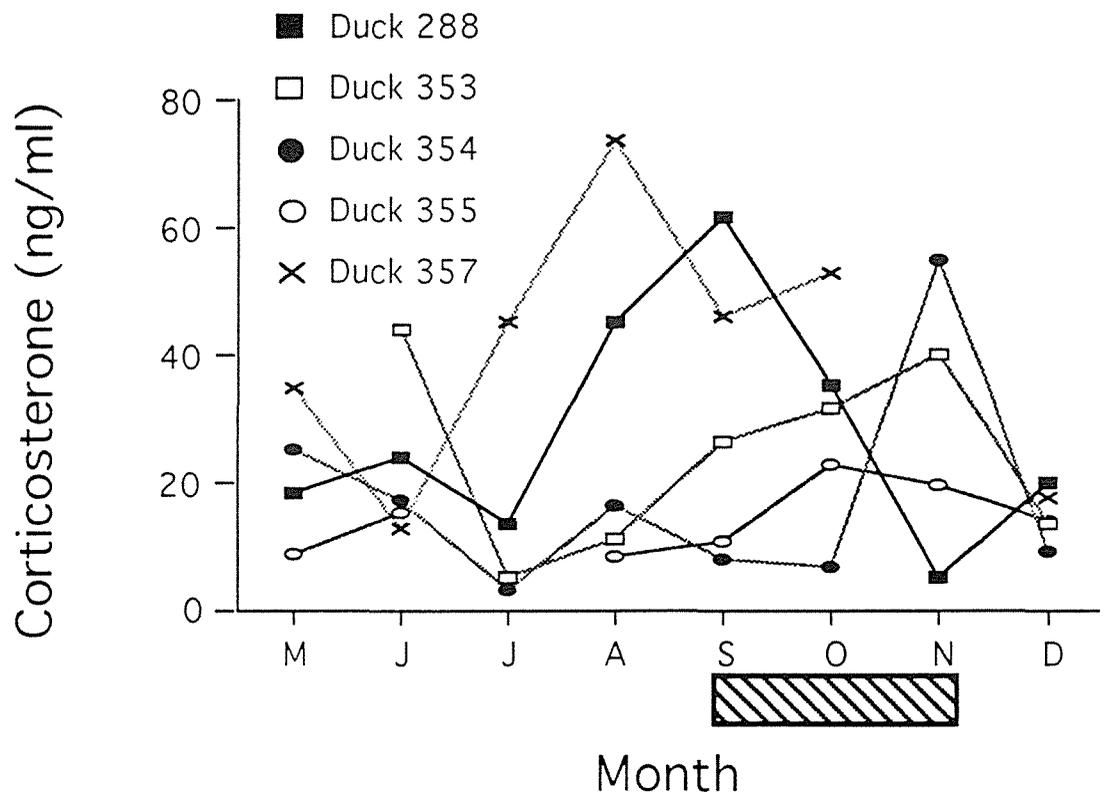


Figure 3.03: Plasma corticosterone levels in 5 male grey ducks sampled monthly from May to December. Horizontal bar indicates breeding season.

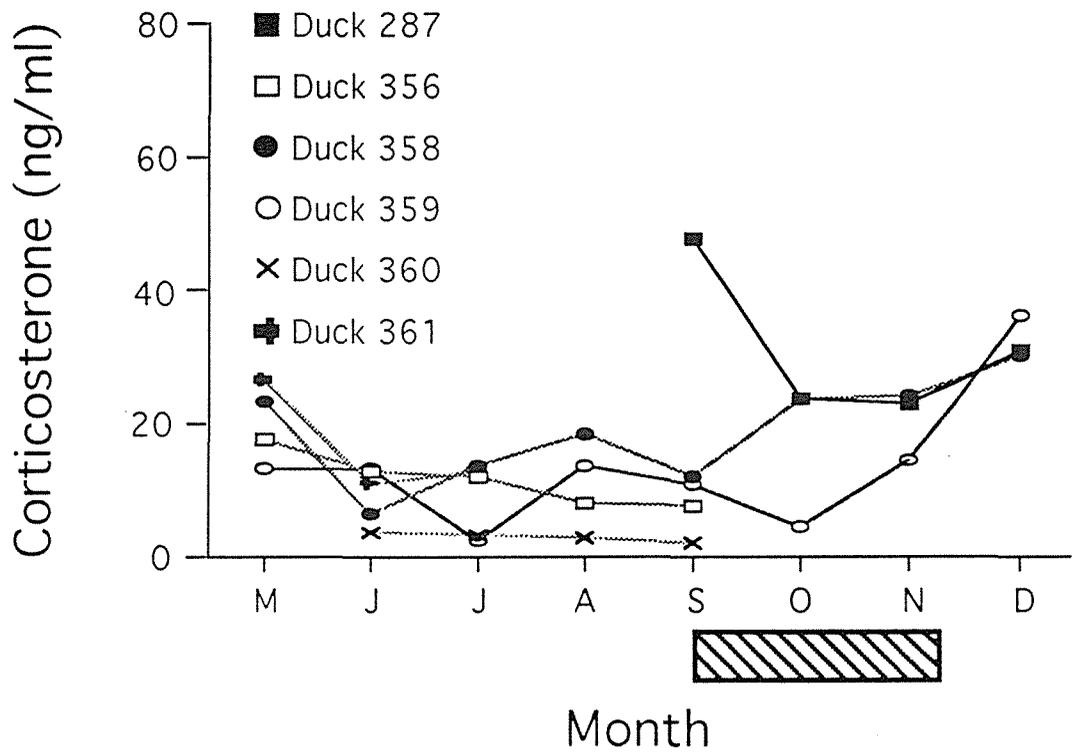


Figure 3.04: Plasma corticosterone levels in 6 female grey ducks sampled monthly from May to December. Horizontal bar indicates breeding season.

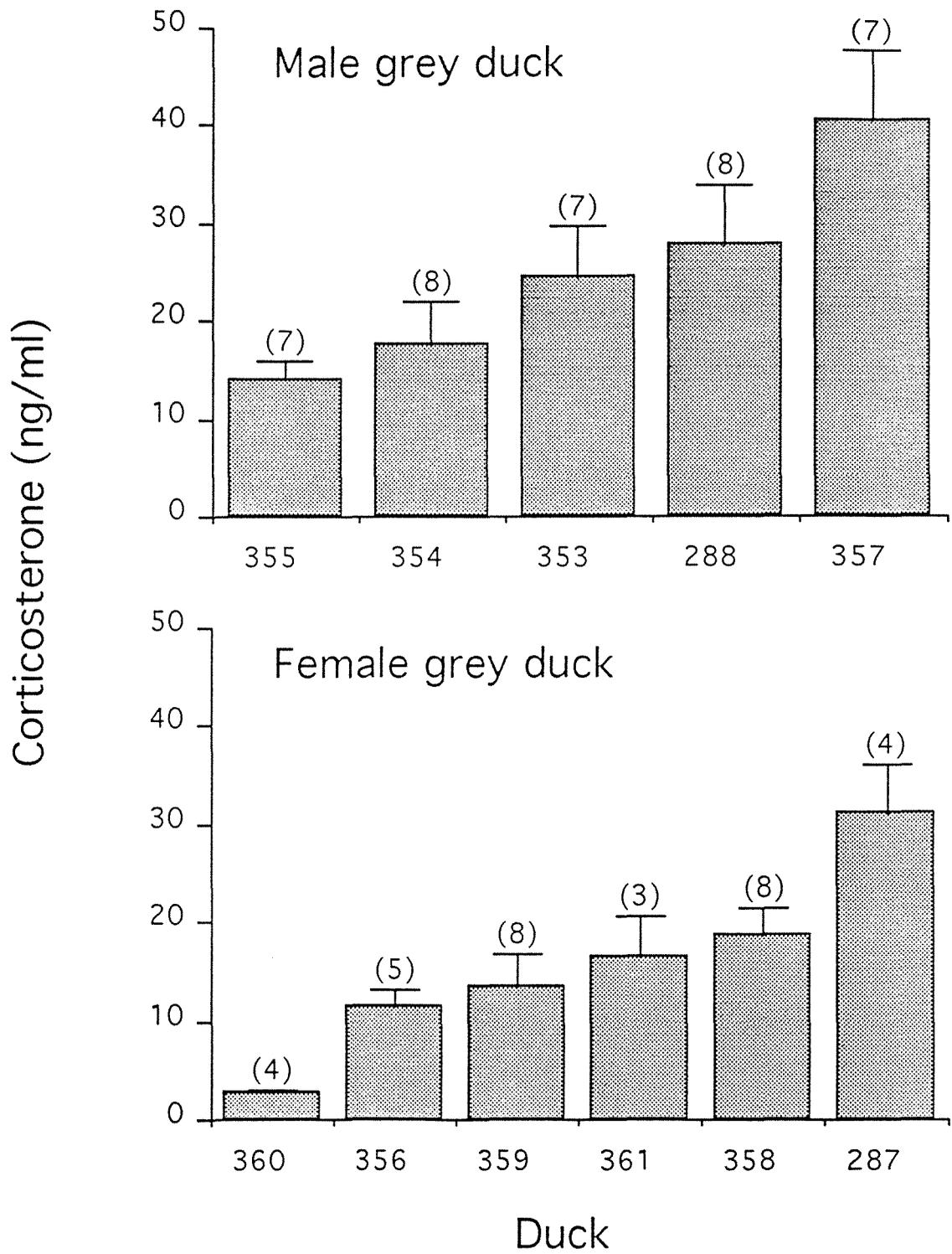


Figure 3.05: Mean plasma corticosterone levels in 5 male and 6 female grey ducks. Number of samples collected from each duck are in brackets above each column.

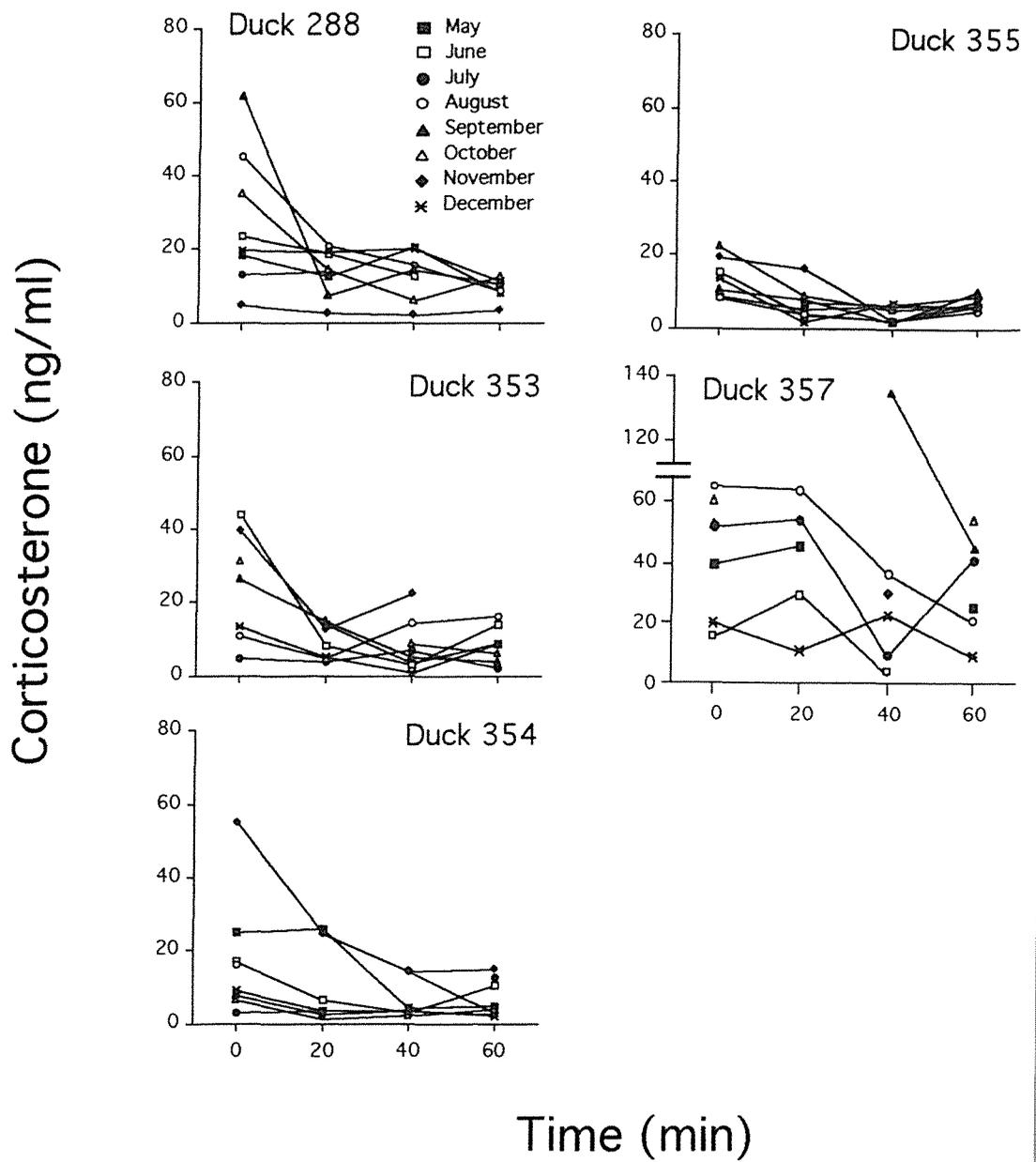


Figure 3.06: Plasma corticosterone levels in 5 male grey ducks sampled at 20 min intervals for 1 h monthly from May to December.

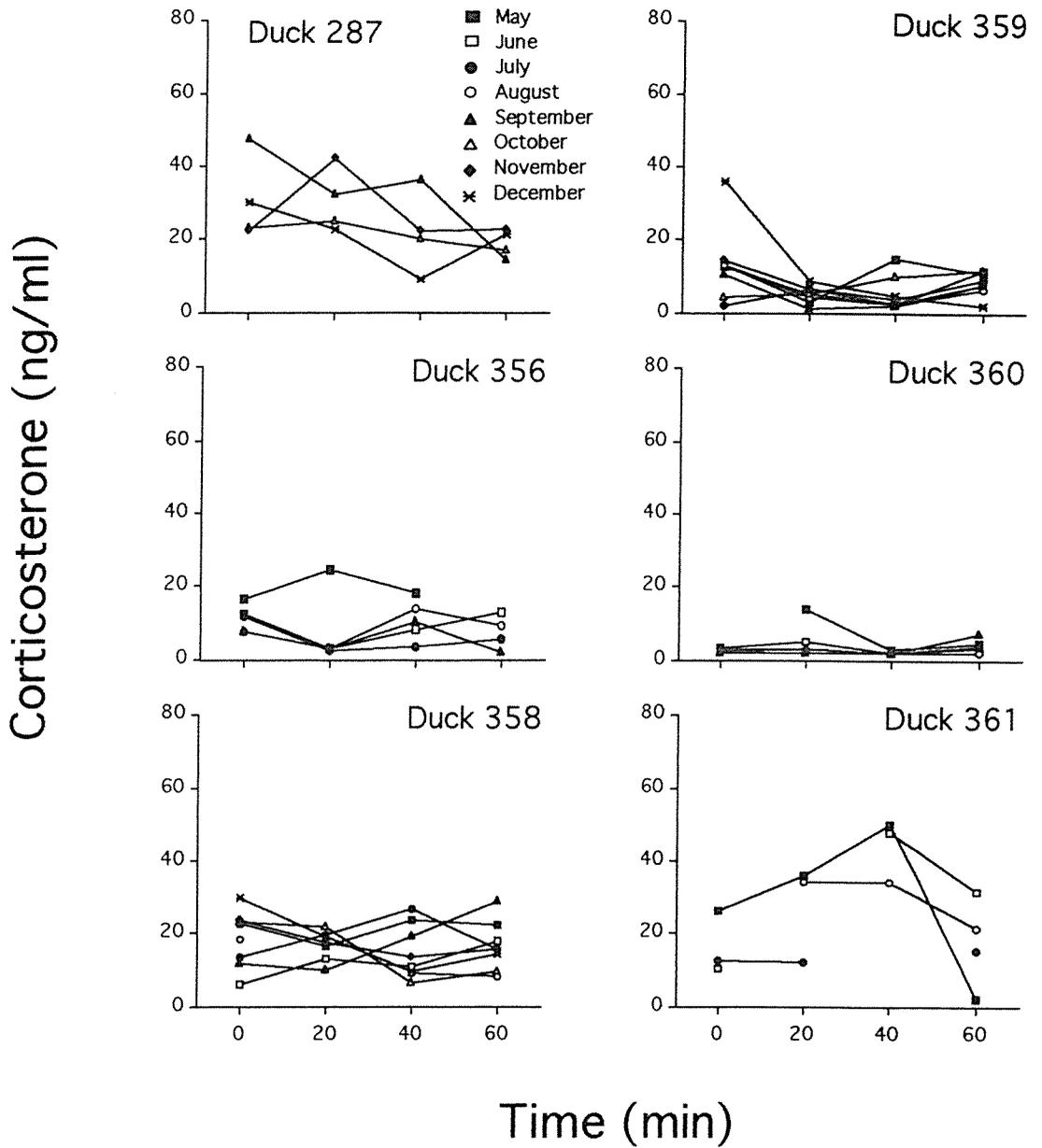


Figure 3.07: Plasma corticosterone levels in 6 female grey ducks sampled at 20 min intervals for 1 h monthly from May to December.

### 3.3.2 Possible causes of the variation in plasma corticosterone levels and responses to sampling stress

#### 3.3.2.1 Effects on plasma corticosterone levels of the time taken to obtain a blood sample and of the order of sampling

There was no effect of time on corticosterone levels when samples were collected within 90 sec of a duck being picked up ( $p=0.341$ ; Fig 3.08). There was also no relationship between the order in which a duck was picked up for sampling and its corticosterone level ( $p=0.205$ ; Fig 3.09), so the removal of a duck from its group did not have any significant effect on the corticosterone levels in the remaining ducks of that group.

#### 3.3.2.2 Relationship between plasma corticosterone levels and the social status of individual male grey duck

The social status of male grey duck was generally consistent during the breeding season months of September to November (Fig 3.10). A comparison of the social status of individual males and corticosterone levels in the first sample did not reveal any clear relationship. For example the most submissive male duck (#353) had the median corticosterone level of the group of male ducks sampled during the months of September to November (Fig 3.03). The most aggressive duck (#288) had the highest and fourth highest corticosterone levels in September and October respectively before having the lowest level in November which coincided with a fall in social status. The duck (#355) who replaced #288 as the most aggressive duck in November had little change in corticosterone levels in November compared with levels in September and October.

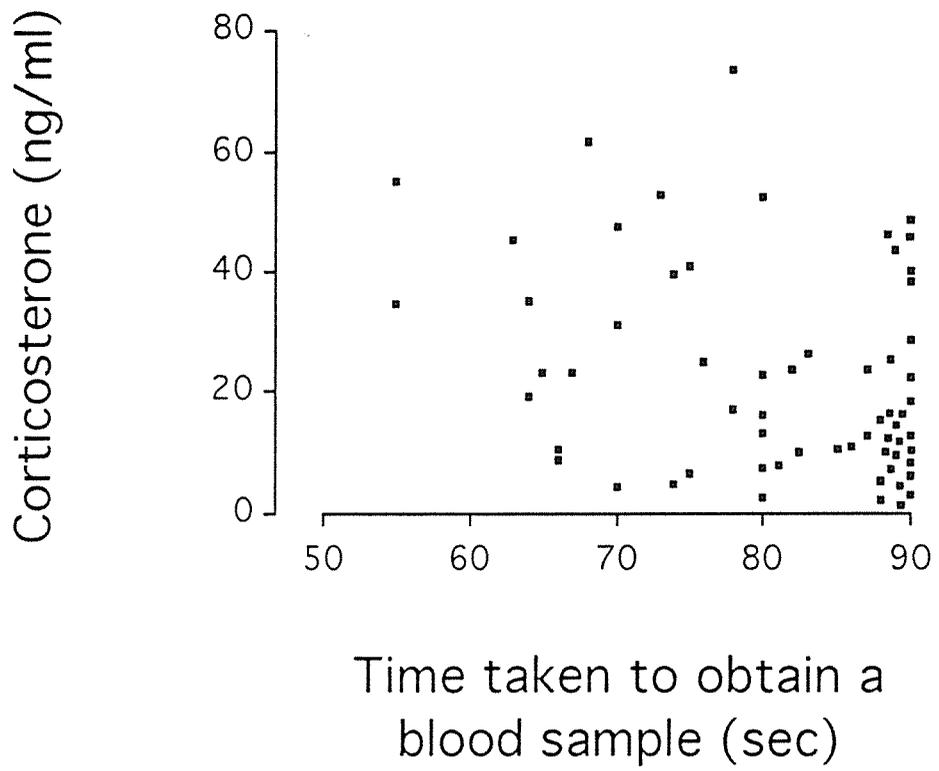


Figure 3.08: Relationship between plasma corticosterone levels and the time taken to obtain the first blood sample in grey ducks.

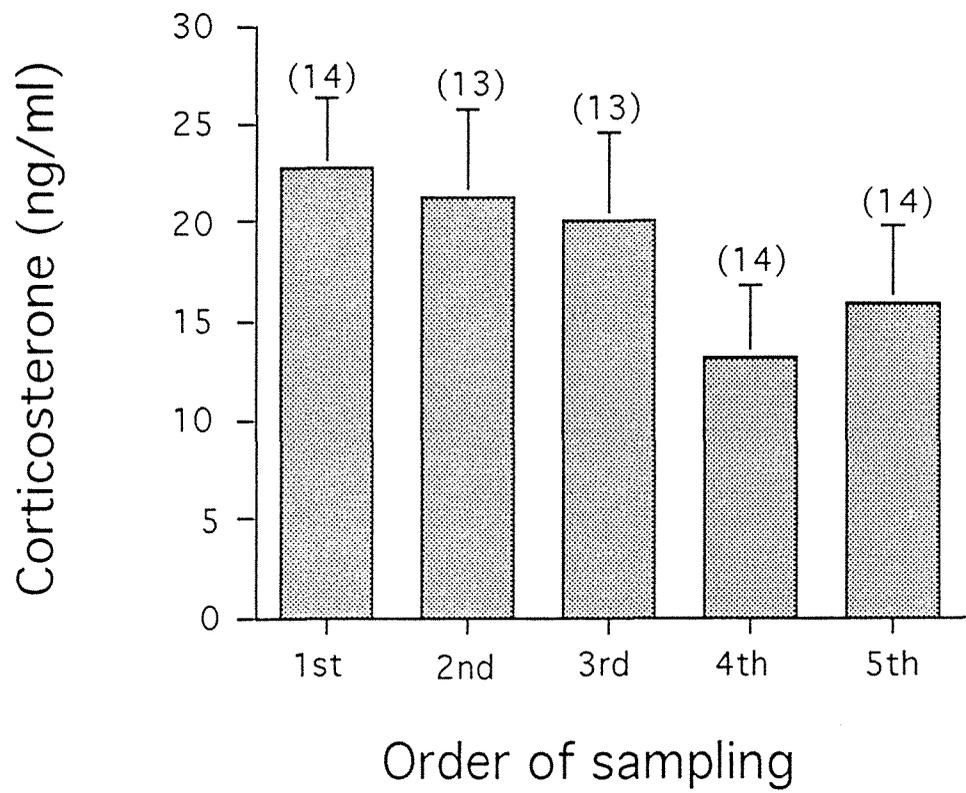


Figure 3.09: Relationship between plasma corticosterone levels in the first sample and the order of sampling in grey ducks.

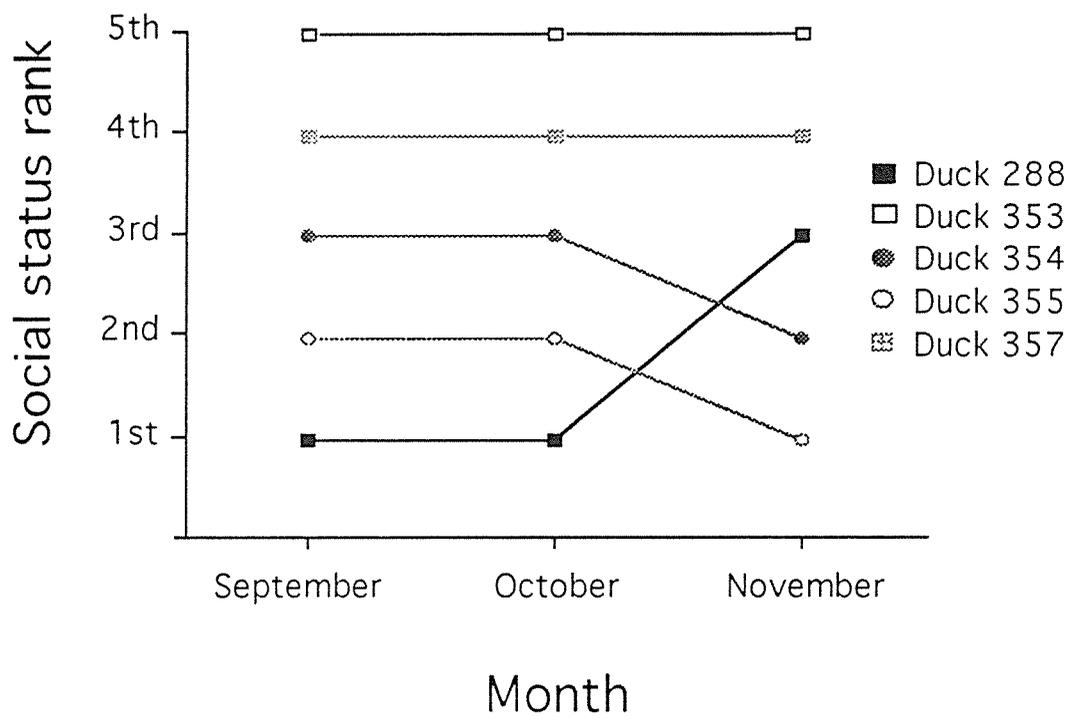


Figure 3.10: Social status or "pecking order" of individual male grey ducks during the breeding season months of September to November.

### 3.3.2.3 Relationship between plasma corticosterone levels and the territorial area of individual grey duck

The area closest to the feeders (the pond area) appears to have been the domain of male ducks #288, 353 and 355 during September, prior to any eggs been laid (Fig 3.11). The range of corticosterone levels in these ducks in September was similar to the range of levels in the other two ducks (Fig 3.03).

The pond area was also frequented by female ducks #356 and 360 during September (Fig 3.12), shortly before these females laid eggs in nestboxes in this area. Ducks #356 and 360 had lower corticosterone levels than the remaining female ducks when sampled in September (Fig 3.04).

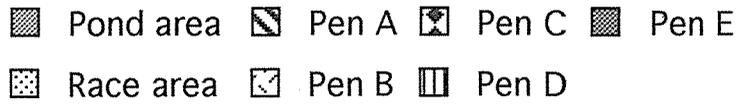
The removal of ducks #356 and 360 and ducklings from nestboxes in the pond area to pens A and B reserved for rearing ducklings coincided with a decrease in the proportion of time the male ducks #288, 353 and 355 spent in the pond area (Fig 3.11). There was no consistent change in corticosterone levels associated with this change in territorial behaviour during November and December (Fig 3.03).

### 3.3.2.4 Relationship between plasma corticosterone levels and the body weight and condition of grey duck

There was a weak inverse relationship between corticosterone levels in the first sample and body weight (Fig 3.13) in male ( $p < 0.01$ ) and female grey duck ( $p < 0.05$ ). For the females, the omission of the record from #360 in September (1.29 kg; less than 1 week before egg laying) changes the correlation coefficient to  $r^2 = 0.217$ .

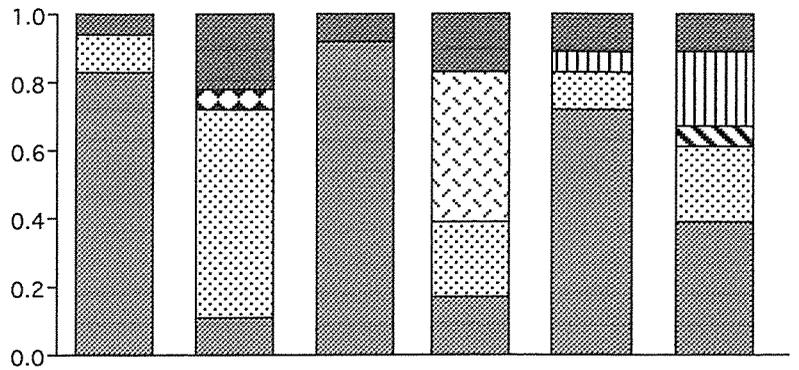
There was a weak inverse relationship between corticosterone levels in the first sample and condition index in male ducks ( $p < 0.05$ ; Fig 3.14). Condition indices were only available for the 2 female ducks that produced clutches (Fig 3.14).

# Male grey duck

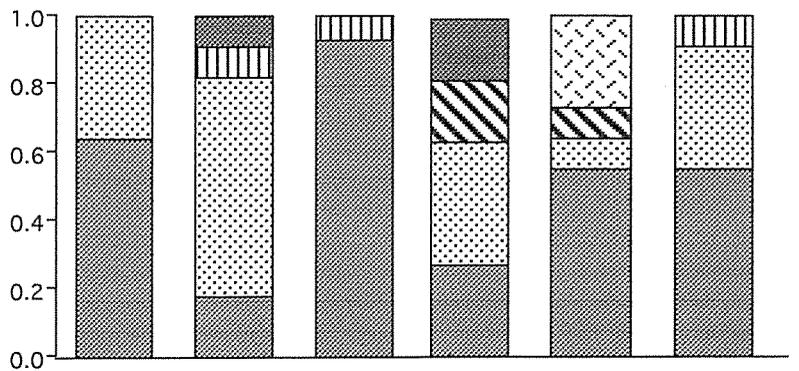


Proportion of times observed in area

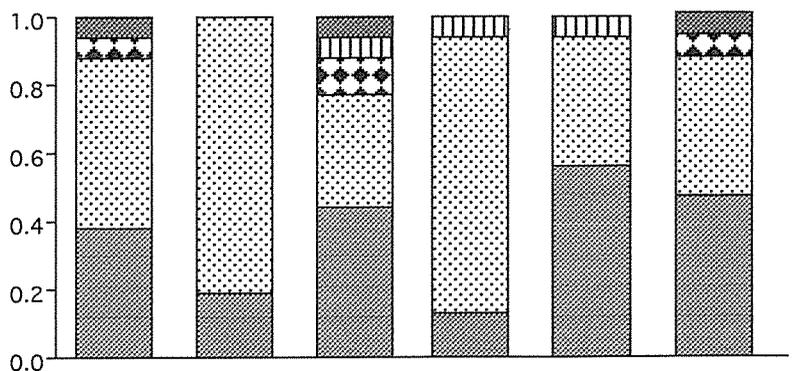
Prior to any eggs being laid (n=18)



During incubation of eggs (n=11)



During rearing of ducklings (n=16)



288      339      353      354      355      357

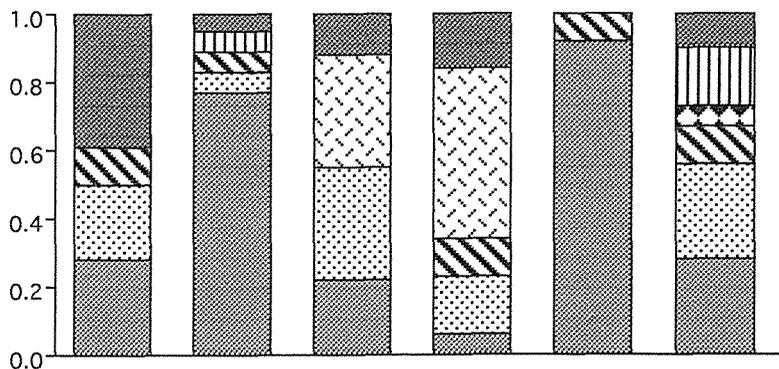
Duck

Figure 3.11: The proportion of times male grey duck were observed in various areas of the outdoor pen (as described in figure 3.01). Number of observations made per duck (n) are in brackets above columns.

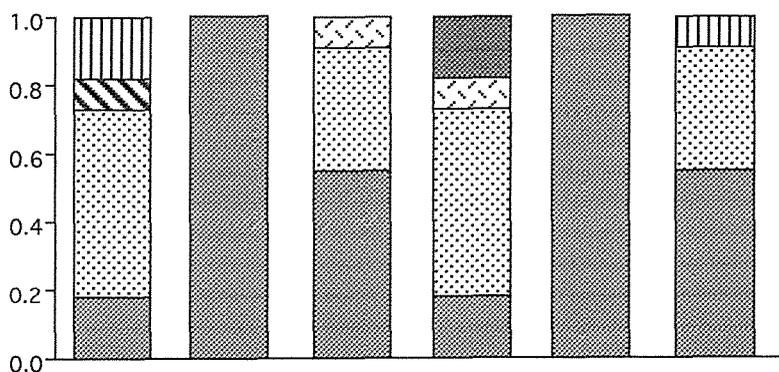
# Female grey duck

Pond area  
  Pen A  
  Pen C  
  Pen E  
 Race area  
  Pen B  
  Pen D

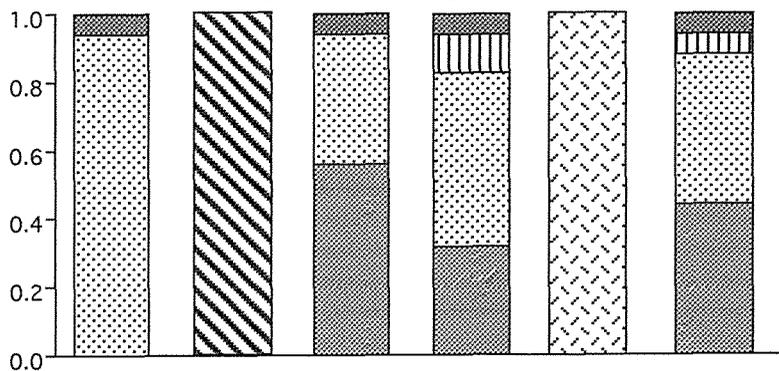
Prior to any eggs being laid (n=18)



During incubation of eggs (n=11)



During rearing of ducklings (n=16)



287      356      358      359      360      361

Duck

Proportion of times observed in area

Figure 3.12: The proportion of times female grey duck were observed in various areas of the outdoor pen (as described in figure 3.01). Number of observations made per duck (n) are in brackets above columns.

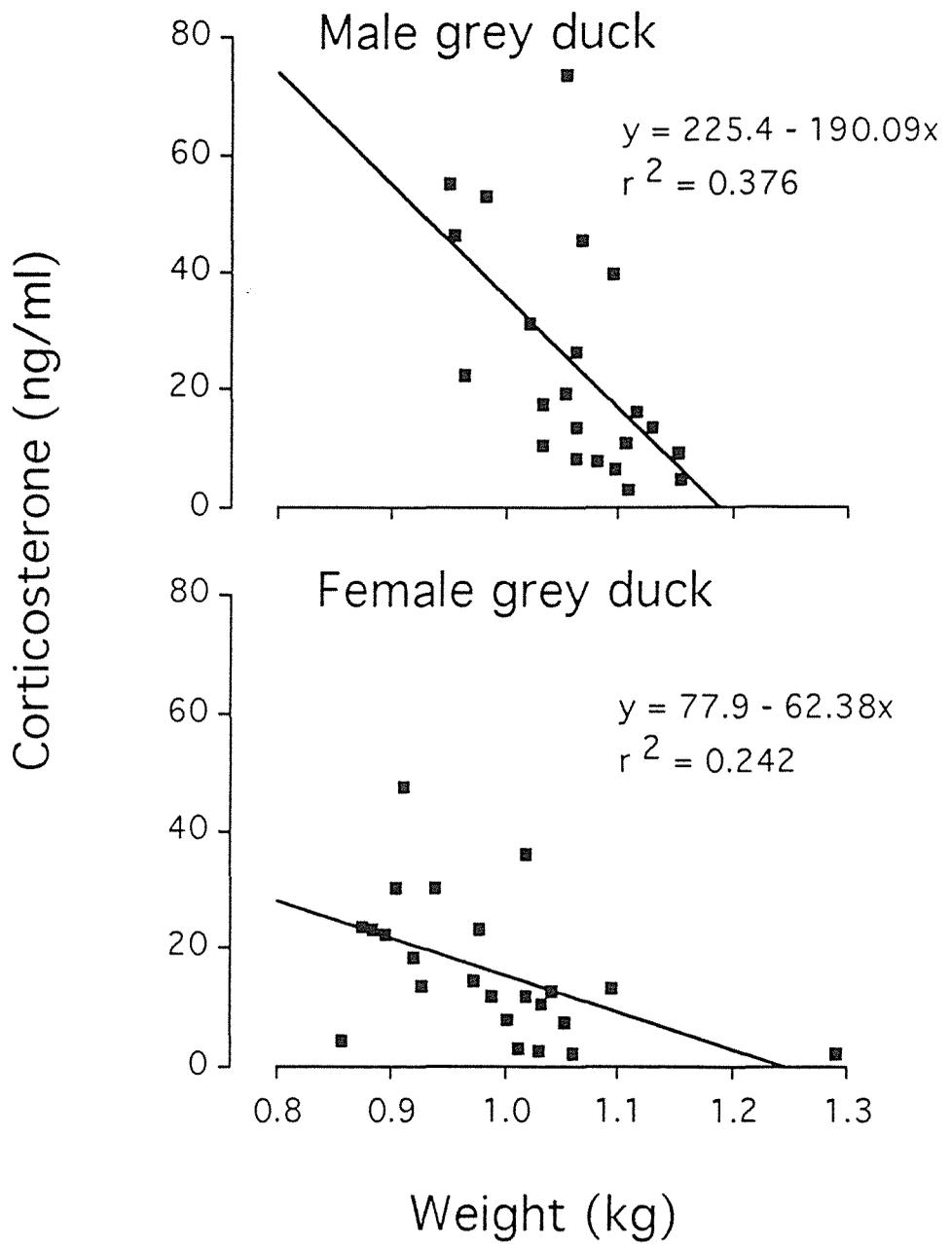


Figure 3.13: Relationship between plasma corticosterone levels in the first blood sample and body weight in male and female grey ducks.

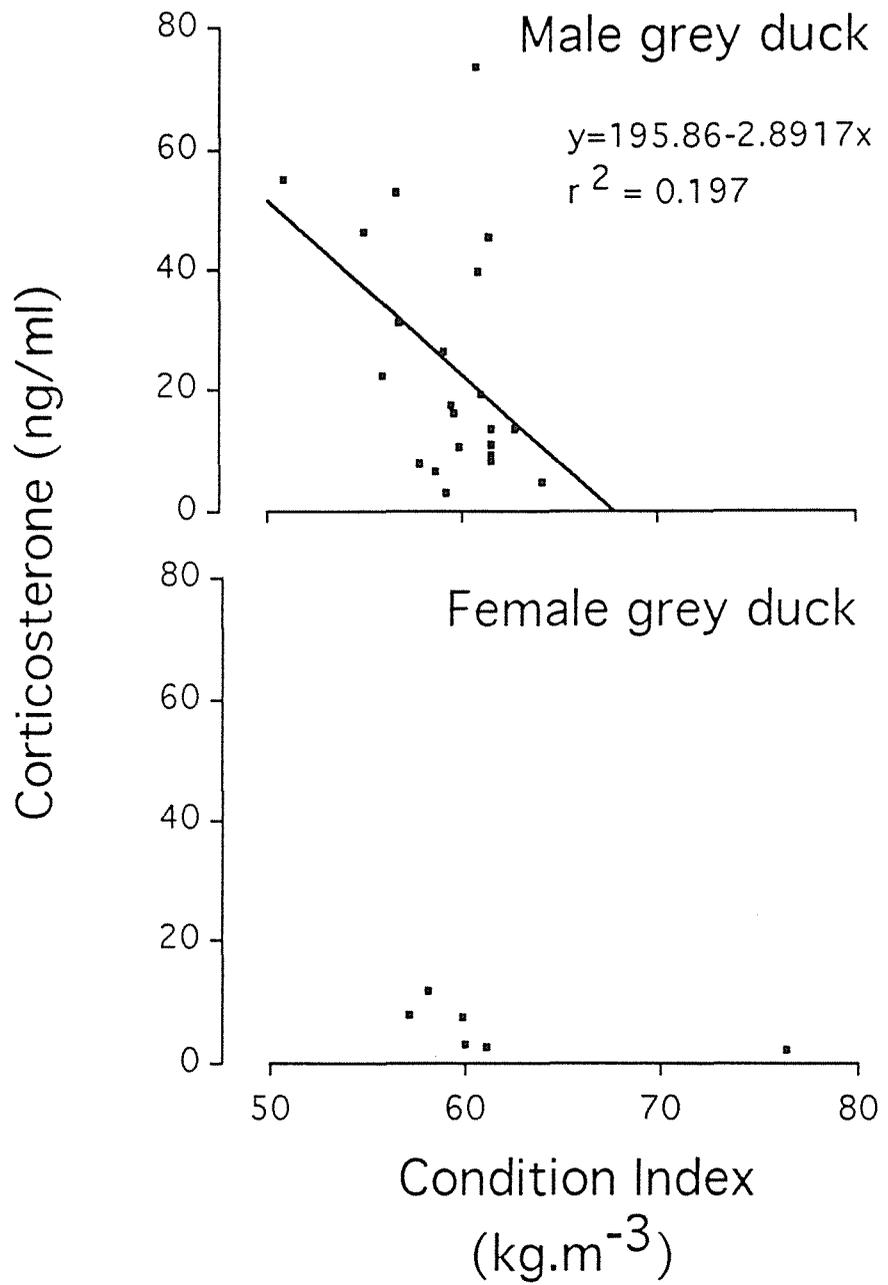


Figure 3.14: Relationship between plasma corticosterone levels in the first blood sample and body condition indices in male and female grey ducks.

### 3.3.3 Monthly variation in plasma corticosterone levels and responses to sampling stress

#### 3.3.3.1 Monthly variation in plasma corticosterone levels

There was no significant monthly variation in corticosterone levels in the first blood samples obtained from male grey ducks ( $p=0.926$ ; Fig 3.15). Similarly, there were no clear trends in corticosterone levels in individual ducks (Fig 3.03).

There was significant variation between months in corticosterone levels in the first sample obtained from female grey ducks ( $p<0.05$ ; Fig 3.15). However, the removal of ducks #356 and 360 from the calculation of group means from October onwards may have caused the increase in mean levels from August to December.

Corticosterone levels in the first sample obtained from grey duck were significantly higher in males than females when data for all 8 months were combined ( $p<0.05$ ; Fig 3.16). Corticosterone levels in June were significantly higher ( $p<0.05$ ) in males ( $22.58 \pm 5.63$  ng/ml) than in females ( $9.12 \pm 1.85$  ng/ml).

#### 3.3.3.2 Monthly variation in the plasma corticosterone response to sampling stress

In the male grey duck a significant drop in mean corticosterone levels over 1 h occurred in June, September, October and December, though only in June was this drop sustained throughout the sampling period (Fig 3.17). Large variation between individual male ducks in their corticosterone response to sampling stress occurred throughout the 8 months, obscuring any possible monthly variation in the response (Fig 3.18).

In female grey duck December was the only month in which mean corticosterone levels fell below 0 min levels during the 1 h sampling period, this drop occurring 40 min from initial sampling (Fig 3.19). The reduction in group size from 5 to 3 from October onwards and the large variation

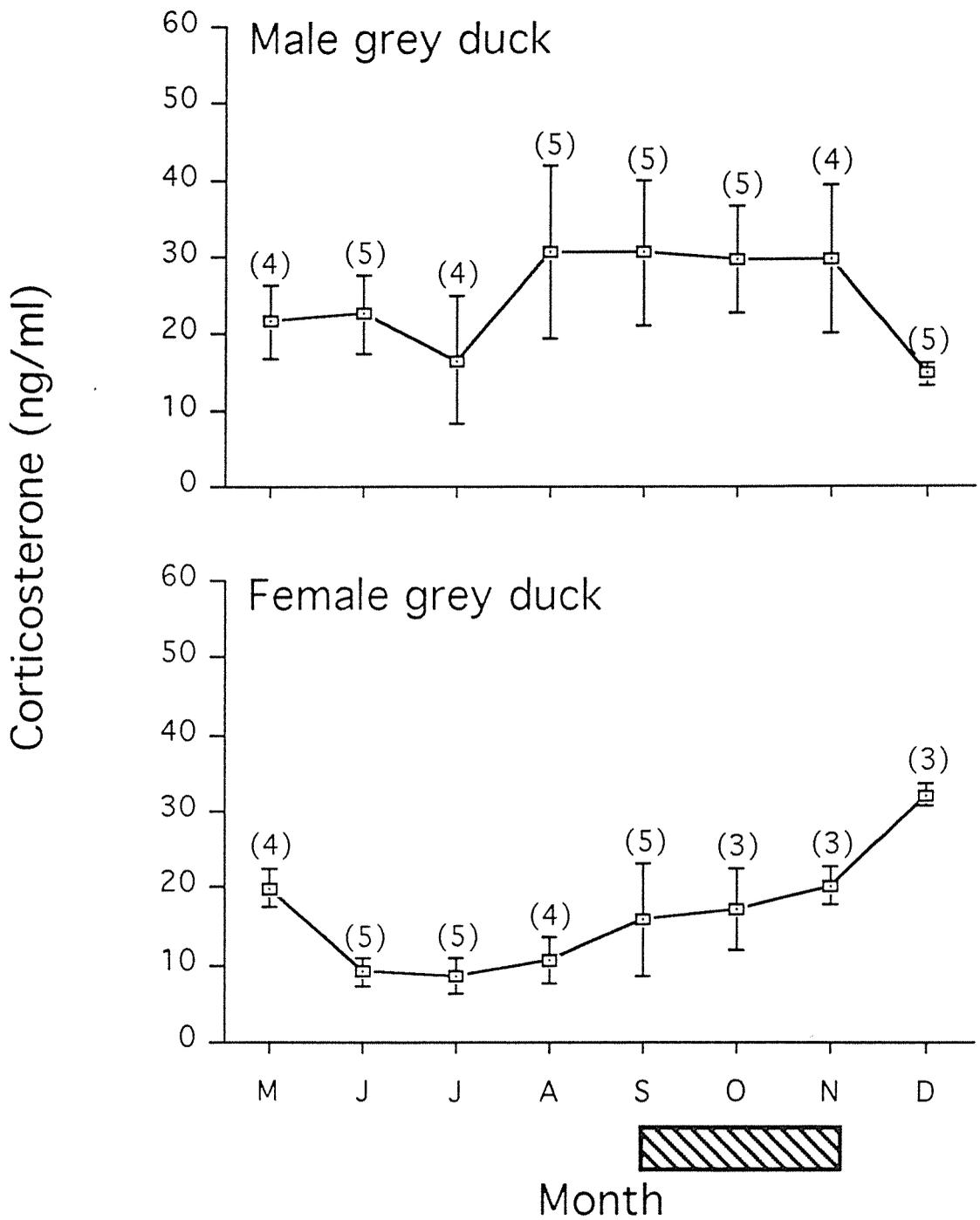


Figure 3.15: Mean plasma corticosterone levels in male and female grey ducks sampled every month from May to December. Group composition changed from October to December with the removal of the two female clutch producing ducks #360 and 356. Horizontal bar indicates breeding season.

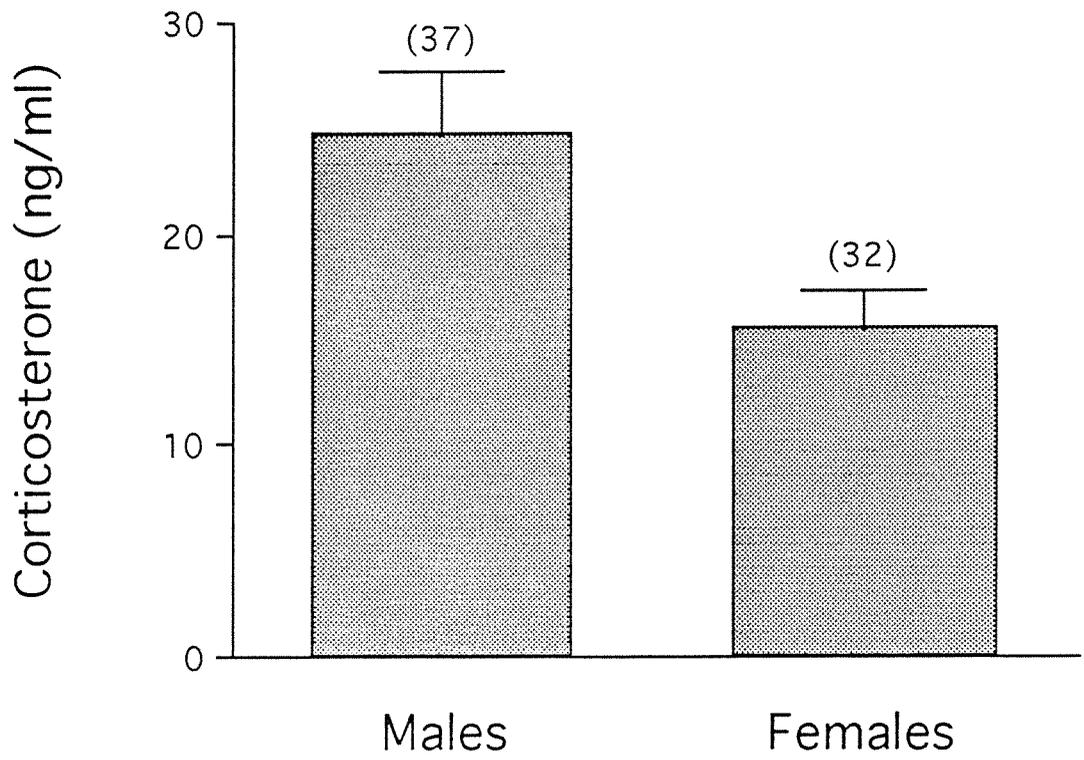


Figure 3.16: Mean plasma corticosterone levels in male and female grey ducks sampled from May to December. Number of ducks sampled are in brackets above columns.

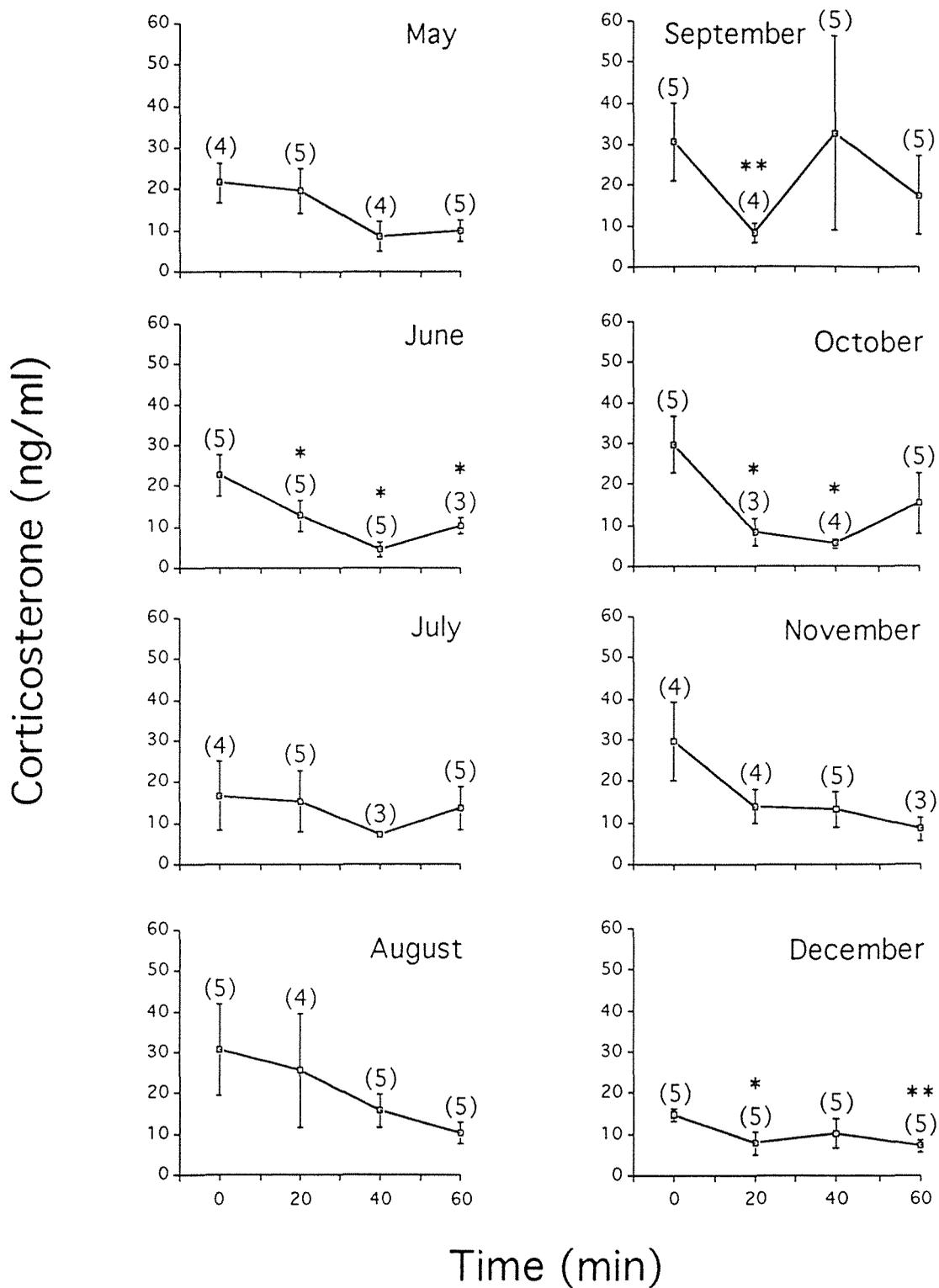


Figure 3.17: Mean plasma corticosterone levels in male grey ducks sampled at 20 min intervals for 1 h every month from May to December. Number of ducks sampled are in brackets above error bars. Statistics relate to changes in corticosterone from starting levels. \* =  $p < 0.05$ , \*\* =  $p < 0.01$  and \*\*\* =  $p < 0.001$ .

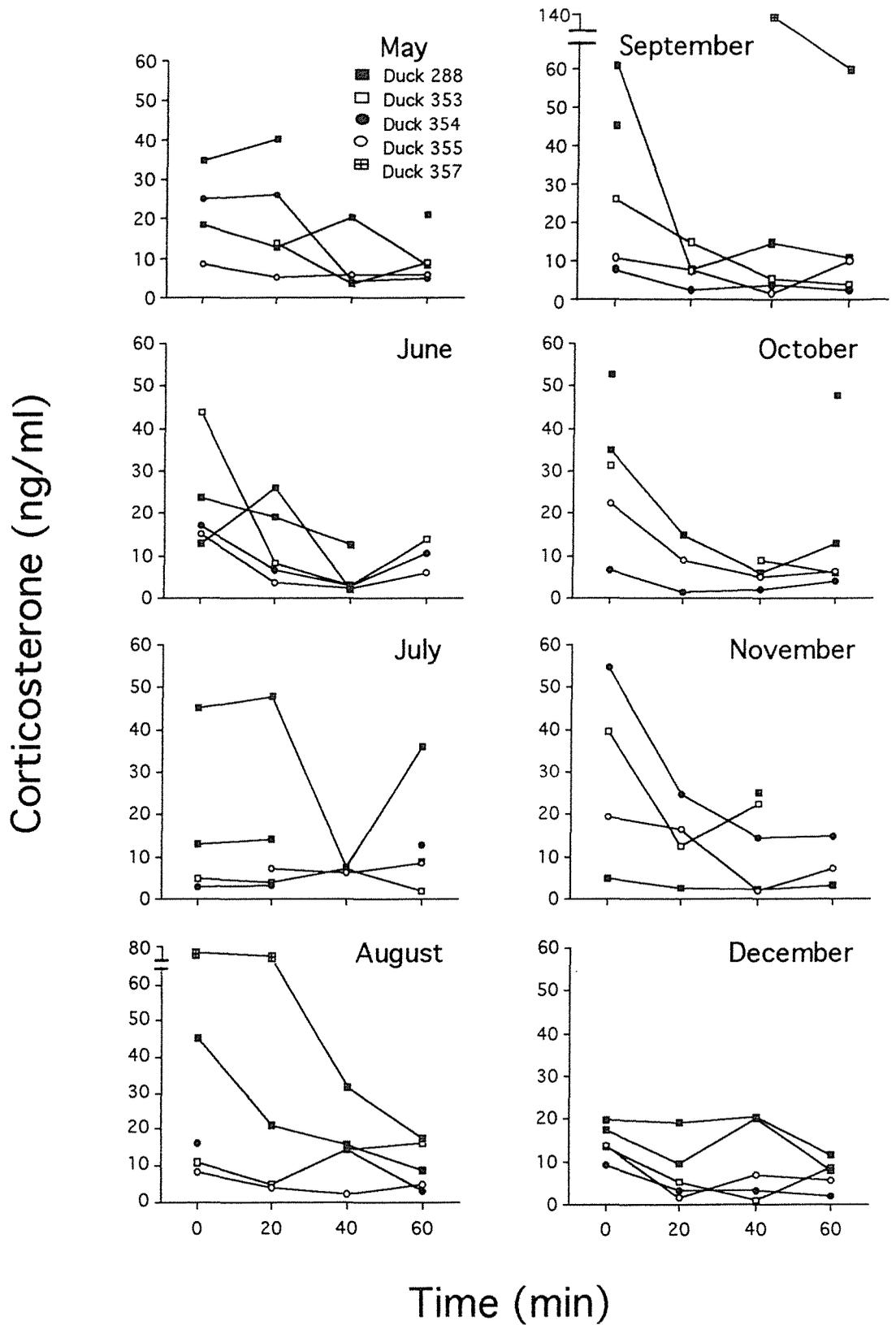


Figure 3.18: Plasma corticosterone levels in male grey ducks sampled at 20 min intervals for 1 h from May to December.

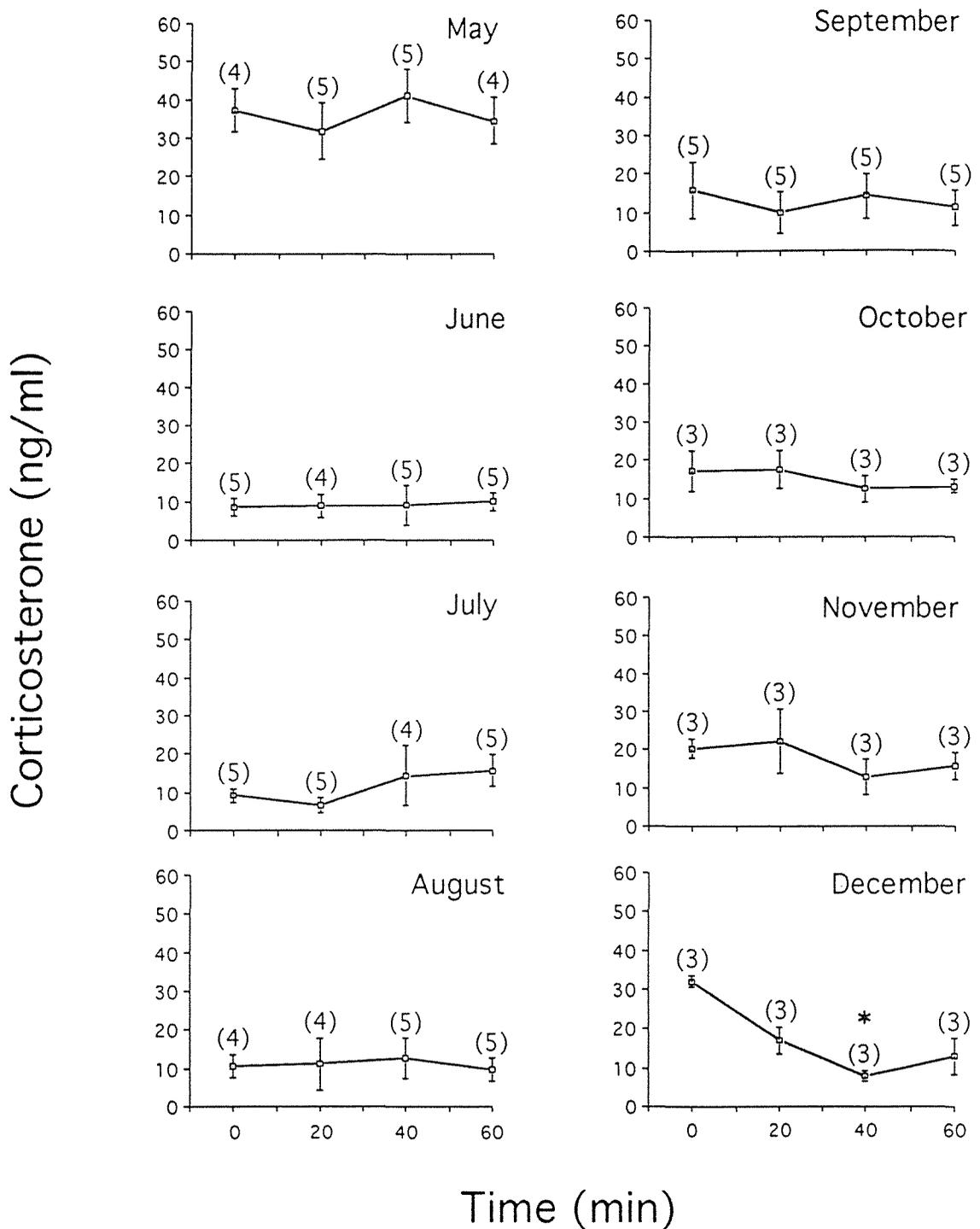


Figure 3.19: Mean plasma corticosterone levels in female grey ducks sampled at 20 min intervals for 1 h every month from May to December. Group composition changed from October to December with the removal of the two clutch producing ducks #356 and 360. Number of ducks sampled are in brackets above error bars. Statistics relate to changes in corticosterone from starting levels.

between individual female ducks obscures any possible monthly variation in the corticosterone response to sampling stress (Fig 3.20).

Sampling of the ducks #356 and 360 directly from their nest in October or from the section of the pen used for the rearing of ducklings in November and December yields larger corticosterone responses than measured in May to September (Fig 3.21).

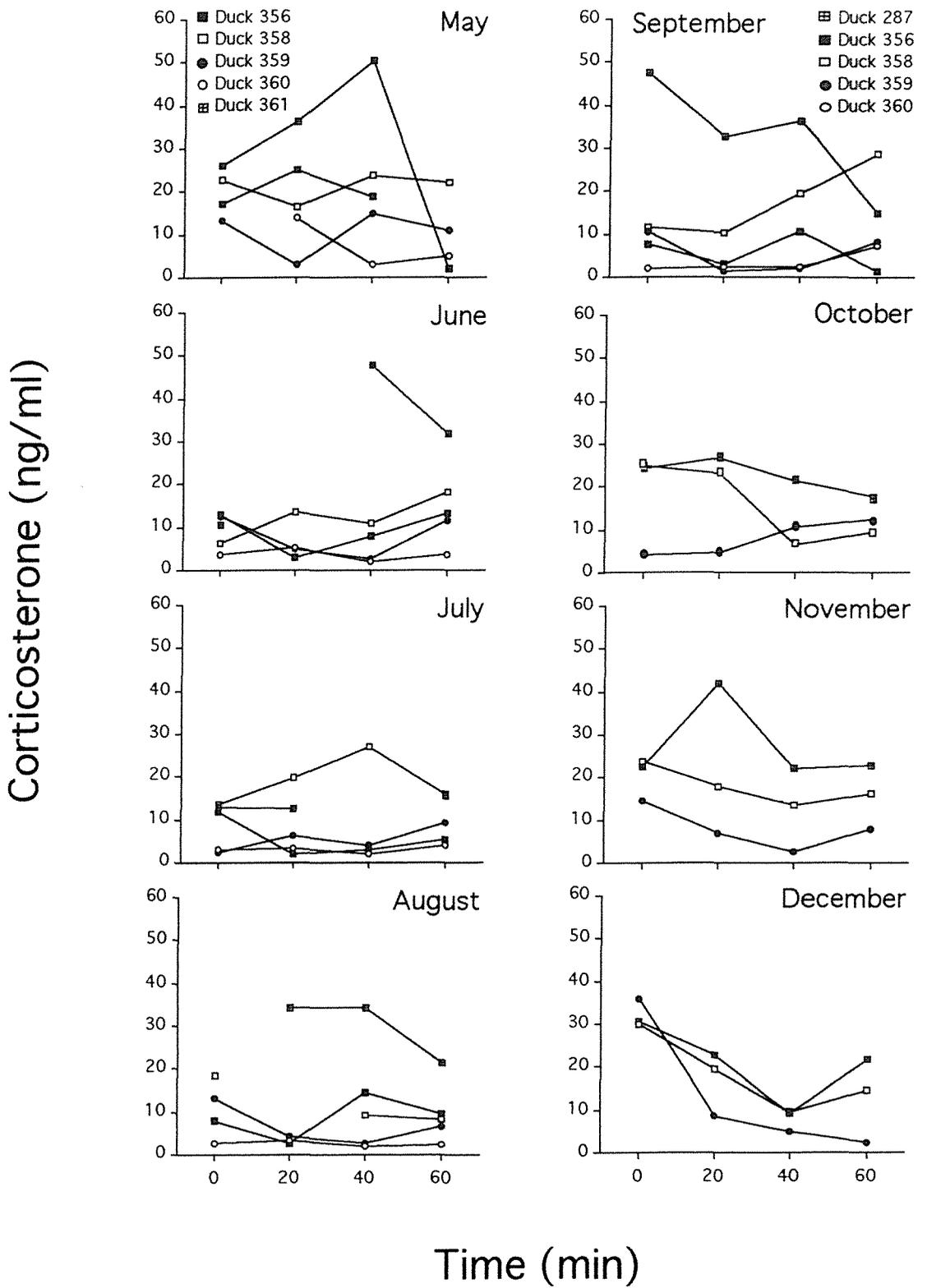


Figure 3.20: Plasma corticosterone levels in female grey ducks sampled at 20 min intervals for 1 h from May to December.

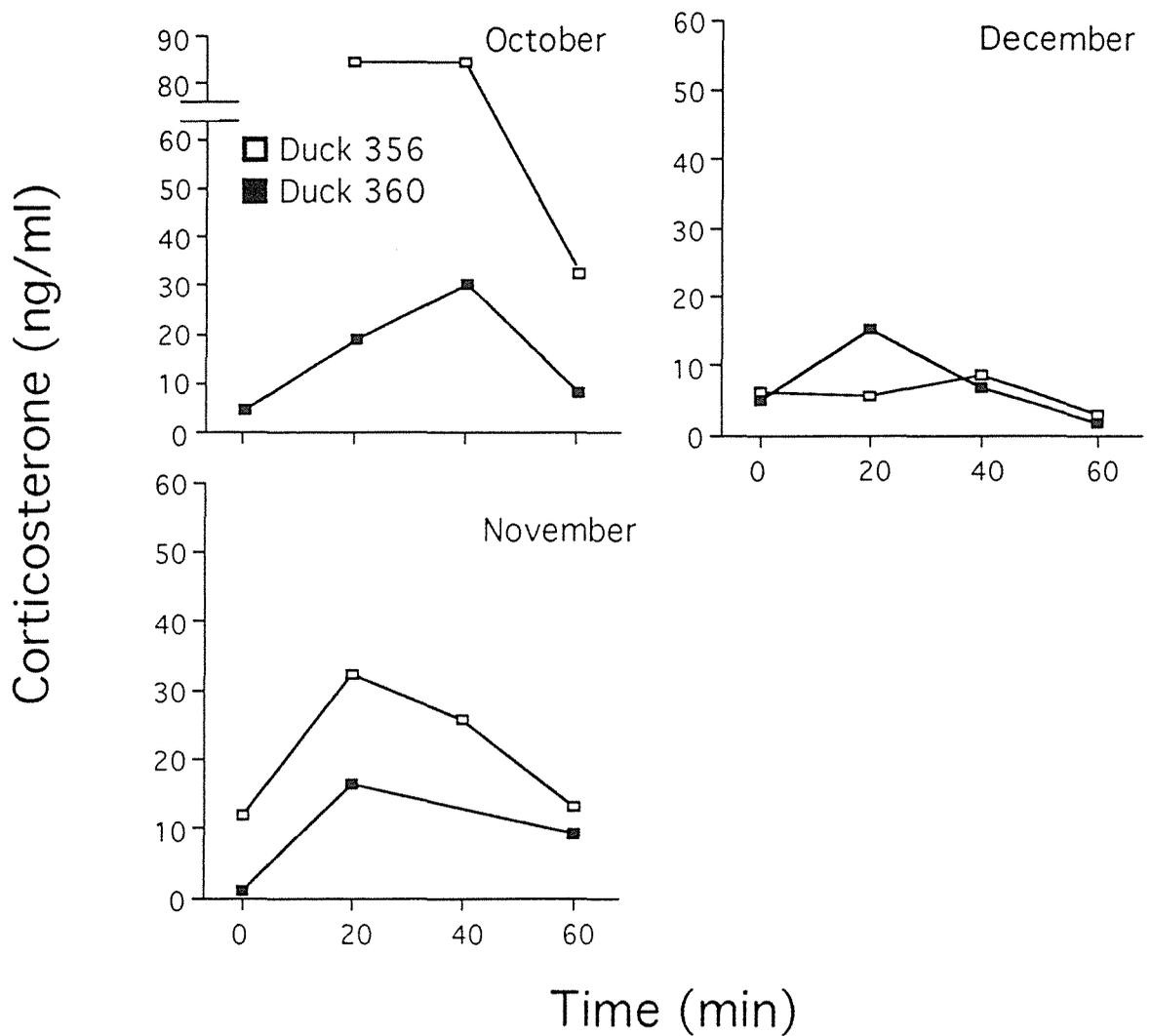


Figure 3.21: Plasma corticosterone levels in two female grey ducks sampled at 20 min intervals for 1 h after being removed from incubating eggs (October) or rearing ducklings (November and December).

### 3.4 Discussion

The individual variation in initial corticosterone levels and responses to sampling stress in grey duck was large, but female ducks that bred consistently had the lowest levels and responses. The social status, territorial area, body weight and body condition of the grey ducks may account for the observed variation in initial corticosterone levels. Consideration of the placement of feeders and overall size and design of enclosures for captive birds could improve breeding success. Furthermore, measurements of either faecal or plasma corticosterone levels may indicate possible stressful stimuli for birds held in captivity.

There was no clear monthly variation in either initial corticosterone levels or responses to sampling stress due to the large individual variation among the grey duck. The difference in initial corticosterone levels between male and female grey duck may have resulted from a difference in the sampling procedures between each sex.

#### 3.4.1 Individual variation in plasma corticosterone levels and responses to sampling stress

##### 3.4.1.1 Individual variation in plasma corticosterone levels

The individual variation in initial corticosterone levels in the female, and possibly also the male, grey ducks is similar to the variation in breeding success observed among these ducks. Of all the ducks #360 consistently had the lowest corticosterone levels prior to the breeding season (Fig 3.04) and with #356 were the only two female ducks to lay eggs. The male ducks paired with these females were #353 and 355. Duck #355 did not consistently have the lowest initial corticosterone levels of the male ducks, but did have the lowest mean level. The mean corticosterone level of duck #353 was the median of the group. There was only a small number of ducks available for this study, and these data provide limited support for the existence of an inverse relationship between corticosterone levels and breeding success in

the grey duck. However high corticosterone levels have been shown experimentally to inhibit the hypothalamo-pituitary-gonadal axis of tree sparrows (Wilson and Follett, 1975) and ducks (Deviche *et. al.*, 1979).

Low corticosterone levels before the breeding season may be indicative of a increased likelihood of breeding. An understanding of the reasons why some ducks have lower levels than others would allow the managers of captive birds to identify those birds most likely to breed, and to provide conditions that are most likely to favour breeding. For example, the most dominant ducks may have the lowest corticosterone levels and also secure the best nesting sites. Furthermore, some ducks may be more suited to captivity and selection of such individuals from a group of wild ducks would improve chances of breeding success. Initial corticosterone levels could not be used to select individuals as differences in levels could be due to some ducks being subjected to environmental stressors prior to capture and sampling which others were not. The capture of ducks, subjecting them to a consistent stressor and then the measurement of the resulting corticosterone response would identify low and high responding individuals that might respond similarly to other stressors such as captivity.

#### 3.4.1.2 Individual variation in the corticosterone response to sampling stress.

The variation in the corticosterone response to sampling stress is a combination of the variation in initial corticosterone levels as measured at 0 min, and the variation in the response to being sampled 20, 40 and 60 min later. Unfortunately there were occasions when one or more of the four samples could not be collected and there were insufficient complete responses available for any statistical analysis of the variation that appears to occur. Individual variation in corticosterone responses to sampling stress has also been observed in chickens (Edens and Siegel, 1975).

Relationships between corticosterone responses and breeding success were similar in the male and female ducks to the relationships between initial corticosterone levels and breeding success. Duck #360 had the lowest

corticosterone responses (Fig 3.06 and 3.07) while the other female duck to produce a clutch (#356) and the male ducks they paired with (#353 and 355) generally had lower responses than the other remaining ducks. If a corticosterone response to sampling stress is indicative of potential breeding success then the response of wild ducks to sampling stress could identify individuals with higher breeding potential. Variation in the corticosterone response of wild mallard ducks to sampling stress has been demonstrated earlier (Ch. 2, section 2.3.2).

### 3.4.2 Possible causes of the variation in plasma corticosterone levels and responses to sampling stress

#### 3.4.2.1 Effects on plasma corticosterone levels of the time taken to obtain a blood sample and of the order of sampling

The large individual variation in initial corticosterone levels observed among the grey ducks (Fig 3.05) could result from variations in the time it took to obtain the blood samples or the order in which a duck is picked up and sampled from a group. The absence of an effect on initial corticosterone levels of the time taken in obtaining a blood sample (Fig 3.08) suggests that taking 55 sec, as compared with 90 sec, to obtain the initial blood sample has not contributed to the individual variation in initial corticosterone levels. The order in which grey ducks were picked up from a group and sampled also had no effect on corticosterone levels (Fig 3.09), suggesting that order of sampling has also not contributed to the observed individual variation in levels.

If the variation in initial corticosterone levels among the grey duck did not result from differences in the way individual ducks were sampled then either such variation is normal or it has been imposed by stimuli which affected some ducks and not others. Initial corticosterone levels of some of the ducks sampled in this study were above 12 ng/ml, a level said to be basal and above which ducks are said to be affected by stressor (Harvey *et. al.*,

1980). Possible stressors that the ducks experienced before initial sampling include; (1) being herded through other duck's territories during the procedures used to confine ducks in the aviary for sampling and (2) close confinement in aviaries with peers who may be dominant. Variation in body weight might also affect basal corticosterone levels (Fig 3.13) and also the magnitude of any corticosterone response to the stressors noted above.

#### 3.4.2.2 Relationship between plasma corticosterone levels and the social status of individual male grey duck

Social interaction is said to affect corticosterone levels and responses to stressors (Siegel, 1980; Compton *et. al.*, 1981; Henry, 1993). In this study, there was no clear relationship between the corticosterone levels of male ducks and their social status during September to November. However, social status was not measured rigorously in this study, and a different measure of status (such as a series of pairwise tests to determine dominance in all combinations of ducks) may have given different results. Hence a dominance affect on corticosterone levels during the close confinement of ducks might have contributed to the variation in levels between individual grey ducks. No relationship between social status and corticosterone levels was found in the Harris hawk (Mays *et. al.*, 1991) and white browed sparrow weaver (*Plocepasser mahali*, Wingfield *et. al.*, 1991), while a negative relationship was demonstrated in the white throated sparrow (*Zonotrichia albicollis*, Schawbl *et. al.*, 1988).

#### 3.4.2.3 Relationship between plasma corticosterone levels and the territorial area of individual grey duck

The procedure used to collect blood samples from the grey ducks consisted of moving all the ducks from the outdoor pen into the aviary and confining them within a 1m diameter ring. Some ducks would therefore have been forced to move into the aviary through the pond area that, in September before eggs were laid, appears to be the territory of male ducks

#288, 353 and 355.

Elevated corticosterone levels have been demonstrated in male piedflycatchers caught outside their territory (Silverin and Wingfield, 1982). However there is no relationship between corticosterone levels and the male ducks #288, 353 and 355 who appeared to occupy the pond area in September (Fig 3.03). Furthermore, other studies on grey duck suggest they are non-territorial (Marchant and Higgins, 1990).

Once within the aviary less dominant ducks were confined in close proximity to the dominant ducks. Either or both the movement of ducks through other ducks territories and close confinement may have lead to higher corticosterone levels in some ducks.

#### 3.4.2.4 Relationship between plasma corticosterone levels and the body weight and condition of grey duck

As suggested previously, the crossing by some grey ducks onto other ducks territories may have been stressful, though this was not confirmed by higher corticosterone levels in ducks herded across others territories prior to sampling (Ch. 3, section 3.3.2.3). All the ducks had to move through the pond area to reach the feeders in the aviary, and it is possible that the more submissive ducks had restricted access to the feeders. This could in turn have lead to the more submissive ducks being lighter.

In this study a weak inverse relationship between body weight and corticosterone levels was found which was stronger for the male than the female grey duck (Fig 3.13). An inverse relationship between corticosterone levels and condition index also exists for the male ducks that is weaker, but confirms, the relationship using body weight (Fig 3.14). A condition index was used to account for variation in size between ducks and is being used in other similar studies of corticosterone levels in birds (J.C. Wingfield, pers. comm.).

Grey ducks with low body weight are more likely to have high corticosterone levels. A similar relationship has been demonstrated in the garden warbler (*Sylvia borin*) where birds with high corticosterone levels

had low storage levels of fat tissue (Schwabl *et. al.*, 1991). However, in another study a relationship between body weight and corticosterone levels was absent in the wild starling (Dawson and Howe, 1983).

In conclusion, variation in corticosterone levels and responses may have been due, in part, to the existence of a dominance hierarchy amongst the grey ducks. Such a hierarchy may have restricted access to feeders for some ducks, lowering body weights and, in elevating corticosterone levels via the close confinement before sampling, yielded the negative relationship between corticosterone levels and body weight. Measurements of corticosterone levels among captive birds may in future contribute information on why partial or no breeding success is achieved. In such studies invasive blood sampling could be avoided by measuring corticosterone levels in faecal samples in the same way estradiol and testosterone have been measured (Cockrem and Rounce, in press).

### 3.4.3 Monthly variation in plasma corticosterone levels and responses to sampling stress

#### 3.4.3.1 Monthly variation in plasma corticosterone levels

In the male grey duck no change in corticosterone levels was seen across the months samples were collected (Fig 3.15), whereas previous studies (also over 8 months) have shown corticosterone levels can vary in ducks across the same seasons as those studied (Assenmacher *et. al.*, 1975). In the female grey duck corticosterone levels fell from May to the winter months of June, July and August, and then rose again in September to December (Fig 3.15). However, the group composition changed from October onwards with the removal of the clutch producing ducks who were incubating eggs or rearing ducklings. As the 2 female duck who were removed previously had the lowest monthly corticosterone levels the monthly variation may be an artefact.

The difference in mean corticosterone levels between male and female grey ducks (Fig 3.16) may have resulted from differences in the way each sex

was sampled. All ducks were herded and confined for two consecutive days each month, with the males sampled on the first day and the females on the second day. This difference in treatments might account for the difference in mean corticosterone levels, since the female ducks may have become accustomed to being herded and confined by the second day and then had a reduced corticosterone response to this procedure. A reduced corticosterone response following habituation to a stressor has been demonstrated in ducks (Harvey and Phillips, 1982).

#### 3.4.3.2 Monthly variation in the plasma corticosterone response to sampling stress

The corticosterone responses of both the male (Fig 3.17) and female (Fig 3.19) grey ducks indicate that, on average, the sampling procedure used neither elevated nor maintained the initial corticosterone levels recorded at 0 min. Instead, corticosterone levels generally declined or remained relatively constant over the 1 h sampling period. As discussed earlier (Ch. 2, section 2.42) ducks with low (less than 12 ng/ml) corticosterone levels when first sampled are likely to show an increase in levels with 20 min sampling, while ducks with high initial levels are likely to show a decrease in levels during subsequent sampling.

Possibly only by obtaining low corticosterone levels in the first sample will any monthly variation in a corticosterone response be seen. In duck #360, a corticosterone response to sampling occurred during the incubating of eggs and rearing of ducklings (Fig 3.21) but not beforehand (Fig 3.20).

In conclusion, as demonstrated earlier in the mallard (Ch. 2. section 2.4.3.2) sampling at 20 min intervals is not frequent enough to sustain or induce a corticosterone response in the grey duck to sampling stress. In future studies on individual or monthly variation in the corticosterone response to sampling stress the placing of ducks into darkened boxes for 1 h after they are first picked up may reduce initial corticosterone levels so a response to sampling stress, if any, can then be observed.

## Chapter 4:

The plasma corticosterone and glucose responses  
to sampling stress and exogenous glucose  
administration in the mallard (*Anas platyrhynchos*)

Abstract

1. The aim of this study was to describe the plasma corticosterone and glucoses responses to blood sampling in the mallard and to investigate the effect of exogenous glucose loads on the corticosterone response to blood sampling.
2. Blood sampling at 5, 10 and 15 min intervals increased plasma corticosterone levels. In one of two groups sampled every 10 min and additionally handled every 2 min for 10 min, corticosterone levels were elevated above those in ducks not additionally handled. No change in glucose levels occurred in any of these groups.
3. Oral doses (10 ml) of 0.84M and 1.38M glucose solutions increased glucose levels while decreasing corticosterone levels. A 30 ml oral dose of a 1.38M glucose solution elevated glucose levels for at least 1 h, but no change in corticosterone levels occurred.
4. Intravenous glucose doses did not change corticosterone levels while a drop in glucose levels occurred 40 min after 3 ml of a 1.94M glucose dose had been given.
5. It is concluded that an oral glucose load may lower previously elevated corticosterone levels, and that the frequency of handling or blood sampling affects the magnitude of any resulting corticosterone response.

#### 4.1 Introduction

At certain times the capture and handling of wild birds is necessary for conservation purposes. When such times occur, every effort should be made to reduce any resulting stress, such as using darkened boxes to lower arousal levels when transporting wild birds (Kilgour and Dalton, 1984). Some groups involved in the capture and handling of wild birds give the birds an oral glucose solution in the belief it reduces stress (Cockrem per. comm.). This belief is based on the adrenal gland hormone corticosterone's involvement in increasing plasma glucose levels by inducing hepatic gluconeogenesis and glycogenolysis (Holmes and Phillips, 1976; Hazelwood, 1986). As corticosterone is a hormone whose levels increase under stressful situations, augmenting the effects of corticosterone by giving exogenous glucose may help the bird cope with the stress and possibly result in a reduced corticosterone response. As elevated corticosterone levels have been suggested to hinder reproductive events (Wingfield, 1988), reducing a corticosterone response to stress by exogenous glucose may be advantageous.

Though corticosterone has been said to induced hepatic gluconeogenesis and glycogenolysis (Holmes and Phillips, 1976 and Hazelwood, 1986), increases in glucose levels as a result of a stress-induced elevation in corticosterone levels have been inconsistent. Increases in both corticosterone and glucose levels as a consequence of blood sampling have been demonstrated in the duck (*Anas platyrhynchos*, Harvey and Phillips, 1982) while in the chicken (*Gallus domesticus*) neither corticosterone or glucose levels changed with blood sampling (Culbert and Wells, 1975). Freeman and Manning (1977) did demonstrate an increase in glucose levels during repeated blood sampling in the chicken but did not measure corticosterone levels. In another species, the pigeon (*Columbia livia*), Jeronen *et. al.* (1976) showed a rise in corticosterone levels with cold temperature stress while a small increase in glucose levels occurred at the same time.

Indirect support for an effect of corticosterone on hyperglycaemia comes from Peaker *et. al.* (1971) and Holmes and Phillips (1976), who

demonstrated that glucose levels in ducks increased when they were given exogenous ACTH (Adrenocorticotrophic hormone), the hormone that increases corticosterone levels. ACTH has also been shown to induce hyperglycaemia in the chicken (Heald *et. al.*, 1965; Culbert and Wells, 1975; Freeman and Manning, 1977).

Other hormones besides corticosterone and ACTH that may increase glucose levels in birds include adrenaline and glucagon (Hazelwood, 1986). Levels of both adrenaline and glucagon have been shown to increase in response to stress (Bloom, 1973; Freeman and Manning, 1976; Rees *et al.*, 1984) and these hormones may be responsible for increases in glucose levels that occur during stress. Adrenaline may not increase glucose levels directly, but instead act via a stimulation of glucagon release (Freeman and Manning, 1976, Sitbon *et. al.*, 1980). Studies by Mahata and Ghosh (1991) on the pigeon, parakeet and crow, by Grande and Prigge (1970) on geese and ducks and by Heald *et. al.* (1965) and Freeman and Manning (1977) on chickens have demonstrated increases in plasma glucose within 15 min of the birds been given exogenous glucagon.

Glucagon release from the pancreas is influenced by plasma glucose levels as low glucose levels stimulate glucagon release and high glucose levels inhibit the release of glucagon, as in mammals (Sitbon *et. al.*, 1980). Corticosterone levels also increase when glucose levels drop and decline when normal levels of glucose are restored (Culbert and Wells, 1975). The increase in glucose levels in this situation could, in part, be due to the glyceamic effects of corticosterone.

It is not known whether glucose levels have a negative feedback relationship with corticosterone in a similar manner to glucagon. If such a relationship does exist between corticosterone and glucose then high glucose levels may reduce corticosterone responses to stressors such as capture and handling. The presence of a negative feedback relationship gives a possible route for artificially reducing a corticosterone stress response by raising glucose levels via an exogenous dose of glucose. Impeding the corticosterone response to stress may avoid any suppression of reproductive events.

In this study to investigate whether glucose levels do rise in response to stress, both glucose and corticosterone levels are measured in mallards subjected to blood sampling. Blood sampling was also used in the second part of this study to investigate whether either oral or intravenous exogenous glucose reduces any corticosterone response to sampling stress.

## 4.2 Materials and methods

Seventy five mallards (*Anas platyrhynchos*) from 9 to 11 months old were used in this study. The ducks were reared at the Avian Physiology Unit, Massey University, and had access to food (duck pellets, J.F. Cockrem, Massey University) and water *ad libitum*. The ducks were housed in aviaries containing single sex groups of 15 ducks. The aviaries were open to the ambient temperature and photoperiod. All samples were obtained over a 6 week period in mid-winter, with sampling starting at 9.30am and finishing by 11.00am to avoid any diurnal rhythm effect. Due to limited numbers, female ducks were used to study the effect of handling and sampling on corticosterone and glucose levels and male ducks were used for the remaining experiments on the effect of exogenous glucose.

Most samples were collected by final year students during the research component of their science degree in physiology under the supervision of Dr. J.F. Cockrem and myself.

### 4.2.1 Sample collection and exogenous glucose administration

A maximum of 10 of the 15 ducks housed per aviary were blood sampled at one time. The ducks were rounded up within their aviary into a darkened confined space approximately 1m in diameter, individually removed from the group and taken to another room where a blood sample (0.5 to 2.0 ml) was taken from the brachial vein. Due to the inexperience of the students in collecting blood samples no time limit was placed on sample collection, though all samples were obtained in less than 5.5 min after the duck was picked up. The time taken to obtain a blood sample after the duck was picked up was recorded to the nearest minute. After a blood sample had been obtained the ducks were placed into individual darkened boxes and further samples collected as necessary.

The relationship between exogenous glucose and the plasma corticosterone response to sampling was investigated using oral or intravenous glucose solutions. All glucose solutions were made from

Analar D-glucose in 0.154M isotonic saline. Oral glucose doses were given by a crop tube and intravenous glucose solutions were injected into the brachial vein. In both cases the first blood sample was collected before any glucose solution was administered. Blood samples were collected in heparinised tubes, centrifuged and the plasma frozen (-20°C) for later analysis.

#### 4.2.2 Experiments

##### 4.2.2.1 Plasma corticosterone and glucose responses to sampling and handling stress

In this initial experiment female mallards were sampled at 5, 10 or 15 min intervals as shown in Table 4.1 (groups A to E). This experiment investigated the effects of sampling on plasma corticosterone levels and whether any change in corticosterone levels coincided with a change in plasma glucose levels. The time taken to obtain the first blood sample from the moment a duck is picked up and the order in which a duck is picked up from a group and sampled were also investigated in relation to possible effects on corticosterone levels.

Two groups, D and E, were handled every 2 min for 10 or 20 min in addition to being sampled at 10 min intervals to investigate whether additional stress imposed by handling elevates corticosterone (and possibly glucose) levels more than sampling alone. Additional handling involved picking up the duck from its individual darkened box and restraining the duck in the sampling position for approximately 10 sec before returning the duck to its box.

##### 4.2.2.2 Exogenous glucose administration and the plasma corticosterone and glucose responses to sampling stress

In the second experiment male mallards were blood sampled and then given either an oral or intravenous dose of a glucose solution or isotonic

Table 4.1: Experimental groups

Group	Sex	n	Sampling protocol	Additional handling	Oral fluids	Intravenous fluids
A	F	10	0, 5, 10 & 15 min	None	None	None
B	F	5	0, 10 & 20 min	None	None	None
C	F	5	0, 15 & 30 min	None	None	None
D	F	5	0, 10 & 20 min	Every 2 min from 0 to 10 min	None	None
E	F	5	0, 10 & 20 min	Every 2 min from 0 to 20 min	None	None
F	M	10	0, 30 & 60 min	None	10 ml saline	None
G	M	5	0, 30 & 60 min	None	10 ml 0.28M glucose solution	None
H	M	5	0, 30 & 60 min	None	10 ml 0.56M glucose solution	None
I	M	5	0, 30 & 60 min	None	10 ml 0.84M glucose solution	None
J	M	5	0, 30 & 60 min	None	10 ml 1.38M glucose solution	None
K	M	4	0, 20, 40 & 60 min	None	30 ml saline	None
L	M	4	0, 20, 40 & 60 min	None	30 ml 1.38M glucose solution	None
M	M	4	0, 20, 40 & 60 min	None	None	3 ml saline
N	M	4	0, 20, 40 & 60 min	None	None	3 ml 0.56M glucose solution
O	M	4	0, 20, 40 & 60 min	None	None	3 ml 1.38M glucose solution
P	M	4	0, 20, 40 & 60 min	None	None	3ml 1.94M glucose solution

saline (groups F to P, Table 4.1). Blood samples were collected at 20 or 30 min intervals after glucose or saline administration. Varying doses of glucose solutions were given either orally or intravenously to investigate which method and dose would elevate glucose levels and reduce any corticosterone response to sampling stress.

#### 4.2.3 Measurement of plasma glucose

Glucose concentrations were measured in triplicate 100µl plasma aliquots by the glucose oxidase method using commercial reagents (Serva reagents, Feinbiochemica GmbH & Co., Heidelberg). Glucose oxidase catalyses the oxidation of beta-D-glucose to D-gluconic acid and hydrogen peroxide. The hydrogen peroxide then reacts with toluidine to produce an oxidized, coloured by-product whose concentration is determined spectrophotometrically (Brown, 1987).

#### 4.2.4 Measurement of plasma corticosterone

Corticosterone was assayed using the same procedure as outlined in Chapter 2, Section 2.2.3. As stated previously, the intra- and inter-assay coefficients of variation were 6.5% and 9.3% respectively while the minimum detectable corticosterone concentration was 0.40 ng/ml.

#### 4.2.5 Statistical analyses

The effect of time, or sampling, on corticosterone and glucose levels within each group of mallards was investigated by one-way ANOVA with Bonferroni adjusted probability levels used for subsequent post-hoc tests. Where the Bartlett's test indicated heterogeneity of variance the effect of sampling on corticosterone and glucose levels within each group was investigated by Friedman nonparametric ANOVA.

Comparisons between means of individual groups was also investigated using one-way ANOVA with Bonferroni adjusted probability

levels used for subsequent post-hoc tests. Where the Bartlett's test indicated heterogeneity of variance comparisons of corticosterone and glucose levels between groups was by Friedman nonparametric ANOVA (Wilkinson, 1990).

The relationship between corticosterone levels and either the time taken to obtain the first blood sample or the order of sampling of individual mallards from a group was investigated using the Kruskal-Wallis nonparametric ANOVA.

Log transformed data was used for statistical analysis. Where error bars are shown the error used was the standard error of the mean (SEM).

## 4.3 Results

### 4.3.1 Plasma corticosterone and glucose responses to sampling and handling stress

#### 4.3.1.1 Effect on plasma corticosterone levels in the first blood sample of the time taken to obtain a blood sample and of the order of sampling

Corticosterone levels in ducks from this study did not exhibit any significant trend when samples were obtained within 330 sec of the duck being handled ( $p=0.106$ , Fig 4.01). The removal of a duck from its group did not have any significant effect on the corticosterone levels in the remaining ducks of that group, because there was no relationship between the order in which a duck was picked up for sampling and its corticosterone level ( $p=0.972$ , Fig 4.02).

#### 4.3.1.2 Plasma corticosterone and glucoses responses to sampling stress

When ducks were sampled at 5, 10 or 15 min intervals an increase in corticosterone from levels measured in the first sample occurred (Fig 4.03). In ducks sampled every 5 min for 15 min (group A) corticosterone levels had increased significantly ( $p<0.01$ ) 5 min after the ducks were first picked up (Fig 4.03). Corticosterone levels increased above 0 min levels on at least one occasion between 5 and 15 min in all 10 ducks (Fig 4.04). Ducks sampled at 10 min (group B) intervals demonstrated a steady rise in corticosterone levels with levels at 20 min significantly higher ( $p<0.01$ ) than 0 min levels (Fig 4.03). Corticosterone levels increased in all 5 ducks sampled over the 20 min period of observation (Fig 4.04). Corticosterone levels in ducks sampled at 15 min intervals (group C) also rose over the 30 min of observation with levels at both 15 and 30 min being significantly higher ( $p<0.05$ ) than levels at 0 min (Fig 4.03). Corticosterone levels increased in 4 of the 5 ducks over the

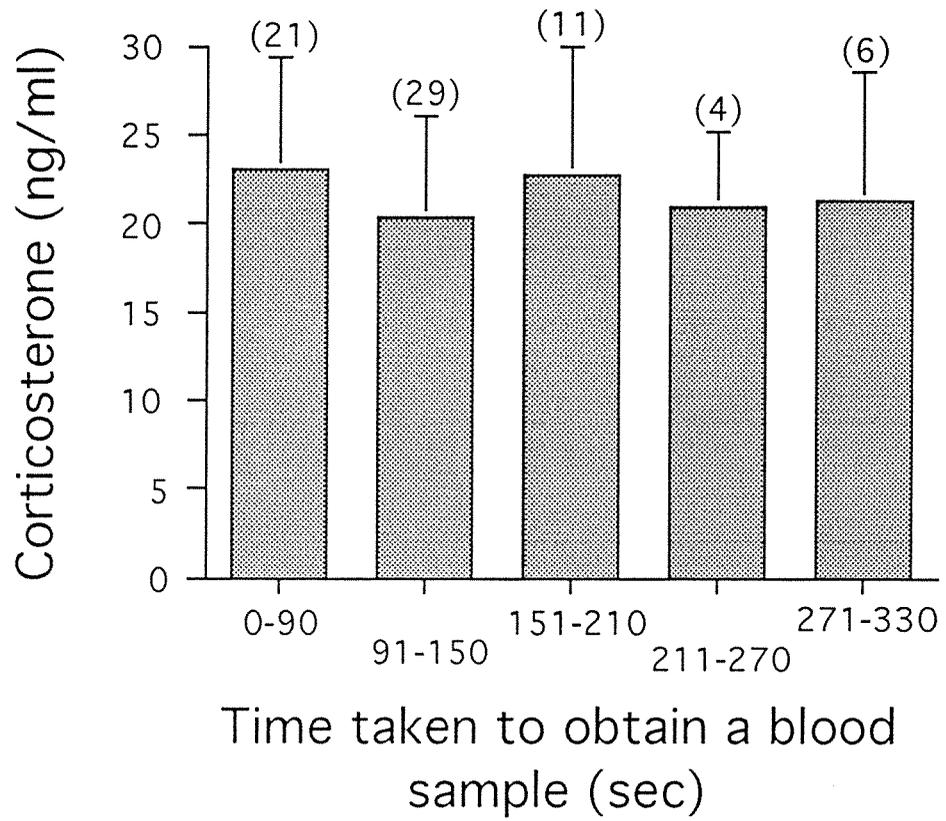


Figure 4.01: Mean plasma corticosterone levels in relation to the time taken to obtain the first blood sample in ducks. Number of ducks sampled are in brackets above columns.

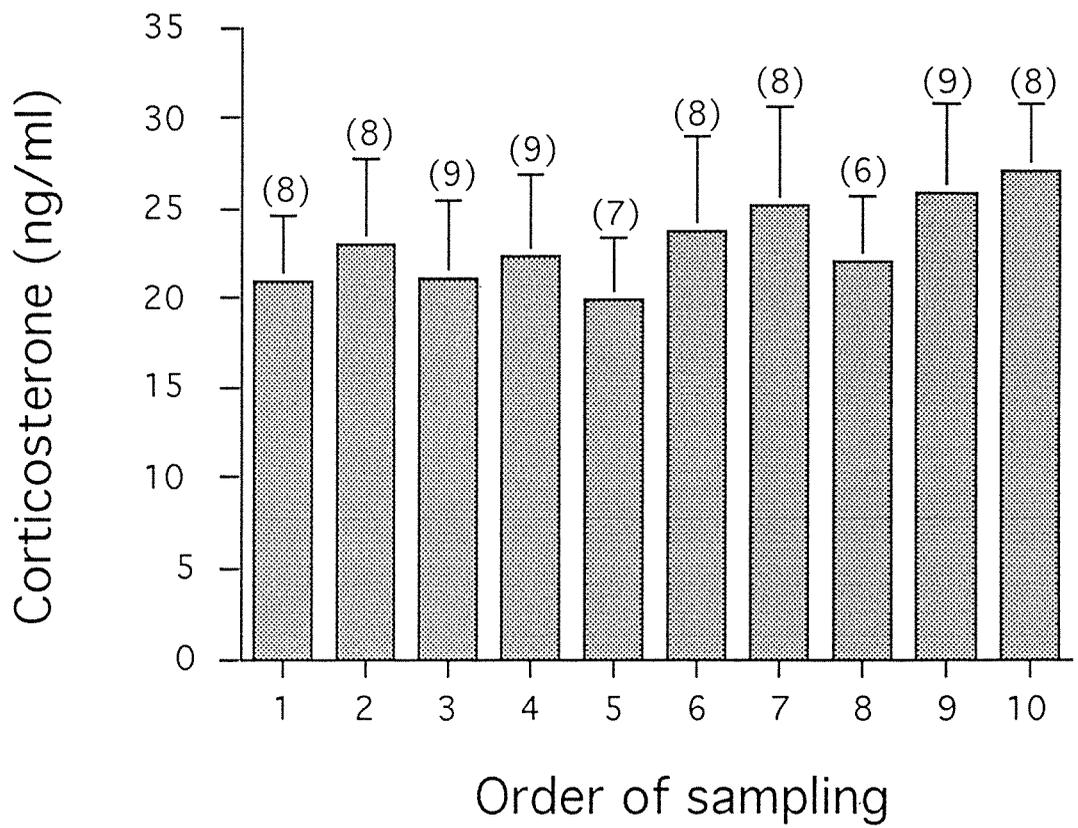


Figure 4.02: Relationship between plasma corticosterone levels in the first blood sample and the order in which ducks were sampled. Number of ducks sampled are in brackets above columns.

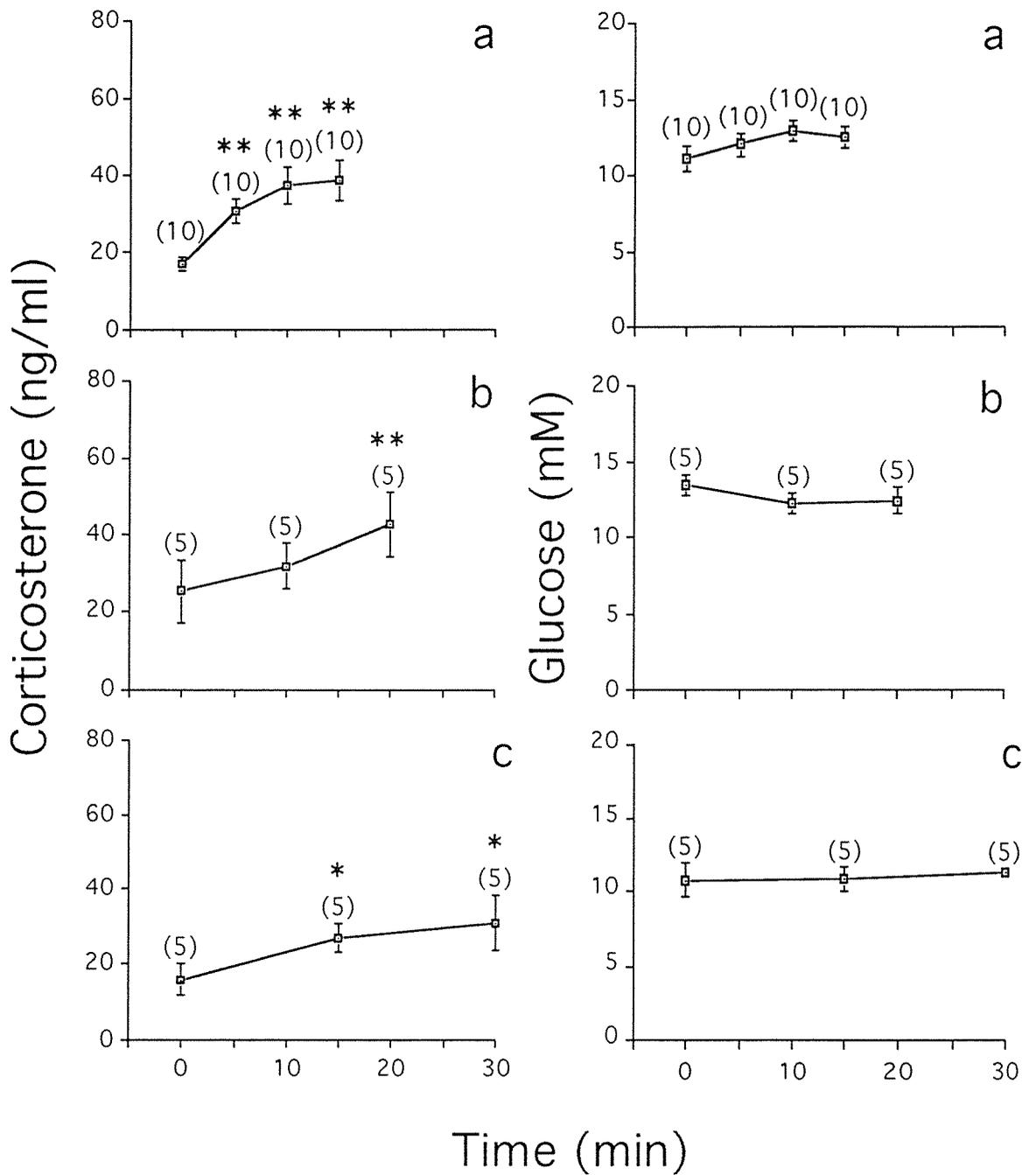


Figure 4.03: Mean plasma levels of corticosterone and glucose in ducks sampled at (a) 5 min (group A), (b) 10 min (group B) or (c) 15 min (group C) intervals. Number of ducks sampled are in brackets above error bars. Statistics relate to changes in corticosterone from starting levels. \*= $p < 0.05$ , \*\*= $p < 0.01$  and \*\*\*= $p < 0.001$

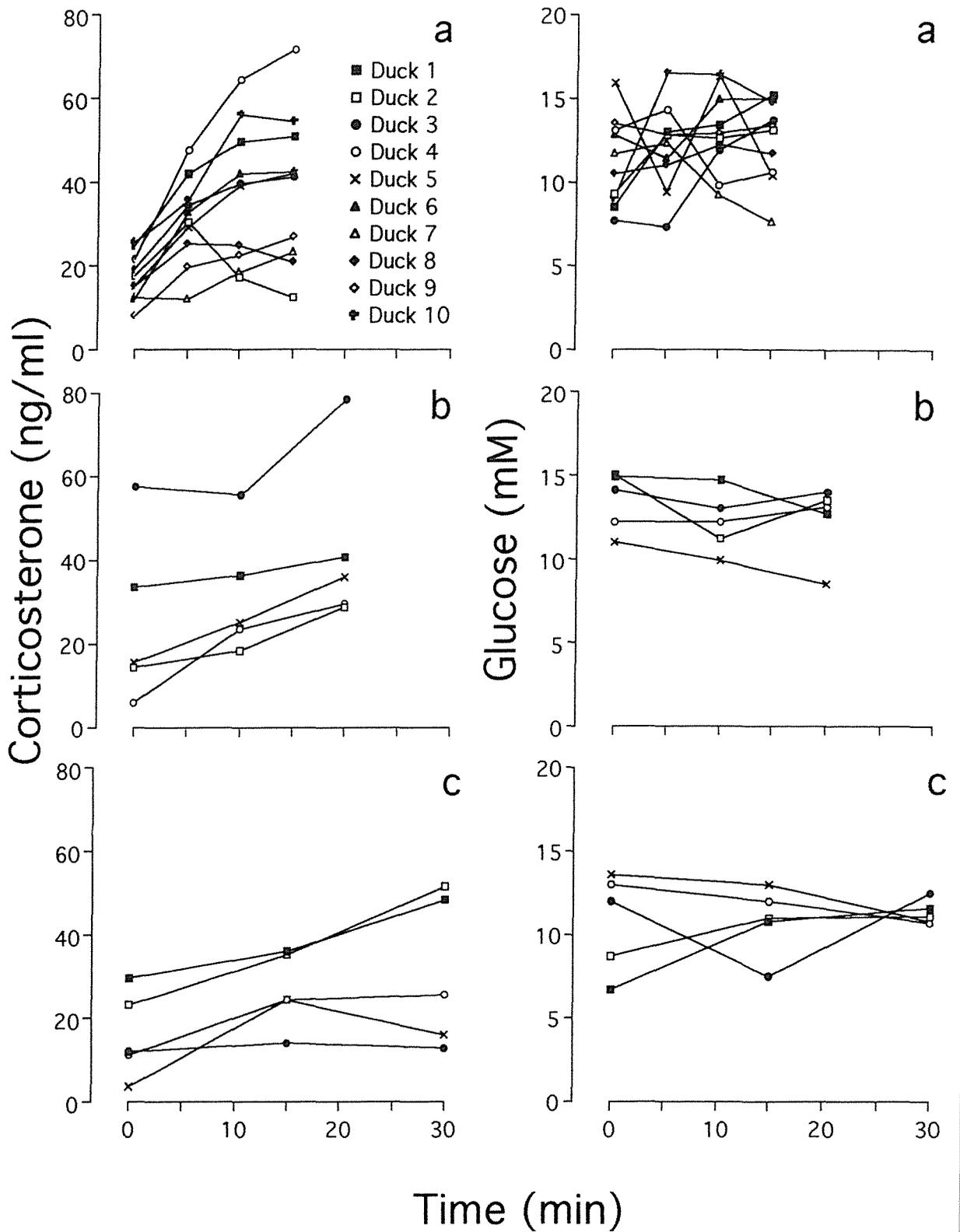


Figure 4.04: Plasma levels of corticosterone and glucose in ducks sampled at (a) 5 min (group A), (b) 10 min (group B) or (c) 15 min (group C) intervals.

30 min period of observation (Fig 4.04).

There were no significant changes in plasma glucose levels in any of the groups subjected to repeated sampling at various intervals (Fig 4.03), though there was a trend for glucose levels to increase between 0 and 10 min in ducks sampled at 5 min intervals (group A). There was no clear observable relationship between changes in corticosterone and glucose levels over the periods sampled in any of the ducks (Fig 4.04).

The corticosterone levels at 0 min (Fig 4.05) were not significantly different between group A, B and C ducks ( $p=0.108$ ). After 10 min corticosterone levels had risen more in the ducks sampled twice (group A) than in ducks sampled once (group B, Fig 4.06), but this difference was not significant ( $p=0.153$ ). After 15 min corticosterone levels in ducks sampled three times (group A) had risen more than levels in ducks sampled once (group C, Fig 4.07), but again the difference was not significant ( $p=0.097$ ).

#### 4.3.1.3 Plasma corticosterone and glucose responses to sampling and additional handling stress

Corticosterone levels were higher at 10 min than at 0 min in both groups of ducks handled every 2 min (Fig 4.08), with this elevation in levels significant in group D ( $p<0.01$ ) but not group E ducks ( $p=0.217$ ). Corticosterone levels then declined in both groups, despite the continued handling of group E ducks every 2 min. An increase in corticosterone levels between 0 and 10 min was seen in 4 of the 5 ducks in each group, while a drop in levels between 10 and 20 min was seen in all of the group D ducks and 3 of 4 group E ducks (Fig 4.09).

These results can be compared with those from group B ducks who were sampled at 10 min intervals but not handled every 2 min. These birds had a steady rise in corticosterone levels over the 20 min period (Fig 4.08), as described earlier.

There were no changes in plasma glucose levels in any of the two groups of ducks handled in addition to being sampled (Fig 4.08). There was no observable relationship between changes in both corticosterone and

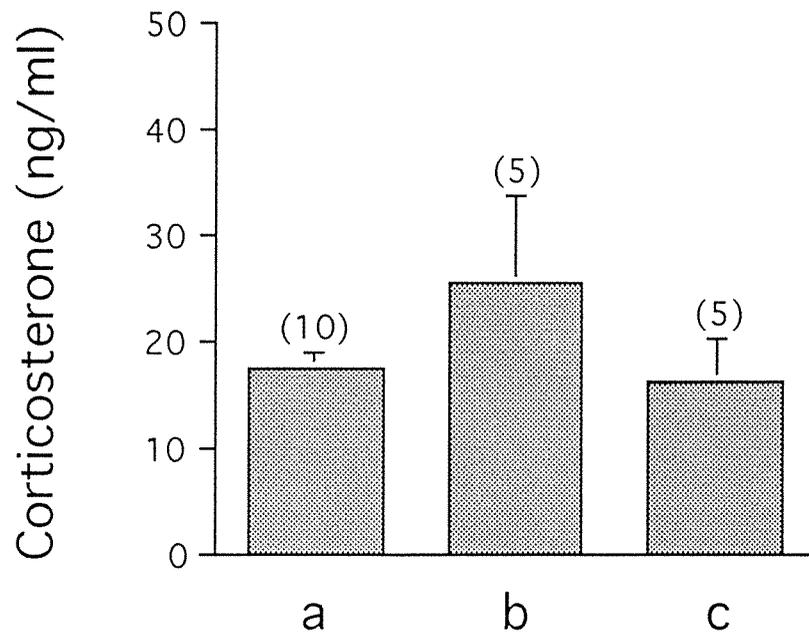


Figure 4.05: Mean plasma corticosterone levels in ducks sampled at (a) 5 min (group A), (b) 10 min (group B) or (c) 15 min (group C) intervals at 0 min. Number of ducks sampled are in brackets above columns.

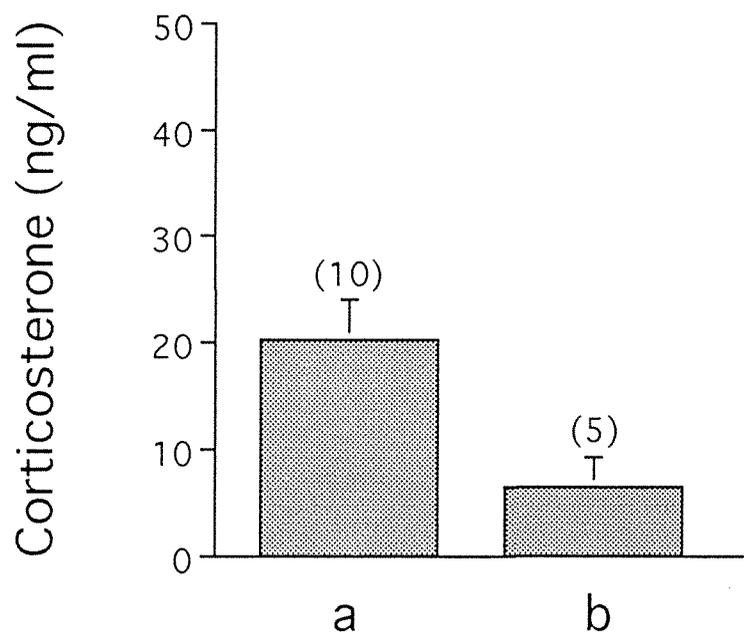


Figure 4.06: Change in mean plasma corticosterone levels 10 min after the first sample in ducks sampled at (a) 5 min (group A) or (b) 10 min (group B) intervals. Number of ducks sampled are in brackets above columns.

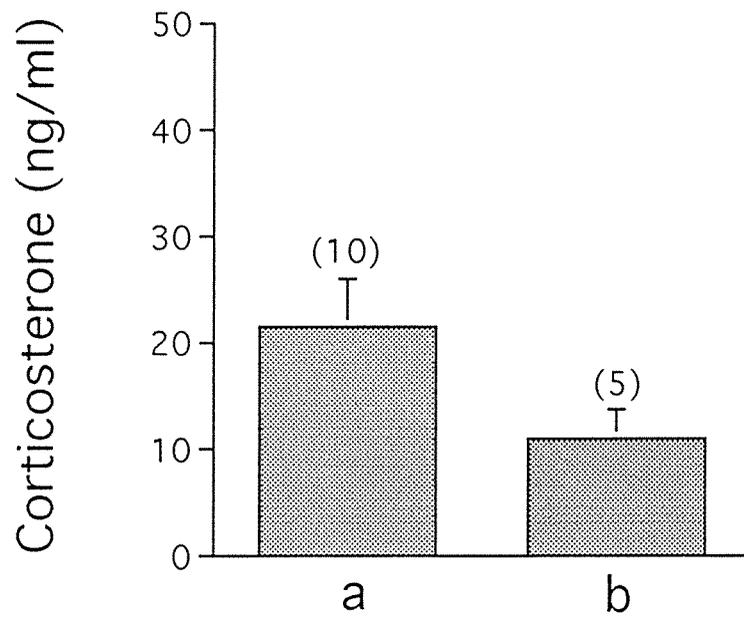


Figure 4.07: Change in mean plasma corticosterone levels 15 min after the first sample in ducks sampled at (a) 5 min (group A) or (b) 15 min (group C) intervals. Number of ducks sampled are in brackets above columns.

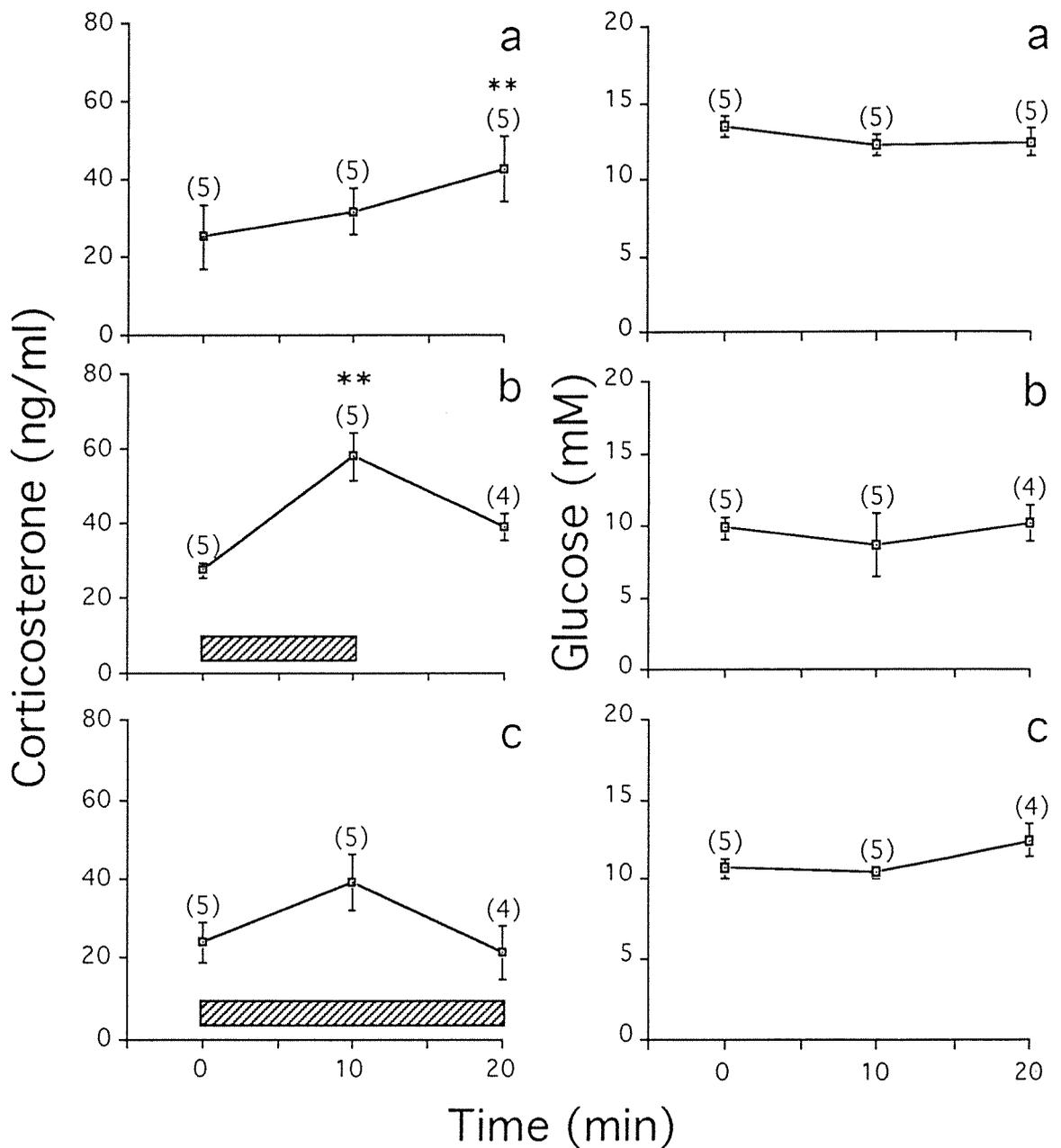


Figure 4.08: Mean plasma levels of corticosterone and glucose in ducks sampled every 10 min with either (a) no additional handling (group B), (b) additional handling every 2 min for 10min (group D) or (c) additional handling every 2 min for 20 min (group E). Horizontal bar indicates period in which handling at 2 min intervals occurred. Number of ducks sampled are in brackets above error bars. Statistics relate to changes in corticosterone from starting levels.

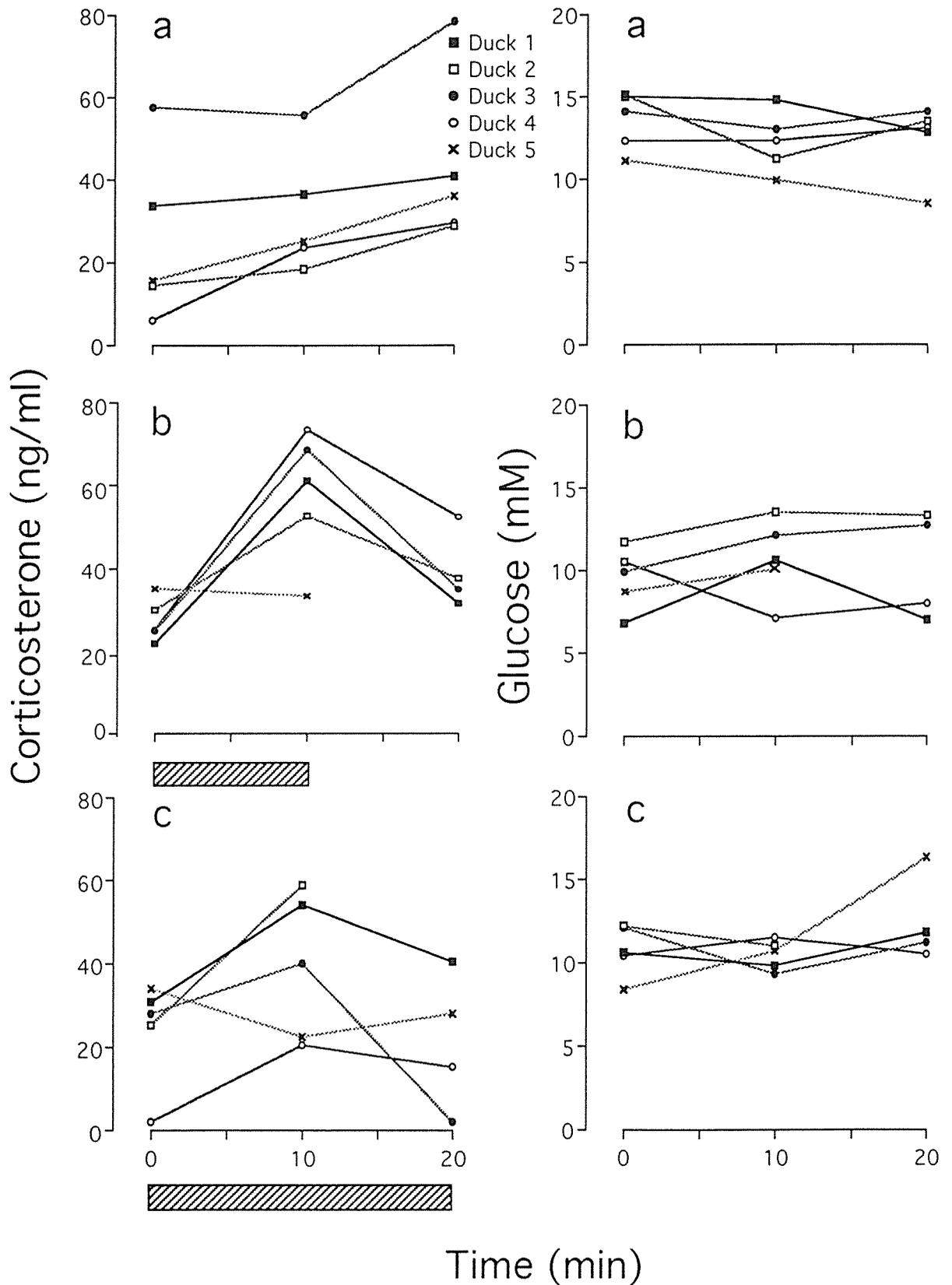


Figure 4.09: Plasma levels of corticosterone and glucose in ducks sampled every 10 min with either (a) no additional handling (group B), (b) additional handling every 2 min for 10 min (group D) or (c) additional handling every 2 min for 20 min (group E). Horizontal bar indicates period in which handling at 2 min intervals occurred.

glucose levels over the period sampled in any of the ducks (Fig 4.09).

The corticosterone levels at 0 min did not differ between group B, D and E ducks (Fig 4.10,  $p=0.759$ ). At 10 min (Fig 4.11) corticosterone levels had risen significantly more ( $p<0.01$ ) in one of the two groups of ducks handled in addition to being sampled (group D) than levels in ducks sampled only (group B). Corticosterone levels in the remaining group of ducks also handled in addition to being sampled (group E) were not different to levels in group B ducks sampled only ( $p=0.758$ ). By 20 min (Fig 4.12) there was no difference in corticosterone levels between group B and D ducks ( $p=0.931$ ) nor between group B and E ducks ( $p=0.281$ ).

#### 4.3.2 Exogenous glucose administration and the plasma corticosterone and glucose responses to sampling stress

##### 4.3.2.1 Oral glucose administration and the plasma corticosterone and glucose responses to sampling stress

When solutions (10 ml) of increasing glucose strengths were administered to ducks by crop tube glucose levels at 30 min (Fig 4.13) were significantly higher than 0 min levels in ducks given 0.84M (group I,  $p<0.01$ ) and 1.38M glucose solutions (group J,  $p<0.001$ ). By 60 min glucose levels in group I and J ducks were similar to levels measured at 0 min. Glucose levels rose and fell in 3 of 4 group J ducks and in all of the group I ducks (Fig 4.14). Of the ducks given saline (group F), 0.28M glucose (group G) and 0.56M glucose (group H) solutions only 1 duck in group F, 3 ducks in group G and 2 ducks in group H had a rise and fall in glucose levels between 0 and 60 min.

The rise and fall in glucose levels in group I and J ducks also coincided with a inverse fall and rise in corticosterone levels (Fig 4.13). Corticosterone levels at 30 min were significantly lower than levels at 0 min in both group I and J ducks ( $p<0.05$ ) while by 60 min levels were similar to those measured at 0 min. Corticosterone levels fell and rose in 2 of 3 the group I ducks to have all 3 blood samples collected from them and in 4 of the 5 group J ducks (Fig 4.14). Of the group F, G and H ducks, only 1 duck in either group F, G or

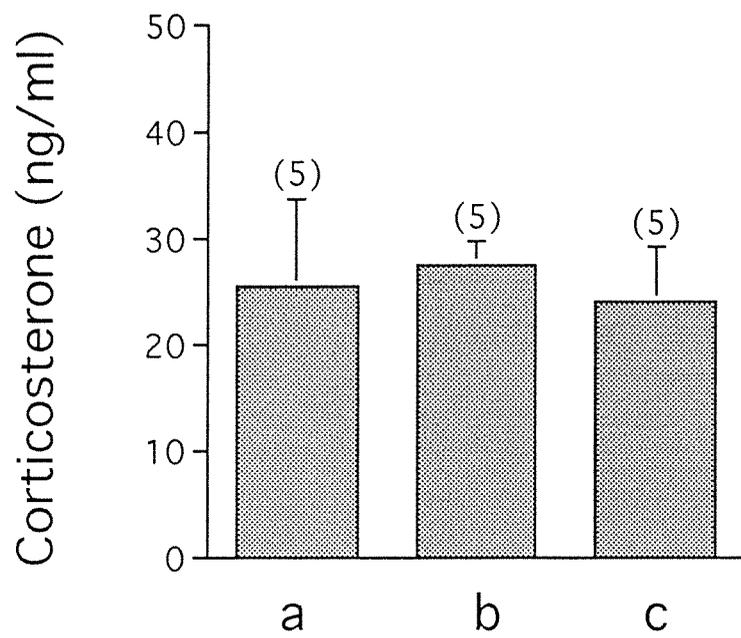


Figure 4.10: Mean plasma corticosterone levels at 0 min in ducks sampled every 10 min with either (a) no additional handling (group B), (b) additional handling every 2 min for 10 min (group D) or (c) additional handling every 2 min for 20 min (group E). Number of ducks sampled are in brackets above columns.

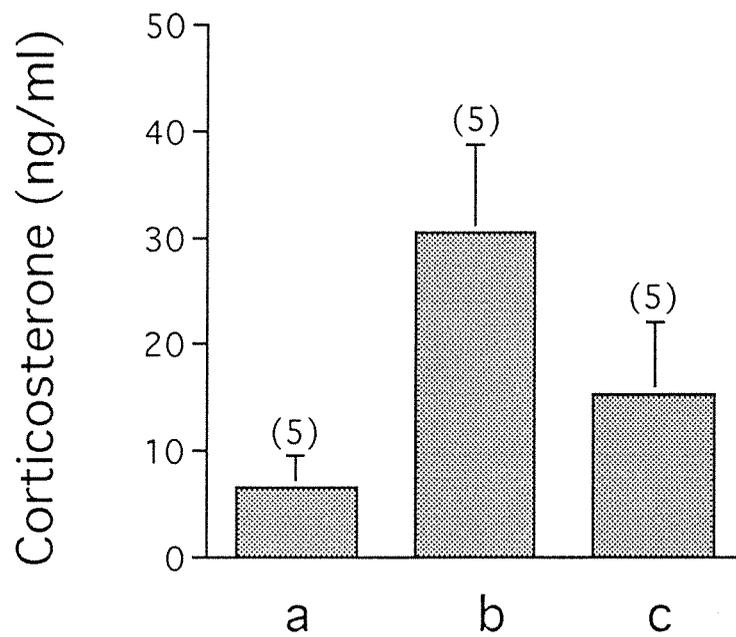


Figure 4.11: Change in mean plasma corticosterone levels 10 min after the first sample in ducks sampled every 10 min with either (a) no additional handling (group B), (b) additional handling every 2 min for 10 min (group D) or (c) additional handling every 2 min for 20 min (group E). Number of ducks sampled are in brackets above columns.

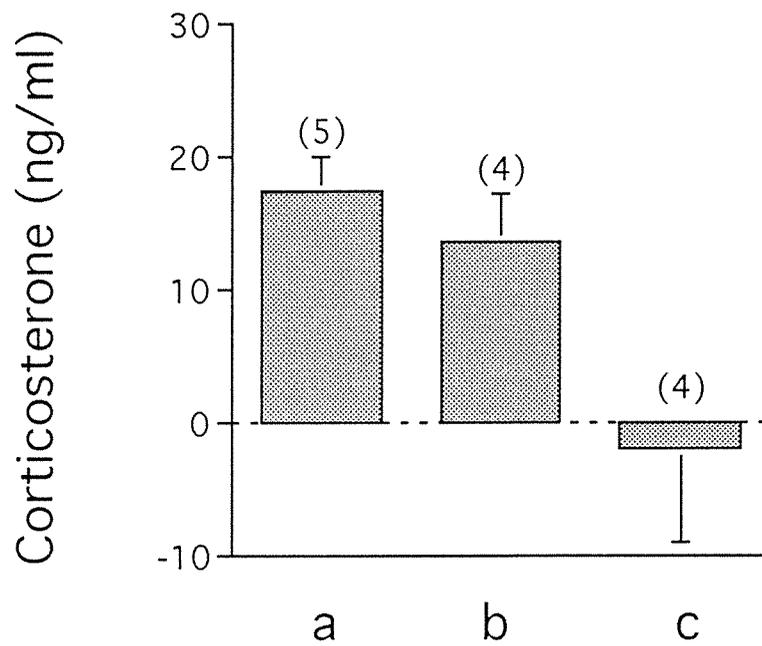


Figure 4.12: Change in mean plasma corticosterone levels 20 min after the first sample in ducks sampled every 10 min with either (a) no additional handling (group B), (b) additional handling every 2 min for 10 min (group D) or (c) additional handling every 2 min for 20 min (group E). Number of ducks sampled are in brackets above columns.

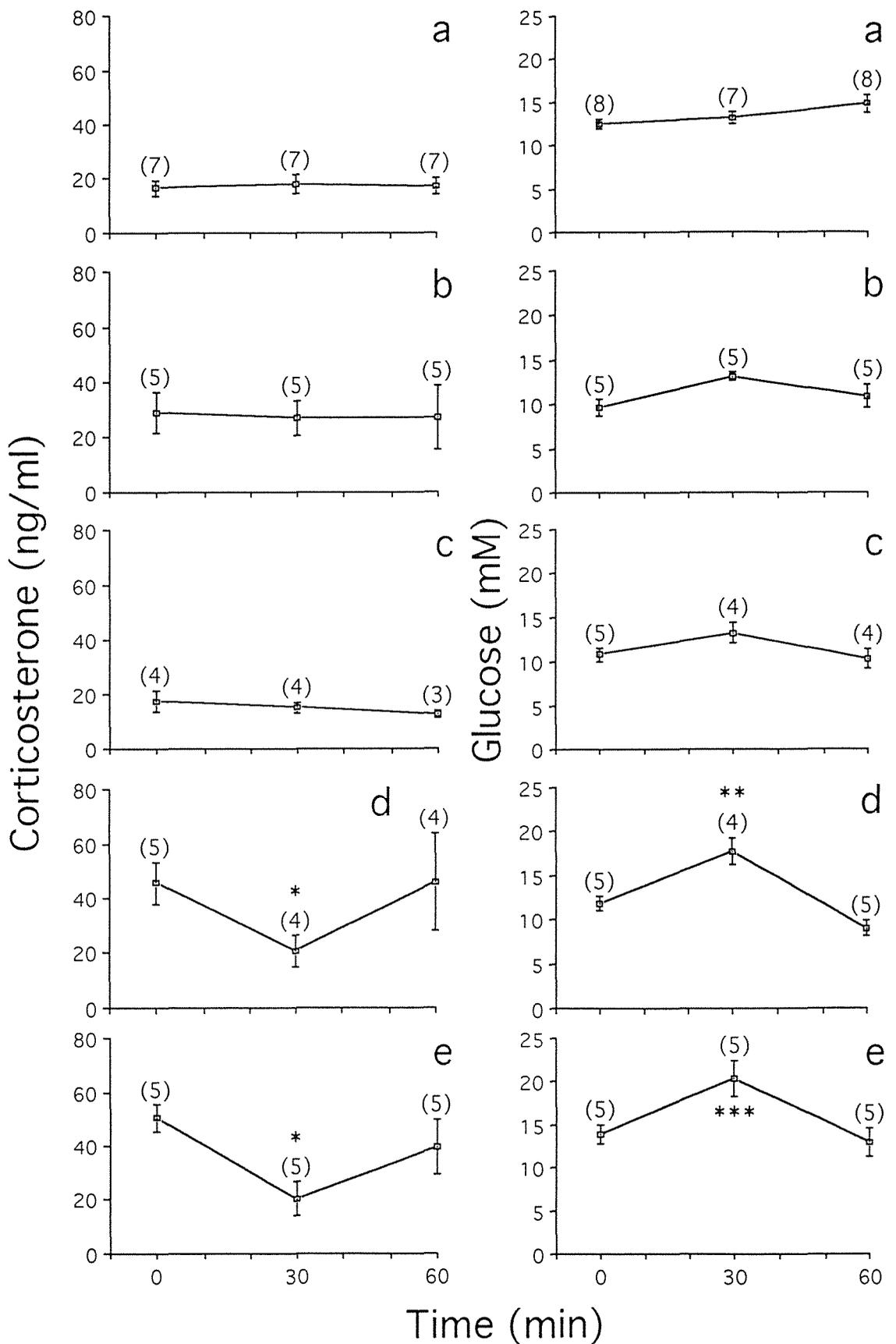


Figure 4.13: Mean plasma levels of corticosterone and glucose in ducks sampled every 30 mins and given a 10 ml oral dose of either; (a) saline (group F), (b) 0.28M glucose solution (group G), (c) 0.56M glucose solution (group H), (d) 0.84M glucose solution (group I) or (e) 1.38M glucose solution (group J). Number of ducks sampled are in brackets above error bars. Statistics relate to changes in corticosterone or glucose from starting levels.

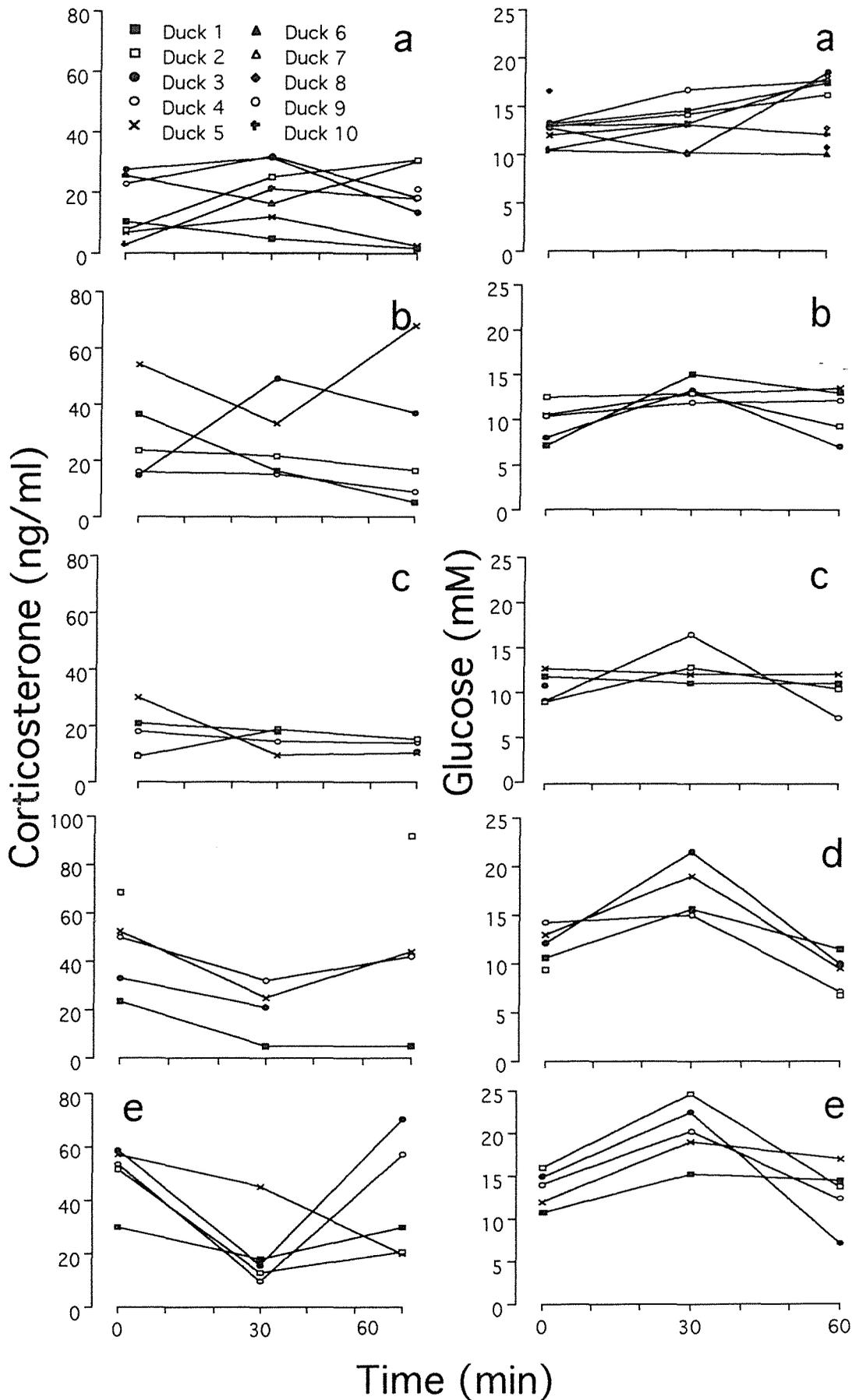


Figure 4.14 Plasma levels of corticosterone and glucose in ducks sampled every 30 mins and given a 10 ml oral dose of either; (a) saline (group F), (b) 0.28M glucose solution (group G), (c) 0.56M glucose solution (group H), (d) 0.84M glucose solution (group I) or (e) 1.38M glucose solution (group J).

H demonstrated a fall and rise in corticosterone levels between 0 and 60 min.

Glucose levels at 0 min (Fig 4.15) were not significantly different between group F to I ducks ( $p=0.368$ ). At 30 min (Fig 4.16) glucose levels had risen significantly more in the ducks from group J than levels in group F ducks ( $p<0.05$ ) while levels in group I ducks had almost risen significantly more than levels in group F ducks ( $p=0.085$ ). By 60 min (Fig 4.17) the change in glucose levels from 0 min was not significantly different between either group F and I ducks ( $p=0.481$ ) or group F and J ducks ( $p=0.853$ ).

The actual glucose levels measured at 30 min of  $12.11 \pm 1.32$  mM,  $17.73 \pm 1.51$  mM and  $20.30 \pm 2.05$  mM in group F, I and J ducks respectively, were significantly different between group F and I ducks ( $p<0.05$ ) and group F and J ducks ( $p<0.01$ ). At 60 min glucose levels were not different between group F, I and J ducks.

Corticosterone levels at 0 min (Fig 4.18) were significantly higher in group I ( $p<0.01$ ) and J ducks ( $p<0.01$ ) than in other groups of ducks given weaker glucose solutions or saline. At 30 min (Fig 4.19) corticosterone levels had fallen significantly more in the ducks from group I ( $p<0.05$ ) and group J ( $p<0.01$ ) than levels in group F ducks ( $p<0.05$ ). By 60 min (Fig 4.20) the change in corticosterone levels from 0 min was not significantly different between either group F and I ducks ( $p=0.975$ ) or group F and J ducks ( $p=0.819$ ).

The actual corticosterone levels measured at 30 min of  $16.56 \pm 3.19$  ng/ml,  $20.80 \pm 5.73$  ng/ml and  $20.36 \pm 6.42$  ng/ml in group F, I and J ducks respectively, were not significantly different between either group F and I ducks ( $p=0.968$ ) or group F and J ducks ( $p=0.983$ ). By 60 min corticosterone levels in group I and J ducks were significantly higher ( $p<0.05$ ) than levels in other groups of ducks.

Sustained elevation of glucose levels for 1 h was achieved in ducks given a larger 30 ml dose of a 1.38M glucose solution (group L) while ducks given 30 ml of saline (group K) had no change in glucose levels (Fig 4.21). Glucose levels rose in all 4 of the group L ducks with levels at 60 min still

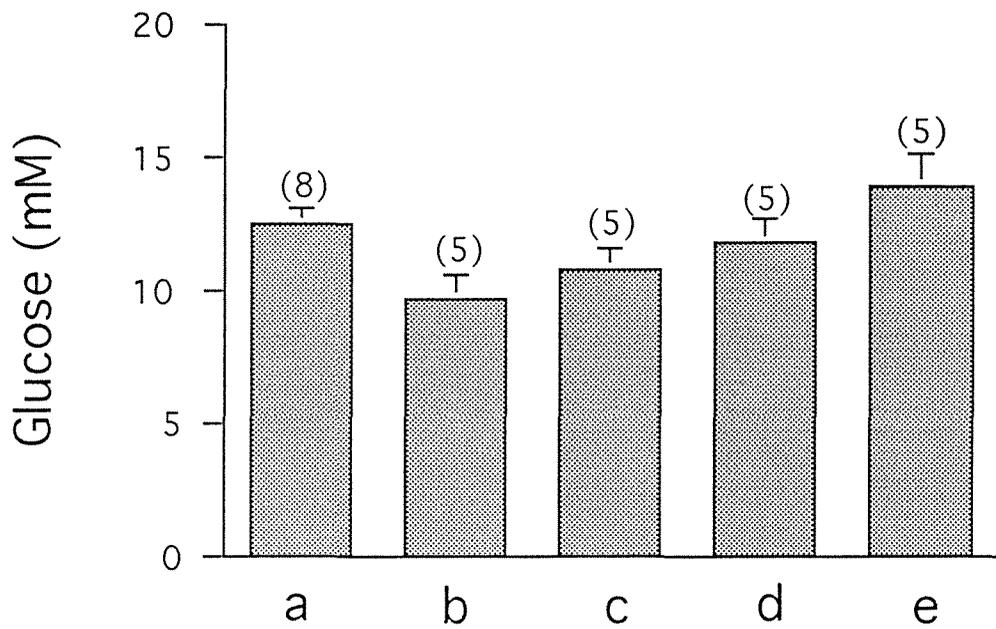


Figure 4.15: Mean plasma glucose levels at 0 min in ducks sampled every 30 mins and given a 10 ml oral dose of either; (a) saline (group F), (b) 0.28M glucose solution (group G), (c) 0.56M glucose solution (group H), (d) 0.84M glucose solution (group I) or (e) 1.38M glucose solution (group J). Number of ducks sampled are in brackets above columns.

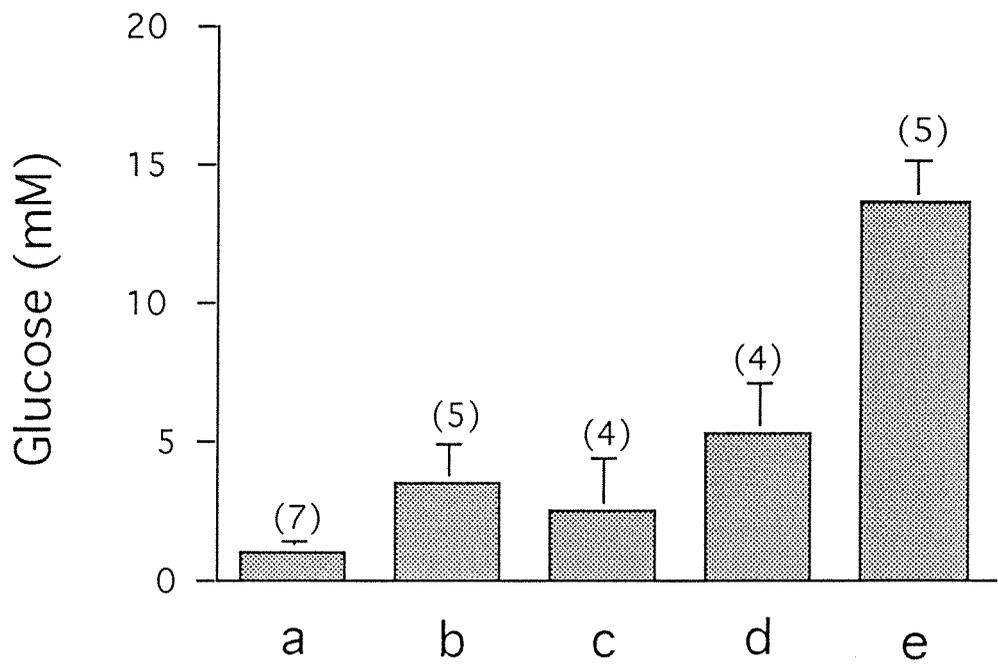


Figure 4.16: Change in mean plasma glucose levels 30 min after the first sample in ducks given a 10 ml oral dose of either; (a) saline (group F), (b) 0.28M glucose solution (group G), (c) 0.56M glucose solution (group H), (d) 0.84M glucose solution (group I) or (e) 1.38M glucose solution (group J). Number of ducks sampled are in brackets above columns.

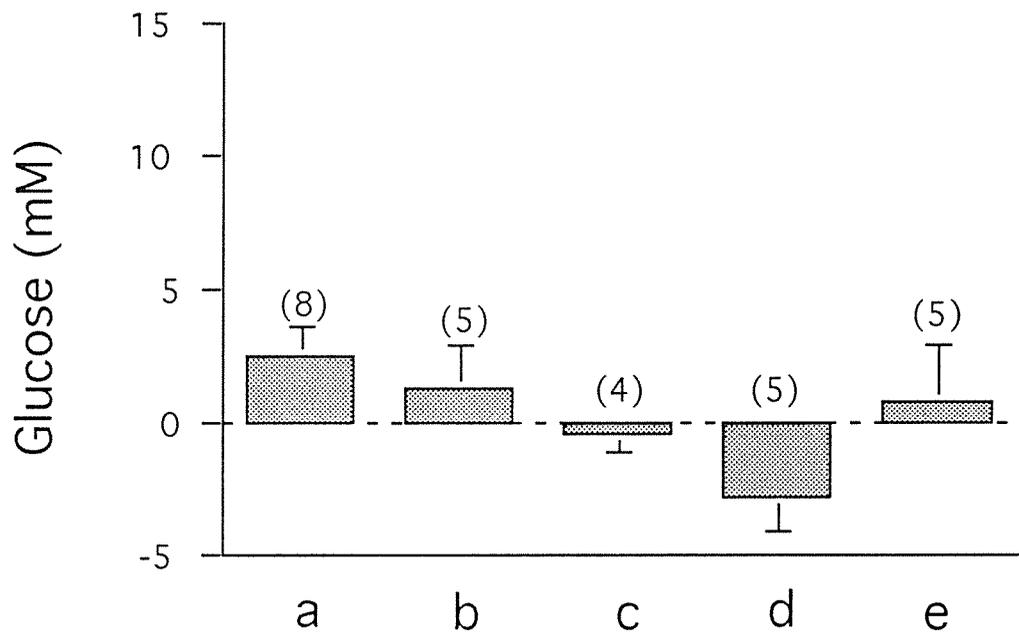


Figure 4.17: Change in mean plasma glucose levels 60 min after the first sample in ducks given a 10 ml oral dose of either; (a) saline (group F), (b) 0.28M glucose solution (group G), (c) 0.56M glucose solution (group H), (d) 0.84M glucose solution (group I) or (e) 1.38M glucose solution (group J). Number of ducks sampled are in brackets above columns.

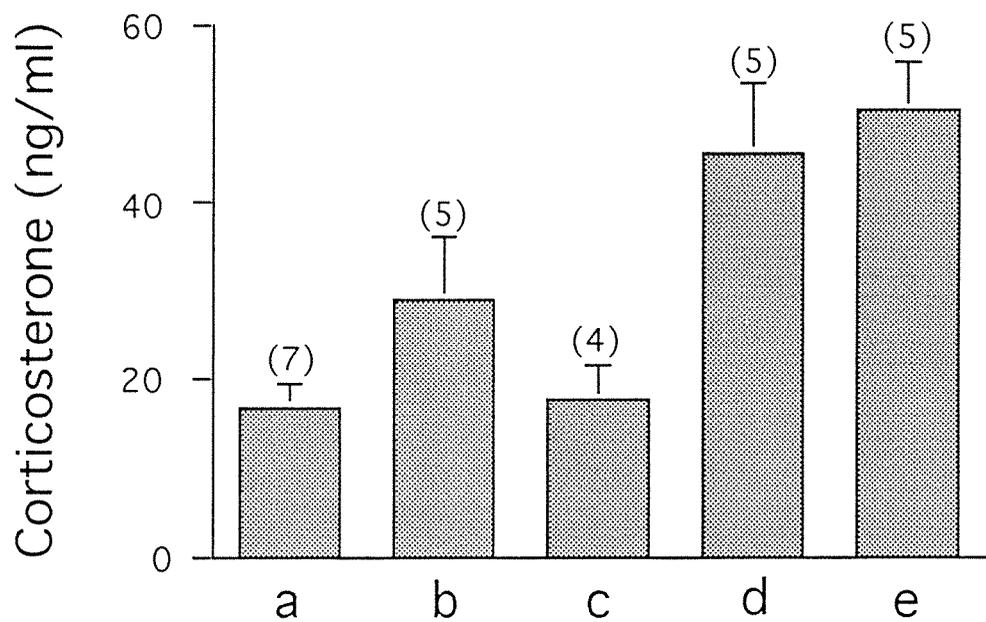


Figure 4.18: Mean plasma corticosterone levels at 0 min in ducks sampled every 30 mins and given a 10 ml oral dose of either; (a) saline (group F), (b) 0.28M glucose solution (group G), (c) 0.56M glucose solution (group H), (d) 0.84M glucose solution (group I) or (e) 1.38M glucose solution (group J). Number of ducks sampled are in brackets above columns.

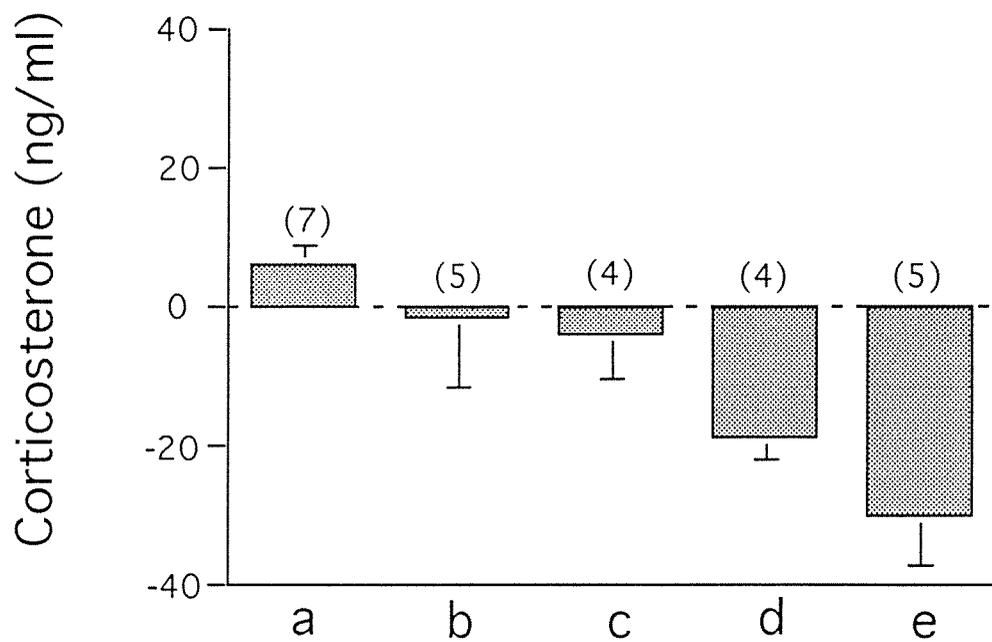


Figure 4.19: Change in mean plasma corticosterone levels 30 min after the first sample in ducks given a 10 ml oral dose of either; (a) saline (group F), (b) 0.28M glucose solution (group G), (c) 0.56M glucose solution (group H), (d) 0.84M glucose solution (group I) or (e) 1.38M glucose solution (group J). Number of ducks sampled are in brackets above columns.

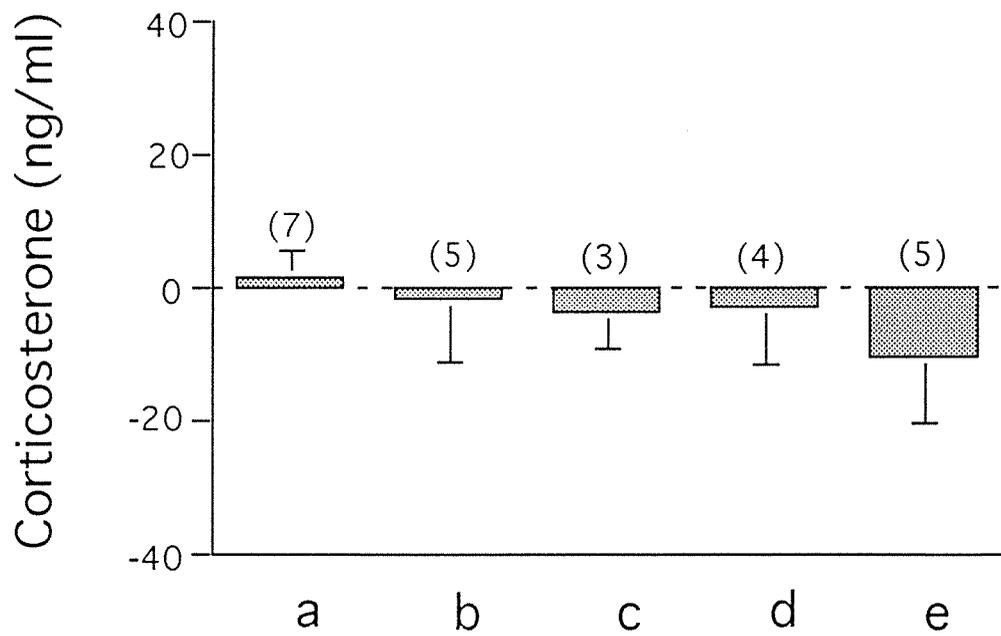


Figure 4.20: Change in mean plasma corticosterone levels 60 min after the first sample in ducks given a 10 ml oral dose of either; (a) saline (group F), (b) 0.28M glucose solution (group G), (c) 0.56M glucose solution (group H), (d) 0.84M glucose solution (group I) or (e) 1.38M glucose solution (group J). Number of ducks sampled are in brackets above columns.

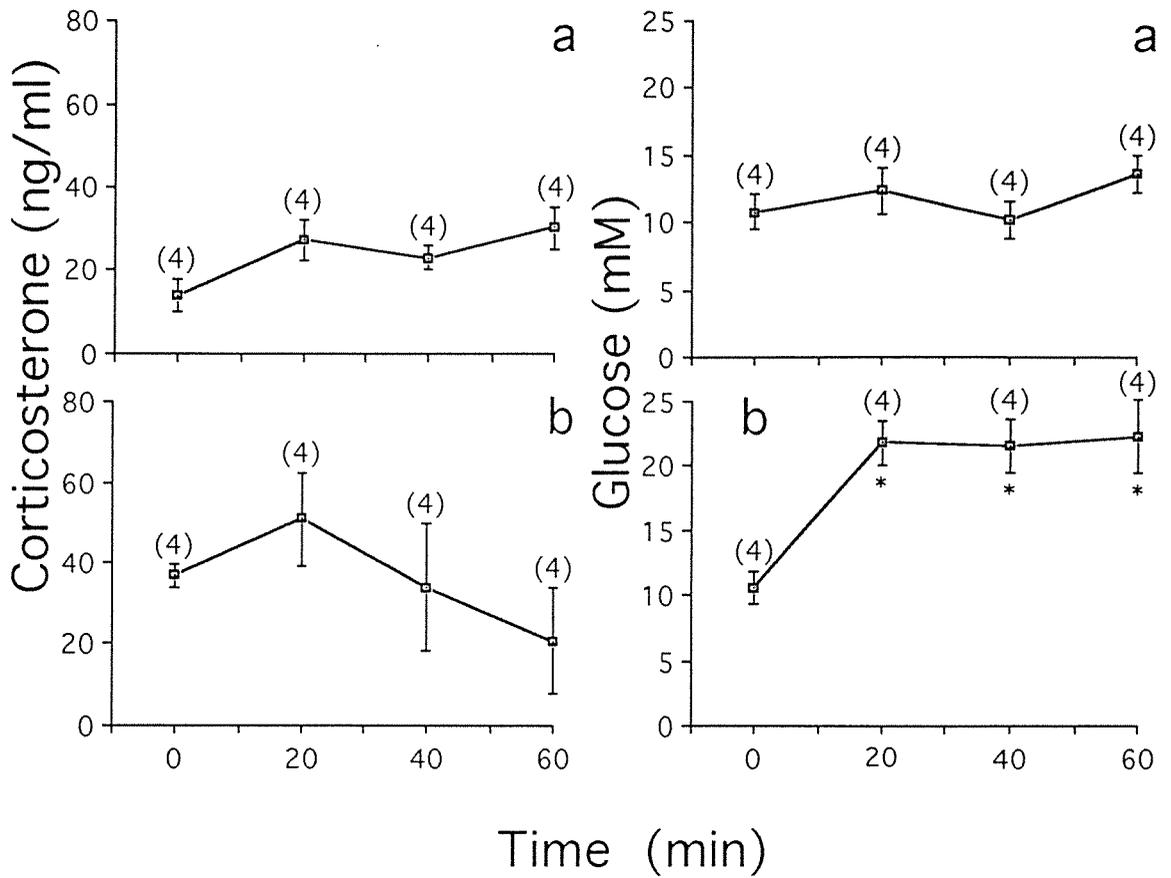


Figure 4.21: Mean plasma levels of corticosterone and glucose in ducks sampled every 20 min and given a 30 ml oral dose of (a) saline (group K) or (b) 1.38M glucose solution (group L). Number of ducks sampled are in brackets above error bars. Statistics relate to changes in glucose from starting levels.

higher than those measured at 0 min (Fig 4.22). Unlike ducks in group I and J who were given smaller doses of glucose solutions, corticosterone levels in group L ducks did not fall significantly ( $p=0.184$ ) when glucose levels were elevated (Fig 4.21). Corticosterone levels at 60 min were lower however, than levels measured at 0 min in 3 of the 4 group L ducks, although 1 of the 3 ducks had an increase in levels at 20 min (Fig 4.22).

Glucose levels at 0 min were not significantly different ( $p=0.915$ ) between group K and L ducks, though by 20, 40 and 60 min levels were significantly higher in group L ducks given glucose than group K ducks given saline ( $p<0.01$ ,  $p<0.01$  and  $p<0.05$  respectively). Corticosterone levels at 0 min were significantly higher ( $p<0.01$ ) in ducks given glucose ( $36.78 \pm 2.88$  ng/ml) than ducks given saline ( $13.90 \pm 3.95$  ng/ml), but there was no significant difference in levels at 20 min ( $p=0.109$ ), 40 min ( $p=0.515$ ) or 60 min ( $p=0.531$ ).

#### 4.3.2.2 Intravenous glucose administration and the plasma corticosterone and glucose responses to sampling stress

When solutions (3 ml) of increasing glucose strengths were administered to ducks intravenously via the brachial vein only the 1.94M glucose solution (group P) significantly ( $p<0.05$ ) altered glucose levels from those measured at 0 min (Fig 4.23). By 40 min glucose levels in all 4 of the group P ducks had fallen below levels measured at 0 min (Fig 4.24). Glucose responses of the other two groups of ducks given an intravenous glucose solution (groups N and O) were variable, as were the glucose responses of the ducks given saline (group M).

Corticosterone levels did not significantly change from levels measured at 0 min in any group (Fig 4.23), although levels in individual ducks tended to increase rather than decrease (Fig 4.24).

At no time were glucose or corticosterone levels significantly different between ducks given saline (group M) and ducks given a glucose solution (group N, O and P), though glucose levels at 40 min ( $7.81 \pm 0.37$  mM) in

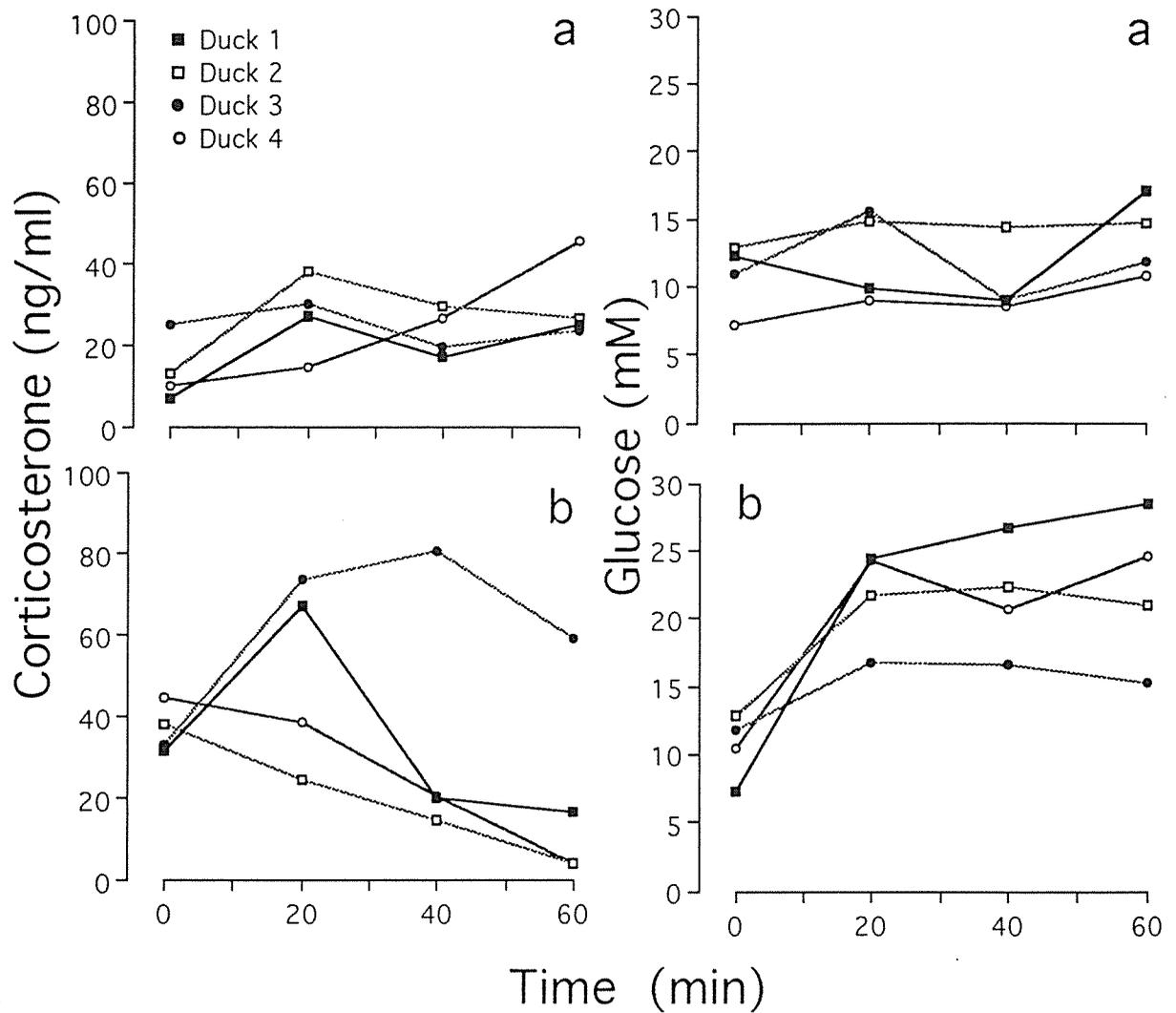


Figure 4.22 Plasma levels of corticosterone and glucose in ducks sampled every 20 min and given a 30 ml oral dose of (a) saline (group K) or (b) 1.38M glucose solution (group L).

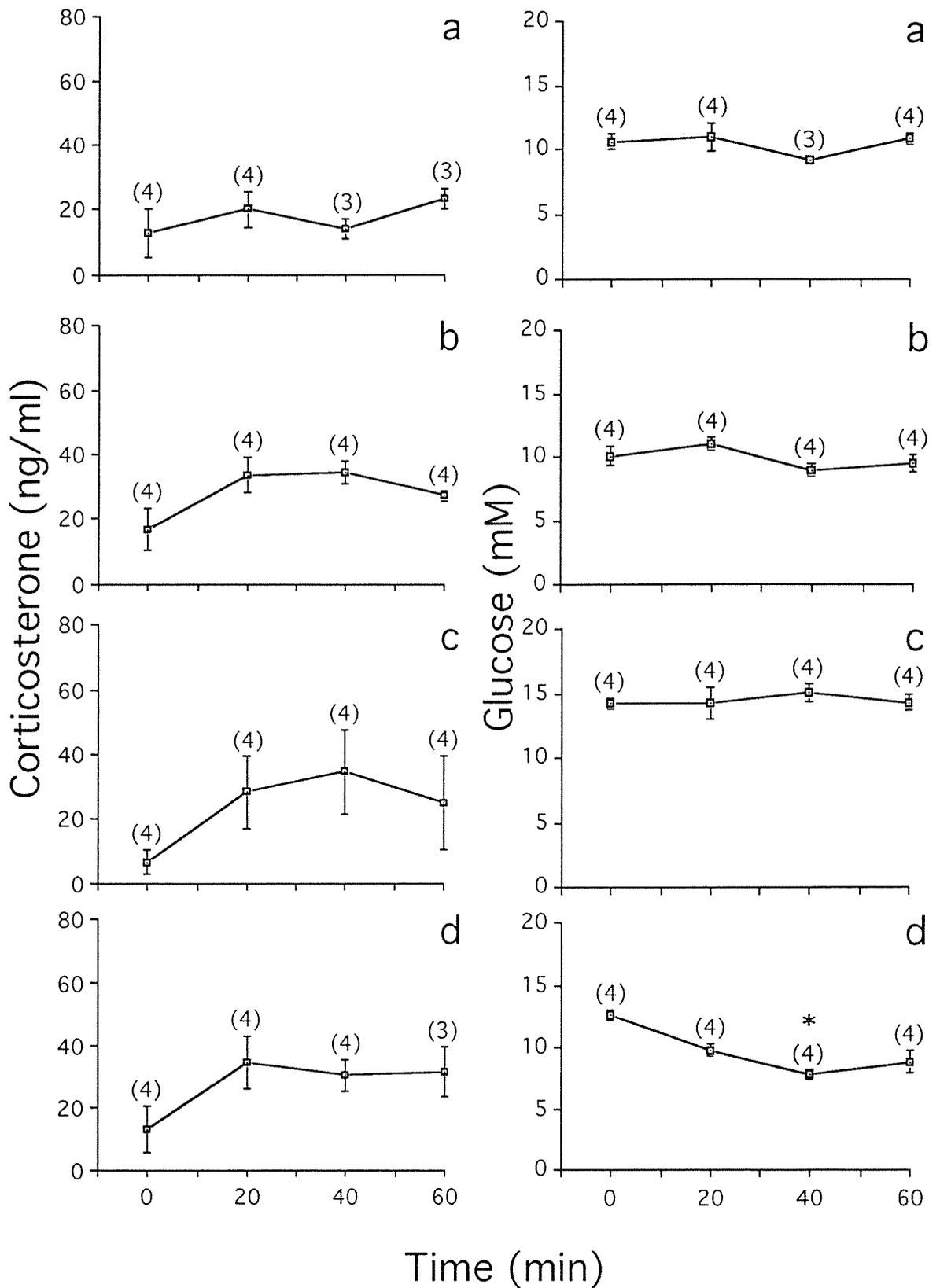


Figure 4.23 Mean plasma levels of corticosterone and glucose in ducks sampled every 20 min and given a 3 ml intravenous dose of either; (a) saline (group M), (b) 0.56M glucose solution (group N), (c) 1.38M glucose solution (group O) or (d) 1.94M glucose solution (group P). Number of ducks sampled are in brackets above error bars. Statistics relate to changes in glucose from starting levels.

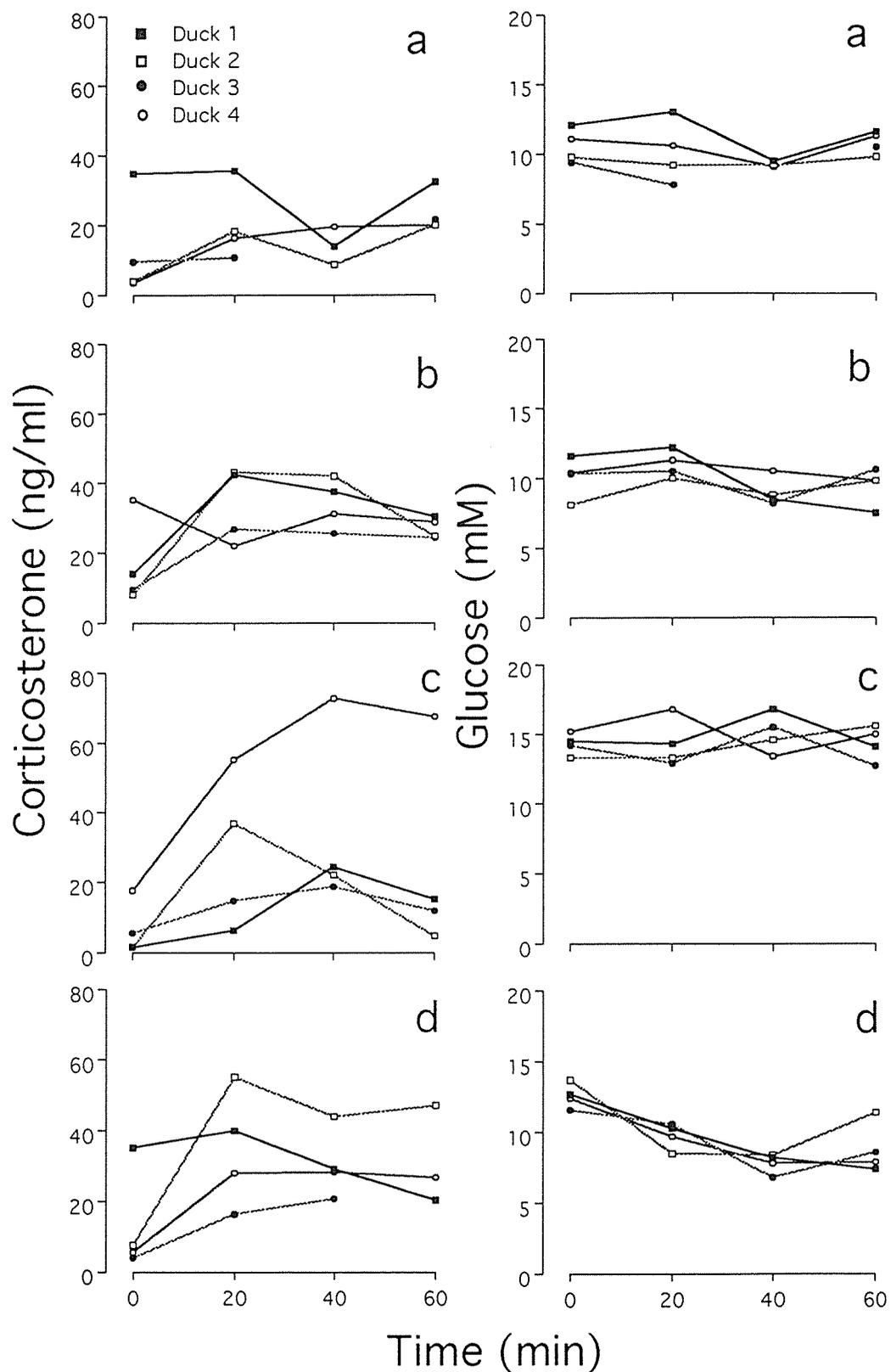


Figure 4.24 Plasma levels of corticosterone and glucose from ducks sampled every 20 min and given a 3 ml intravenous dose of either; (a) saline (group M), (b) 0.56M glucose solution (group N), (c) 1.38M glucose solution (group O) or (d) 1.94M glucose solution (group P).

group P ducks were almost significantly lower ( $p=0.072$ ) than glucose levels in group M ducks ( $9.27 \pm 0.12$  mM).

## 4.4 Discussion

In summary, the absence of any relationship between corticosterone levels and either the time taken to obtain a blood sample or the order in which a duck was sampled from a group may have been due to elevated levels in the ducks prior to sampling. Corticosterone levels increased when sampling stress was applied despite the high levels before sampling, with the magnitude of the increase depending on the frequency of sampling. Handling alone augmented, and could have possibly initiated, the observed corticosterone response to sampling stress. No evidence of a plasma glucose response to sampling stress was seen, despite the observable increase in corticosterone levels. This may have been because any stress induced rise in glucose levels in this study was too slight to detect.

The lowering of high corticosterone levels in ducks given an oral glucose dose that was strong enough to increase glucose levels after 30 min suggests that oral glucose administration may reduce a corticosterone response to stress.

### 4.4.1 Plasma corticosterone and glucose responses to sampling and handling stress

#### 4.4.1.1 Effects on plasma corticosterone levels in the first blood sample of the time taken to obtain a blood sample and of the order of sampling

In this study, corticosterone levels did not increase during the sampling period, despite samples being collected up to 5.5 min after the ducks were picked up. The contrast between this result and a previous report that corticosterone levels can increase after 1 min (Harvey *et. al.*, 1980) may be due to the high corticosterone levels in the present study. Harvey *et. al.* (1980) considered corticosterone levels of less than 12 ng/ml to be basal, whereas mean levels in the first samples collected from ducks in this study averaged 20.1 ng/ml. These high initial corticosterone levels indicate that

the ducks were already experiencing stress before they were picked up. The ducks were confined within a 1m diameter ring before sampling so that they could be picked up without being repeatedly chased. The results indicate that this confinement was probably stressful for the ducks, so that any corticosterone response to being handled for up to 5.5 min for blood sample collection was masked by a response to confinement. Rees *et. al.* (1985b) showed a similar effect in chickens where one stressor (starvation) masked the corticosterone response to another stressor (treadmill exercise).

The placement of ducks into individual darkened boxes for 1 h has been shown to lower high corticosterone levels (Ch. 2, section 2.4.3.2) and could be used in future studies to lower levels prior to sampling. Furthermore, blood samples should be collected in less than 90 sec since corticosterone levels rise within 5 min after ducks are initially sampled (group A, Fig 4.03).

No increase in corticosterone levels was seen among ducks as their peers were removed from the group (Fig 4.02). However, a sampling order effect on corticosterone levels could have been obscured due to elevated levels prior to sampling. Other studies have shown no effect of sampling order on corticosterone levels (Dusseau and Meier, 1971; Harvey *et. al.*, 1980; Harvey and Phillips, 1982; Jones and Harvey, 1987; Lagadic *et. al.*, 1990), although Dittami (1981) did find such an effect in geese.

#### 4.4.1.2 Plasma corticosterone and glucose responses to sampling stress

Sampling ducks at 5 min intervals increased corticosterone levels in this (Fig 4.03) and other studies (e.g. Harvey *et. al.*, 1980). In this study mean corticosterone levels reached a plateau between 10 and 15 min at 38 ng/ml. These may be the maximum mean corticosterone levels that can be induced by sampling. Harvey *et. al.* (1980) also demonstrated a maximum corticosterone response of 25 to 40 ng/ml in ducks sampled at 2 min intervals.

The higher corticosterone levels at 10 and 15 min in ducks sampled at 5

min intervals compared with corresponding levels in ducks sampled at 10 or 15 min intervals suggest that the frequency of sampling determines the rate of rise of any increase in corticosterone levels. Furthermore, if the severity of the stressor determines the maximum corticosterone levels, then less frequent sampling may reduce the severity of the stress experienced and a lower maximum corticosterone level will result. In other studies maximum corticosterone levels in ducks were related to the sampling frequency (Harvey *et. al.*, 1980; Rees *et. al.*, 1983).

No corticosterone response to sampling occurred when ducks given saline in the second experiment (Fig 4.13, 4.21 and 4.23) were sampled at 20 or 30 min intervals. It may be that any increase in corticosterone levels as a result of the initial sampling and glucose administration had diminished by the time of the next sample. The metabolic half-life of corticosterone is 10 to 12.5 min, indicating that a decline from 40 ng/ml to 10 ng/ml is possible within 20 to 30 min (Bradley and Holmes, 1971; Harvey *et. al.*, 1980).

The ability of corticosterone to stimulate glycolysis and/or gluconeogenesis is well supported (Hazelwood, 1976; Sitbon *et. al.*, 1980) though in this experiment glucose levels did not rise in conjunction with the demonstrated increases in corticosterone levels. In the only study to report increases in both corticosterone and glucose levels with repeated sampling, Harvey and Phillips (1982) found that glucose levels in the duck increased 2.1mM from 12.3mM after 90 min of repeated sampling at 15 min intervals. In this study an average increase in glucose levels of 1.9mM from 11.2mM was seen in the ducks sampled at 5 min intervals, but was not significant due to large variation between ducks. Furthermore, as Harvey and Phillips (1982) recorded an increase in glucose levels after 90 min the absence of a increase in glucose levels in this study could be due to the shorter period samples were collected.

In future studies sampling regime of 5, 10 or 15 min intervals should allow the detection of any change in glucose levels with sampling stress as similar regimes have done so (Freeman and Manning, 1977; Harvey and Phillips, 1982). One problem in investigating the glyceamic effects of corticosterone are the similar actions of glucagon and the catecholamines

(Jeronen *et. al.*, 1976; Freeman and Manning, 1976 and 1978). The determination of which hormone is causing any increase in glucose levels may require studies using specific hormone antagonists.

#### 4.4.1.3 Plasma corticosterone and glucose responses to sampling and additional handling stress

In one of two groups of ducks additional handling every 2 min between the collection of blood samples at 10 min intervals temporarily elevated corticosterone levels above levels in ducks not subjected to additional handling (Fig 4.08). Holmes *et. al.* (1990a) states handling alone may even induce a corticosterone response similar to that seen with sampling, indicating that it may be the handling of the bird rather than the venipuncture which is imposing the stress upon the bird.

Possible reasons for the absence of any increase in glucose levels despite a increase in corticosterone levels with sampling and additional handling are similar to those discussed in the previous section. In future studies of the relationship between corticosterone and glucose the duration of sampling may have to be increased, since the only study to demonstrate a rise in both corticosterone and glucose levels collected samples for 90 min at 15 min intervals (Harvey and Phillips, 1982). For the mallard we chose to limit the number of samples collected from each duck to four. However, handling alone could be used to increase corticosterone levels allowing samples to be collected for a greater duration than 45 min which sampling at 15 min intervals would only allow.

#### 4.4.2 Exogenous glucose administration and the plasma corticosterone and glucose responses to sampling stress

##### 4.4.2.1 Oral glucose administration and the plasma corticosterone and glucose responses to sampling stress

The use of oral glucose in lowering corticosterone levels was successful

in ducks given doses of 0.84M (group I) and 1.38M (group J) glucose in which corticosterone levels fell from 0 to 30 min before rising to near 0 min levels by 60 min (Fig 4.13). However, when corticosterone levels at 30 min in group I and J ducks are compared to those in a control group of ducks given saline there was no difference in levels. Corticosterone levels in ducks given saline started and remained around 18 ng/ml, whereas group I and J ducks had significantly higher levels at 0 min (Fig 4.18). These high corticosterone levels decreased at 30 min before returning at 60 min to levels similar to those measured at 0 min. Hence glucose administration can lower previously high corticosterone levels, but it is uncertain whether glucose will inhibit any corticosterone response to sampling stress.

Furthermore, the extent of any reduction in corticosterone levels with oral glucose administration appears to depend on initial corticosterone levels. Ducks with initial corticosterone levels some 20 ng/ml less than group I or J ducks and given an even larger glucose load (group L) had a lower reduction in corticosterone levels despite the larger glucose load (Fig 4.21).

In conclusion, oral glucose administration may be useful in lowering high corticosterone levels, with the duration of any depression in levels depending on how long glucose levels are raised. This indicates that a feedback relationship of glucose on corticosterone release might exist. It is uncertain whether oral glucose administration could stop a corticosterone response from occurring, or whether such a procedure would just temporarily lower corticosterone levels but not reduce the overall corticosterone response. Indeed, the possible stress associated with the procedure of oral glucose administration may even enlarge the overall corticosterone response once temporary inhibition of corticosterone levels has diminished.

In future studies, for oral glucose administration to be shown to reduce stress birds given glucose must have smaller overall corticosterone response than control birds. Low initial levels of corticosterone which then increase with the application of a stressor such as sampling or handling are therefore needed. To allow for individual variation in the corticosterone response the

control or glucose treatments could be swapped in a later re-run and the resulting responses with and without glucose compared for each bird.

In deciding a suitable glucose load to use for future studies information can be gained from the magnitude and the duration for which glucose levels were elevated in this experiment. Glucose levels in group J ducks given 10 ml of a 1.38M glucose solution (containing 13.8 mmoles of glucose) had returned to levels seen before glucose loading by 60 min (Fig 4.13) so presumably 13.8 mmoles can be absorbed and utilised or excreted per hour. The kidneys could excrete 3.3 mmoles of glucose (Shoemaker, 1972) while the remaining 10.5 mmoles could have been utilised by the salt gland, liver, adipose and other tissues.

#### 4.4.2.2 Intravenous glucose administration and the plasma corticosterone and glucose responses to sampling stress

The absence of any increase in glucose levels with intravenous glucose administration indicates either that the glucose dose failed to enter the circulation, or that the glucose given was totally excreted or utilised from the circulation within 20 min.

In a previous study on the duck Peaker *et. al.* (1971) injected a 18 ml solution containing 2.16 mmoles of glucose and raised glucose levels by 2.8mM from 10 min to 3 h after injection. In this experiment 3 ml solutions containing up to 5.82 mmoles of glucose were used indicating that the amount of glucose used was sufficient to raise glucose levels over the period sampled. If the bolus of exogenous glucose given did reach the circulation and temporarily raised glucose levels for less than 20 min then a possible release of insulin which continued after 20 min could explain the decrease in glucose levels observed in all the ducks given a 1.94M intravenous glucose dose.

As only 3 ml was administered it is possible that none of the glucose solutions entered the circulation. Venipuncture often causes haematomas and blood flow distal to the injection site to be blocked from preceding any further. The glucose solutions may have entered the vein but had not been

carried centrally into circulation. The next major vein to intersect the brachial vein does not do so until some 3 cm central to the injection site (Nickel *et. al.*, 1977), so some or all of the 3 ml glucose solution could have stayed in this 3 cm section of the vein for the duration of the experiment. The injection of 3 ml solutions into the brachial vein was complicated by occasional movements by the duck, so accidental injection of part of the solution subcutaneously was also possible.

## Chapter 5:

### General discussion

## 5. General discussion

Stress is a general term which in this study was interpreted to mean the presence of a situation which can compromise the wellbeing of the animal. Stress can be identified by an increase in plasma levels of the hormone corticosterone. Hence the influence of capture and captivity on corticosterone levels in ducks were investigated to see if such procedures are stressful and how any associated stress can be minimised. In the mallard, oral administration of a glucose solution or placing of a duck into a darkened individual box could both reduce previously high corticosterone levels and hence may reduce stress.

Why some birds breed in captivity and others do not was investigated in a group of New Zealand grey duck. Corticosterone levels before the breeding season were lower in breeding ducks than non-breeding ducks, and it appeared that the likelihood of a bird breeding in captivity may be estimated from corticosterone levels. However, the formation of territorial areas by the grey ducks may have inhibited feeder access for the non-breeding ducks, indirectly raising their corticosterone levels and reducing their body weight. Hence measurements of corticosterone levels in captive birds may identify individuals with a decreased likelihood of breeding, or factors which may reduce breeding success.

In investigating changes in corticosterone levels blood samples are obtained and the sampling procedure itself is stressful for the bird. Harvey *et al* (1980) found corticosterone levels increased 60 sec after the picking up a duck for sampling. In this study blood samples were obtained in less than 90 sec from the duck being picked up, with no increase in corticosterone levels occurring during that period. Hence it was assumed that obtaining blood samples in less than 90 sec from the picking up of a duck for sampling yields corticosterone levels reflecting those in the duck before it was picked up. The observation of an increase in corticosterone levels as a result of sampling or any other stress requires the collection of several blood samples. The frequency of sample collection and corticosterone levels in the first sample appear to determine whether a increase in corticosterone levels with

sampling is observed.

### 5.1 Reducing capture stress in wild birds

The capture of wild birds for conservation purposes imposes a stress on the birds which may affect their breeding which is the opposite of what is intended with conservation measures. Hence minimising the stress associated with capture and even transportation of the bird to a new habitat is desirable.

In the mallard, placing the duck in a individual darkened box for 20 min or administering an oral glucose solution lowered previously high corticosterone levels. As the placing of a duck into a darkened box appears to be beneficial the collection of data such as body weight should be done while the bird is in a box. The darkening of the head with a hood might also reduce corticosterone levels and calm the bird, making data collection easier. In the transportation of birds, individual boxes are favoured over communal boxes as other studies have demonstrated higher corticosterone levels in ducks when 2 ducks are held in a box than when ducks are held individually (Cockrem pers. comm.).

The value of oral glucose administration in reducing corticosterone levels and hence stress is unclear. The administration of oral glucose is likely to be a stressful procedure, so the total secretion of corticosterone from the adrenal gland may be greater when glucose is given, despite any temporary reduction in levels, than if glucose was not administered at all.

### 5.2 Selection of individuals with a greater likelihood of successful breeding in captivity

The removal of birds from their natural environment to a captive one should help to increase breeding success by avoiding predation and providing a readily obtainable food supply. However some birds do not breed and are said to be unable to adapt to captivity. In this study, individual variation among both mallard and grey ducks in their corticosterone

responses to consistent stressors suggests that some birds adapt better than others to captivity. Identification of such individuals for selection in captive breeding programmes could improve breeding success.

In this study the corticosterone response to sampling stress after capture by trapping was measured some female mallards, but their subsequent breeding success in captivity was not known. In the grey ducks lower corticosterone responses to sampling stress appeared to occur among the reproductively active ducks, but all ducks may have bred given better access to feeders. Hence if a relationship exists between a corticosterone response to stress and the likelihood of breeding success, it has yet to be demonstrated. In future studies the placing of birds into individual boxes after capture may provide a consistent stressor, with the resulting corticosterone response being measured from either several plasma samples or a single faecal sample. For endangered birds faecal samples provide a non-invasive way of measuring steroid levels and the collection of droppings in the darkened box could potentially allow measurements of corticosterone levels to be made (Cockrem pers. comm.).

### 5.3 Adjustment to captivity and breeding success

When wild birds are brought into captivity corticosterone levels may remain elevated for several days until the birds adjust to their new environment and hopefully breed (Wingfield *et. al.*, 1982). In this study corticosterone levels decreased over several months in mallards caught from the wild.

The environment in which birds are held influences corticosterone levels and hence the time taken to adjust to captivity and whether breeding occurs. In this study, high corticosterone levels were measured in grey ducks who subsequently did not breed. Appreciation that the high corticosterone levels may have resulted from restricted feeder access has led to increases being made in the area available to the grey ducks, and in the number of feeders. This is an example of how the measurement of plasma corticosterone levels, body weight and behavioural observations help

identify problems affecting individual birds which may inhibit them breeding.

#### 5.4 Methods for studying the influence of stress on plasma corticosterone levels

Studying the influence of sampling stress on plasma corticosterone levels can be difficult as corticosterone levels can increase 60 sec after a duck has been picked up for blood sampling (Harvey *et. al.*, 1980). Hence for blood samples to yield corticosterone levels that reflect those in the duck before it was picked up for sampling, a limit of 90 sec to obtain a sample was set which allowed most intended samples to be obtained. Inexperienced students collected the samples used in the study of exogenous glucose effects on corticosterone levels, and some samples took up to 330 sec to obtain. No relationship between corticosterone levels and taking between 30 to 330 sec to obtain the blood samples was demonstrated, but it appears that such a relationship was disguised by the high corticosterone levels in the ducks before they were picked up for sampling.

Despite high corticosterone levels in initial samples a corticosterone response to sampling can be observed if sampling occurs at a high enough frequency. The placing of ducks in a individual darkened box for 1 h before sampling will decrease high corticosterone levels and increase the likelihood of an rise in levels with sampling stress being observed. If birds can be obtained directly from their natural environment and sampled within 90 sec, then the placing of birds into individual darkened boxes would not be required as corticosterone levels will be basal unless the bird was disturbed in its environment beforehand.

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Appendix A: Monthly variation in body weight and condition in grey ducks (*Anas superciliosa*)

The body weights of 5 male and 5 female grey duck were recorded using an electronic balance from July to December. There was no significant change in body weight over these months in either the male ( $p=0.391$ ) or female ducks who did not produce clutches ( $p=0.054$ , Fig 1). However, in both sexes body weight tended to decrease from winter (July to August) to the breeding season in late spring (September to November) before rising again in summer (December). When the body weight data is converted to the body condition measurement outlined in chapter 4, section 4.2.3, a significant decrease in body condition from winter to the breeding season in late spring occurs in male ducks ( $p<0.05$ , Fig 2). Winglength measurements (a component of the body condition measurement) were made in January of the following year when only 2 of the 5 female grey ducks were available, hence body condition indices are only shown for these 2 female ducks.

A lower body weight/condition during the breeding season than before or after could be due to the increased energy expenditure associated with the greater reproductive and territorial behaviour exhibited by the male ducks during the breeding season. A decreased body weight during the breeding season has been found in wild mallards (Hohn, 1947) and starlings (Dawson and Howe, 1983).

The female grey duck #360 started laying eggs 4 days after being weighed in September, hence her high body weight/condition at this time reflects the formation of eggs within her reproductive system.

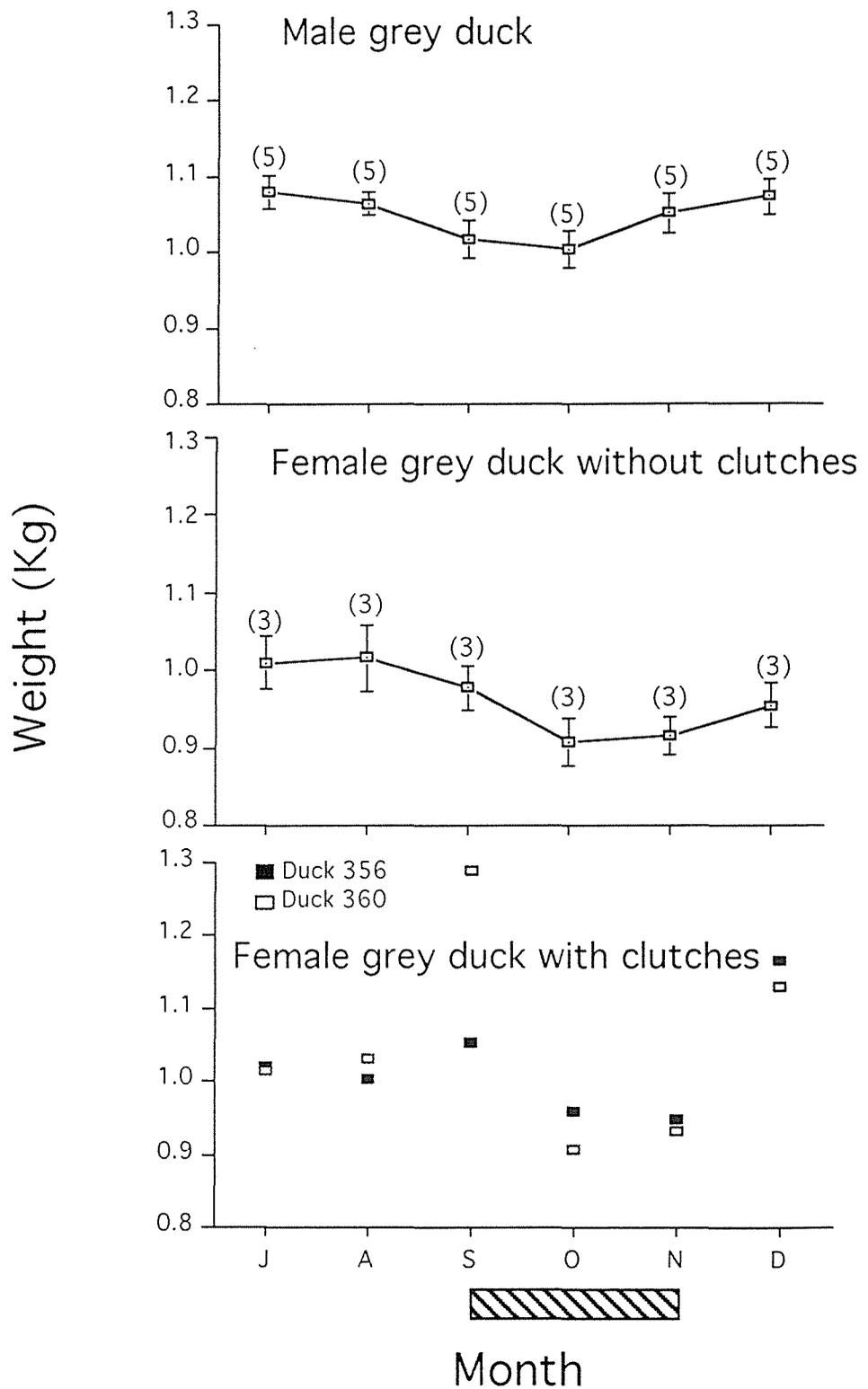


Figure 1: Mean weight of male grey ducks, female grey ducks without clutches and the 2 female grey ducks that did produce clutches. Number of ducks weighed are in brackets above error bars. Horizontal bar indicates breeding season.

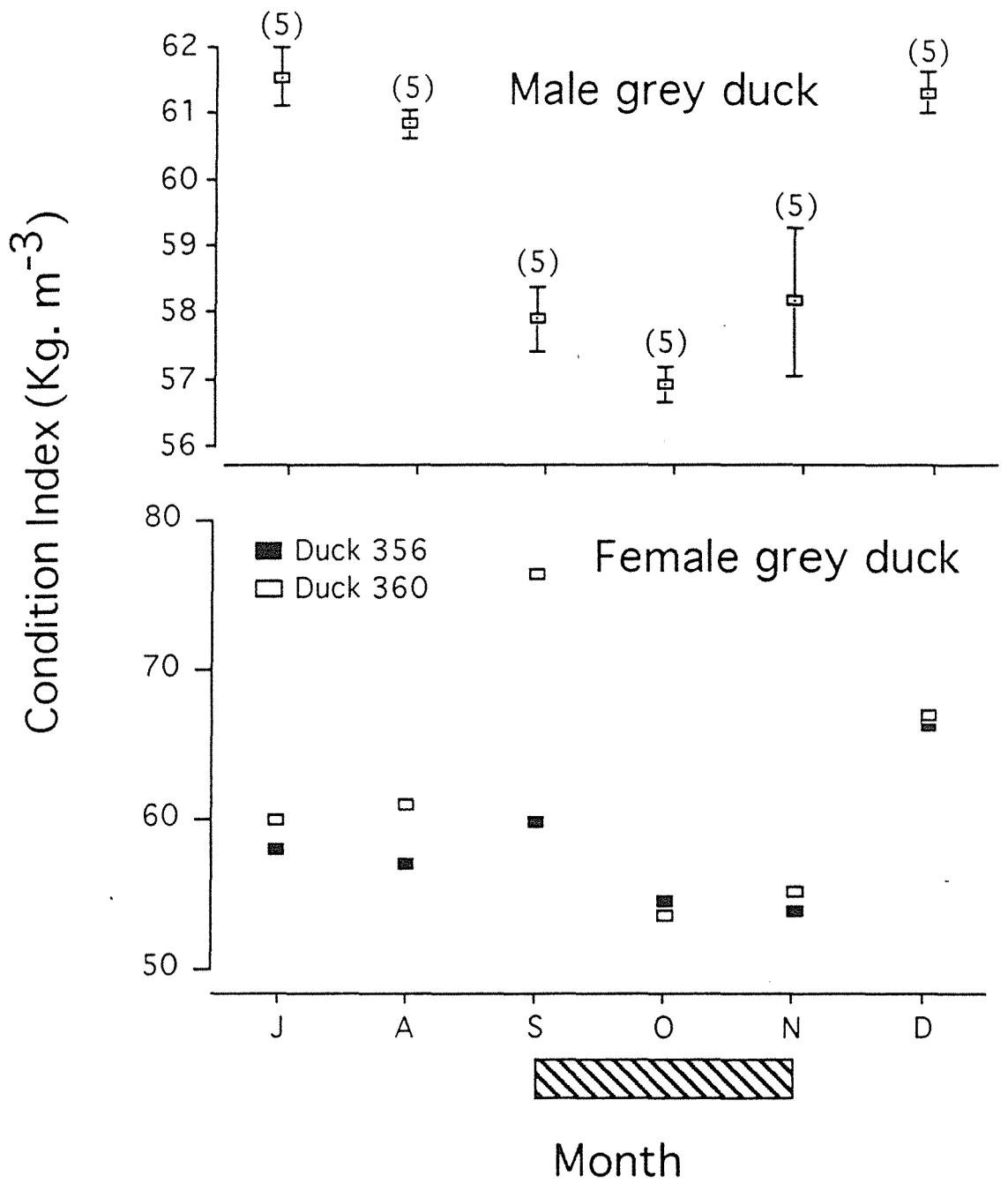


Figure 2: Mean condition indices of male grey ducks and individual condition indices of 2 female grey ducks. Number of male grey ducks measured are in brackets above error bars. Horizontal bar indicates breeding season.