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Anatomy and pathology of adrenal glands in common dolphins: Influence of life history and contaminant burdens

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

Master of Science

in

Zoology

at Massey University, Albany, New Zealand



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2026

Abstract

Adrenal glands play a central role in stress physiology, immune regulation, and endocrine homeostasis, yet their gross morphology, cellular architecture, and pathology remain poorly characterised in delphinids. This study investigated adrenal gland structure, cellular composition, and pathology in common dolphins (*Delphinus delphis*) from New Zealand waters. Ontogeny and cumulative trace element burden were examined as predictors of architecture to establish baseline morphometric and histological reference values in this population, and to investigate contaminant-associated structural remodelling.

Adrenal glands were collected postmortem (2008-2025) from 103 stranded or incidentally bycaught dolphins. Gross morphometrics, digital planimetric histological analysis, and pathological assessment were performed. Hepatic and renal trace element concentrations were determined by inductively coupled plasma mass spectrometry. Linear mixed-effects models examined predictors of adrenal composition and trace element associations with glandular architecture.

Age was the dominant predictor of gross adrenal weight and length, with glands increasing approximately 0.20 g and 0.80 mm per annum, respectively. Bilateral comparisons revealed significant directional asymmetry at the gross level, with left glands heavier and right glands longer, while proportional zonal composition was bilaterally equivalent. Mean cortex-to-medulla ratio was 2.15 ± 0.74 , with females exhibiting significantly higher ratios than males, driven by greater cortical and lower medullary proportions. Capsule proportion increased significantly with age. Prominent medullary band presence was associated with greater medullary proportion, with band prominence corresponding to measurable differences in medullary cross-sectional area. Adrenal pathology was identified in 24.8% of assessed glands, including one case of medullary neoplasia, suggestive of a pheochromocytoma. Pathology prevalence increased with age and sexual maturity.

Bioaccumulative trace element burden (selenium, cadmium, mercury) was significantly associated with greater capsular tissue and reduced cortical proportion, while essential metal status showed opposing directional associations. Mercury-to-selenium molar ratios predicted

capsular expansion independently of life history variables. These associations suggested that bioaccumulative burden drives capsular expansion alongside cortical reduction, a structural pattern not replicated by stress chronicity, disease classification, or pathology presence, which were non-significant predictors of adrenal composition.

This study established the first adrenal morphometric baseline for New Zealand common dolphins and provided novel evidence that bioaccumulative metal burden was associated with structural adrenal remodelling.

Acknowledgements

This thesis would not have been possible without the support and encouragement of many people, and I am deeply grateful to everyone who has contributed to this journey.

First and foremost, I would like to sincerely thank my supervisors, Karen Stockin, Katharina Peters, and Matthew Perrott. The support, opportunities, and intellectual generosity you have extended to me have shaped this thesis and my growth as a researcher. Your mentorship and encouragement throughout this process have been truly invaluable, and I am grateful for the time and care you invested in my work.

I would also like to thank Annie Page and Paula Alonso-Almorox for answering my countless histopathological and HPA-related questions. You have been integral to the core of this thesis, and your generosity, time and expertise made an enormous difference. Thank you to Petru Daniels and Evelyn Lupton for processing my histopathology slides, and to Ramona Eiholzer and Daniel Westlake for digitising them. Thank you to Cathy Harvey and Emma Gulliver for your ongoing histopathological support. Thank you to Sally Gaw, Rob Stainthorpe, and Marie Wong for your support with trace element analyses and for expanding my technical skills.

A huge thank you to the current and past members of the CERG team, without whom this research would not have been possible. I am especially grateful for the contributions of Emma Betty, Odette Howarth, Rebecca Boys, Emily Palmer, Deborah Casano Bally, Hannah Crisp, Denise Poelijoe, Abbey Skilton, and Evi Hanninger, who welcomed me into the group and whose research and dedication paved the way for my thesis. Thank you also to the more recent additions to CERG, who have been equally wonderful in their support: Nicholai Xuereb, Kiera McGarvey, Leo Suarez, Andrea Lattman, Joseph Scott, and Katrin Auer.

This thesis was financially supported by the Massey University Masterate Scholarship, the Wildbase Trust Research Grant, the Society of Integrative and Comparative Biology Grant-in-Aid of Research, and the Royal Society Te Apārangi Hutton Fund, for which I am grateful. I extend particular thanks to Brett Gartrell and Kerri Morgan for their support in this regard. Funding awarded to Karen Stockin through the Royal Society Te Apārangi Rutherford Discovery Fellowship also contributed significantly to the success of this work. I would also like to

acknowledge the BIOCET biobank upon which my project stands and thank Mana Whenua and the Department of Conservation Te Papa Atawhai for supporting the curation of tissues which underpin this project.

I would like to extend my warmest thanks to my colleagues at the University of California, Santa Cruz, in the Biotelemetry and Behavioral Ecology Lab and the broader Coastal Science community. The opportunities and mentorship you have offered me, and the friendships I have built along the way, have meant more than I can express, and I am deeply grateful. I would also like to thank my colleagues at the University of Auckland for welcoming me so warmly, when I arrived in New Zealand. The opportunity to work alongside such a wonderful group came at exactly the right time, and I am grateful for the continued support and camaraderie you have offered me throughout this journey.

To Emily, Deb, and Hannah, thank you for welcoming me into New Zealand with open arms and making it feel like home far sooner than I expected. From camping trips and dinner parties to gym sessions, and pizza nights and nails, you have filled this chapter with warmth, laughter, and the kind of friendship I will carry with me always. To Mazie, Chloe, and Amelia, I cannot express how fortunate I am to have such wonderful friends. The distance never once felt real because of you. Our weekly updates were the thread that kept me tethered to home, and your love and encouragement carried me across every hard stretch of this chapter. I am so lucky to have you, always. I am counting down the days until our beds are head-to-head again.

To my sister, Micaelyn, thank you for the beautiful figures you created for this thesis. Having your art woven into this work is the cherry-on-top, and it means more than I can say. I appreciate your unwavering support, and I am so lucky to have you by my side. I love you tremendously. Finally, to my parents, Mellissa and Marc Mann, thank you for being my greatest supporters. Your constant belief in me kept me going on the hard days, more than you know. Thank you for nurturing my curiosity, instilling in me a deep love of learning, and showing me the joy of hard work and pursuing what you love. I love you both more than words could ever capture.

To everyone named here, and those I may have inadvertently overlooked, this thesis is as much yours as it is mine. Ngā mihi maioha, aroha nui.

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List of Acronyms

Acronym	Definition
°C	degrees Celsius
3 β -HSD	3-beta-hydroxysteroid dehydrogenase
ACTH	adrenocorticotrophic hormone
Al	aluminium
As	arsenic
C:M	cortex-to-medulla ratio
Cd	cadmium
CI	confidence interval
cm	centimetre
Co	cobalt
Cr	chromium
CRH	corticotropin-releasing hormone
CSV	comma-separated value
Cu	copper
CYP17A1	cytochrome P450 17A1
D0-3	disease score: D0, D1, D2, D3
DCC	decomposition condition category
<i>df</i>	degrees of freedom
DHEA	dehydroepiandrosterone
DORM-4	certified fish protein reference material
dw	dry weight
exp	exponential function
Fe	iron
FSH	follicle-stimulating hormone
g	gram
GLG	growth layer group
GSD	geometric standard deviation
H	Kruskal-Wallis test statistic
H&E	hematoxylin and eosin
H ₂ O ₂	hydrogen peroxide
HCl	hydrochloric acid
He	helium
Hg	mercury
Hg:Se	mercury-selenium ratio
IHC	immunohistochemistry
HNO ₃	nitric acid
HPA	hypothalamic-pituitary-adrenal
ICC	intraclass correlation coefficient

ICP-MS	inductively coupled plasma mass spectrometry
ID	identification
IQR	interquartile range
K_bioaccum	kidney bioaccumulative index
K_essential	kidney essential index
kg	kilogram
L_bioaccum	liver bioaccumulative index
L_essential	liver essential index
LH	luteinizing hormone
LMM	linear mixed-effects model
LOD	limits of detection
LOESS	locally estimated scatterplot smoothing
LRT	likelihood ratio test
max	maximum
Mg	magnesium
mg	milligram
min	minimum
mL	millilitres
mm	millimetre
mmol	millimole
Mn	manganese
MΩ	megaohm
<i>n</i>	number
Ni	nickel
O ₂	dioxygen
<i>OR</i>	odds ratio
<i>p</i>	probability value
Pb	lead
PC1	principal component 1
PC2	principal component 2
PCA	principal component analysis
PCBs	polychlorinated biphenyls
PCR	polymerase chain reaction
PromBand	prominent medullary band
Q-Q	quantile-quantile
<i>r</i>	Pearson correlation coefficient
R ²	coefficient of determination
REML	restricted maximum likelihood
ROI	region of interest
SAM	sympathetic-adrenomedullary

SD	standard deviation
SE	standard error
Se	selenium
Sr	strontium
t	t-statistic
T3	triiodothyronine
T4	thyroxine
TSH	thyroid-stimulating hormone
UK	unknown
V	Wilcoxon signed-rank test statistic
V	vanadium
W	Wilcoxon rank-sum test statistic
ww	wet weight
$\times$	multiplied by
z	z-score
ZF	<i>zona fasciculata</i>
ZG	<i>zona glomerulosa</i>
Zn	zinc
ZR	<i>zona reticularis</i>
α	significance threshold (type I error rate)
β	regression coefficient (effect estimate)
Δ AIC	change in Akaike information criterion
μ m	micrometre, micron
ρ	Spearman's rank correlation coefficient
χ^2	Chi-squared

Chapter 1 – Introduction

1.1 Adrenal glands in vertebrates

1.1.1 The endocrine system

The endocrine system supports vital bodily functions and physiological processes including metabolism, growth, development, reproduction, and sexual function (Atkinson et al., 2009; Hiller-Sturmhöfel & Bartke, 1998; Papadimitriou & Priftis, 2009). This system comprises a network of glands and specialised tissues distributed throughout the body that maintain homeostasis through chemical messengers known as hormones. Hormones are produced in endocrine organs, which are dense with vasculature, allowing their efficient release directly into the bloodstream, where they act on distant target tissues. Across vertebrates, endocrine regulation integrates internal physiological states with external environmental cues.

Within this system, the adrenal glands are paired endocrine organs present across vertebrate taxa. They function at the interface of neural and endocrine regulation and play a central role in coordinating responses to physiological challenge (Atkinson et al., 2009; Herman et al., 2016). Due to their integrative role and sensitivity to both environmental and internal stressors, adrenal glands provide valuable insight into organismal health. This becomes particularly relevant in wild species such as marine mammals, exposed to increasing natural and anthropogenic stressors, where physiological responses can reflect these cumulative pressures at both individual and population levels. Such relevance is further emphasised in long-lived species with low reproductive turnover, which often act as bioindicators and sentinels of ecosystem health, integrating environmental impacts over extended temporal scales (Bossart, 2011; Atkinson & Dierauf, 2018).

1.1.2 Neuroendocrine regulation of the adrenal glands

Adrenal gland activity is regulated through two major neuroendocrine pathways: the hypothalamic-pituitary-adrenal (HPA) axis, and the sympathetic-adrenomedullary (SAM) axis. Together, these complementary systems coordinate adrenal hormone synthesis and release in response to acute and long-term internal and external stimuli.

The HPA axis mediates endocrine responses through a hierarchical signalling cascade involving the hypothalamus and pituitary gland. As terminal effector organs within this pathway, the adrenal cortex translates central signals into peripheral hormonal outputs that regulate metabolism, immune function, energy balance and other key physiological processes.

In parallel, the SAM axis, provides rapid neural control of the adrenal medulla via the sympathetic nervous system, enabling immediate responses primarily through the release of catecholamines.

The functional output of the adrenal glands is inherently constrained by their structural organisation. Accordingly, understanding adrenal physiology requires a detailed examination of gross anatomy and histological architecture, which ultimately, will enable or limit endocrine responses to environmental and physiological demands.

1.2 Anatomy of adrenal glands

1.2.1 Gross anatomy

Adrenal glands are present in all mammalian species and function to maintain biochemical and physiological regulation (Cozzi et al., 2017; Hart et al., 2015). In large terrestrial mammals, including humans (*Homo sapiens*), adrenal glands are generally pyramidal. In healthy adult humans, each adrenal gland typically weighs 3.5-5 grams, measures approximately 5 cm in length, 3 cm in width, and 1 cm in thickness (Narayan et al., 2024), and is positioned superior to the upper pole of the respective kidney.

In cetaceans, many adrenal gland characteristics are highly species-specific (Cozzi et al., 2017). Gland shape ranges from pyramidal to oblong or ovular, with substantial interspecific variability in mass and proportions (Clark et al., 2005, 2006, 2008; Vuković et al., 2010; Cozzi et al., 2017). The adrenal glands are positioned cranial to the kidney along the dorsal abdominal cavity (Figure 1.1) and are connected to the kidneys and colon through ligaments. The right adrenal is also connected to the liver through ligaments and lies more cranially than the left adrenal gland (Cozzi et al., 2017).

Adrenal gland mass varies considerably across cetacean species, broadly scaling with body size (Cozzi et al., 2017). In large mysticetes, adrenal glands are substantially larger, with blue whales (*Balaenoptera musculus*) reported to have adrenal masses of 600-1200 g and fin whales (*Balaenoptera physalus*) approximately 600-800 g (Burn et al., 1951). In odontocetes, gland masses are comparatively smaller and vary among species. In Atlantic bottlenose dolphins (*Tursiops truncatus*), mean adrenal mass is approximately 5.0-5.4 g in baseline samples (Clark et al., 2005), with chronically stressed individuals exhibiting significantly greater masses of approximately 11.0 g (Clark et al., 2006). In common dolphins (*Delphinus delphis*) from Ireland, mean left adrenal mass ranged from 5.93 g in acutely stressed individuals to 6.70 g in chronically stressed individuals, with body length and sexual maturity identified as significant predictors of gland mass (Santana et al., 2025). Adrenal mass data for two rarely studied species from New Zealand waters have recently been reported: hourglass dolphins (*Cephalorhynchus cruciger*) with individual gland masses of 7-8 g, and spectacled porpoises (*Phocoena dioptrica*) with gland masses ranging from 7 to 19.2 g across three individuals, though this wide range likely reflects individual variation in body size, age, or health status (Graïc et al., 2025).

Macroscopic coloration also differs between adrenal compartments. In terrestrial and marine mammals, the medulla is characteristically reddish-brown due to the abundance of vascularisation, whereas the cortex is lighter in colour (Cozzi et al., 2017; Myrick & Perkins, 1995). In delphinids, a light yellow, beige, or brown colour of a healthy cortex has been attributed to the high intracellular lipid and cholesterol content (Cozzi et al., 2017). In contrast, darkened, reddish-brown cortices have been reported in delphinids under continuous acute stress, likely due to lipid and cholesterol store depletion and hyperaemia (Myrick & Perkins, 1995).

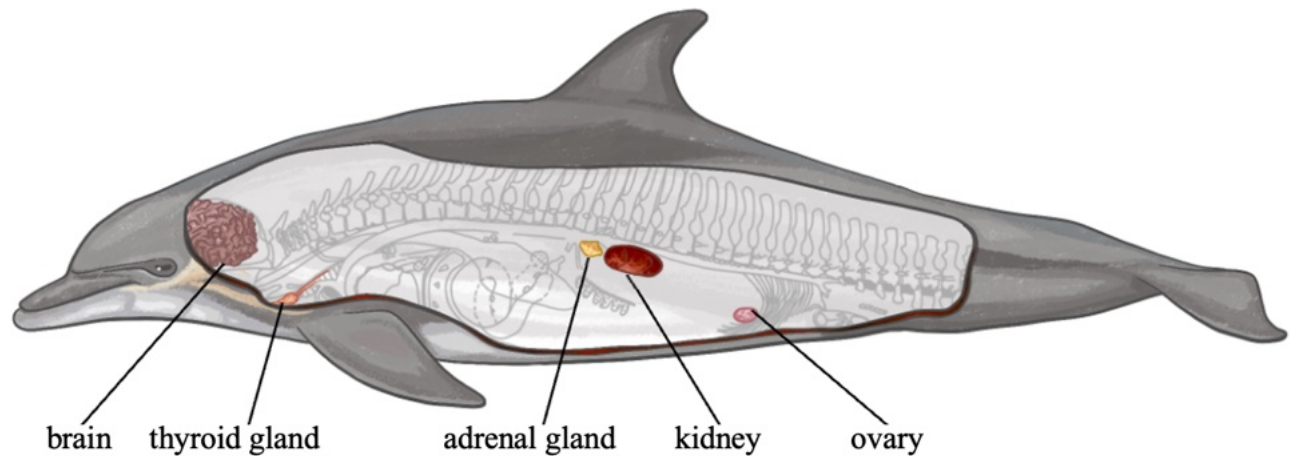


Figure 1.1 Left lateral illustration of common dolphin (*Delphinus delphis*) showing position of the adrenal gland compared to other key organs and glands (illustrated by Micaelyn Mann).

1.2.2 Lateral asymmetry

Lateral asymmetry in paired endocrine organs has been documented across mammalian taxa, including rodents, humans, and cetaceans (Clark et al., 2005, 2006; Hao et al., 2018; Saxu et al., 2023; Bechmann et al., 2024). In mice, the left adrenal gland demonstrates greater tissue mass and higher protein metabolism than the right adrenal, contributing to increased energy expenditure (Saxu et al., 2023). Additionally, neoplasia (tumour) frequency, typically resulting in increased mass, is reported more often in the left adrenal gland than the right, mirroring asymmetry observed in other paired organs such as the mammary glands (Saxu et al., 2023). Conversely, the right adrenal gland is thought to exert greater influence on glucocorticoid (i.e. cortisol) production and secretion (Saxu et al., 2023).

In humans, adrenal neoplasms also occur more frequently in the left adrenal gland more than the right, and individuals with aldosterone dysregulation often exhibit greater mass in the left adrenal relative to the right (Hao et al., 2018; Bechmann et al., 2024). These lateral differences are attributed to aging processes and impaired adrenal function (Hao et al., 2018; Bechmann et al., 2024), raising questions regarding the relative contributions of functional specialisation, disease, and stress to lateral adrenal asymmetry.

In delphinids, bilateral differences in adrenal mass and structure have been less consistently documented. While adrenal neoplasms, including teratomas and adenomas, have been reported in

delphinid species (Newman & Smith, 2006), significant bilateral differences in adrenal mass have not been widely observed (Clark et al., 2005, 2006). Although, in bottlenose dolphins, the left adrenal gland is typically larger than the right, and this asymmetry does not appear to be driven by sex, age, or sexual maturity (Clark et al., 2005, 2006). These findings suggest that functional differentiation or pathological processes may underlie observed asymmetry, though limited species coverage and small sample sizes restrict broader inference. Further investigation incorporating ontogenetic variables and comprehensive health metrics is needed to clarify the drivers of adrenal asymmetry in delphinids.

1.2.3 Histological organisation

At the macroscopic level, mammalian adrenal glands are composed of two parenchymal layers: the outer cortex and the inner medulla, encapsulated by a connective tissue capsule (Figure 1.2; (Clark et al., 2005, 2008; Vuković et al., 2010; Santana et al., 2025)). The cortex consists of steroidogenic cells arranged into discrete zonal layers responsible for the synthesis of mineral corticoids, glucocorticoids, and androgens, while the medulla is composed of chromaffin cells that produce catecholamines. These two compartments differ in embryological origin, cellular composition, and endocrine function, yet are closely integrated through paracrine signalling, dense vascularisation and direct innervation by the sympathetic nervous tissue (Pihlajoki et al., 2015). The structural organisation of these regions defines the gland's functional capacity and underlies hormone synthesis and secretion.

Collectively, the gross and histological organisation of the adrenal glands provides a structural framework through which functional specialisation, stress responsiveness, and pathological susceptibility can be interpreted. Detailed characterisation of adrenal anatomy is therefore essential for understanding the physiological and health-related implications of stress and environmental exposure in delphinid species.

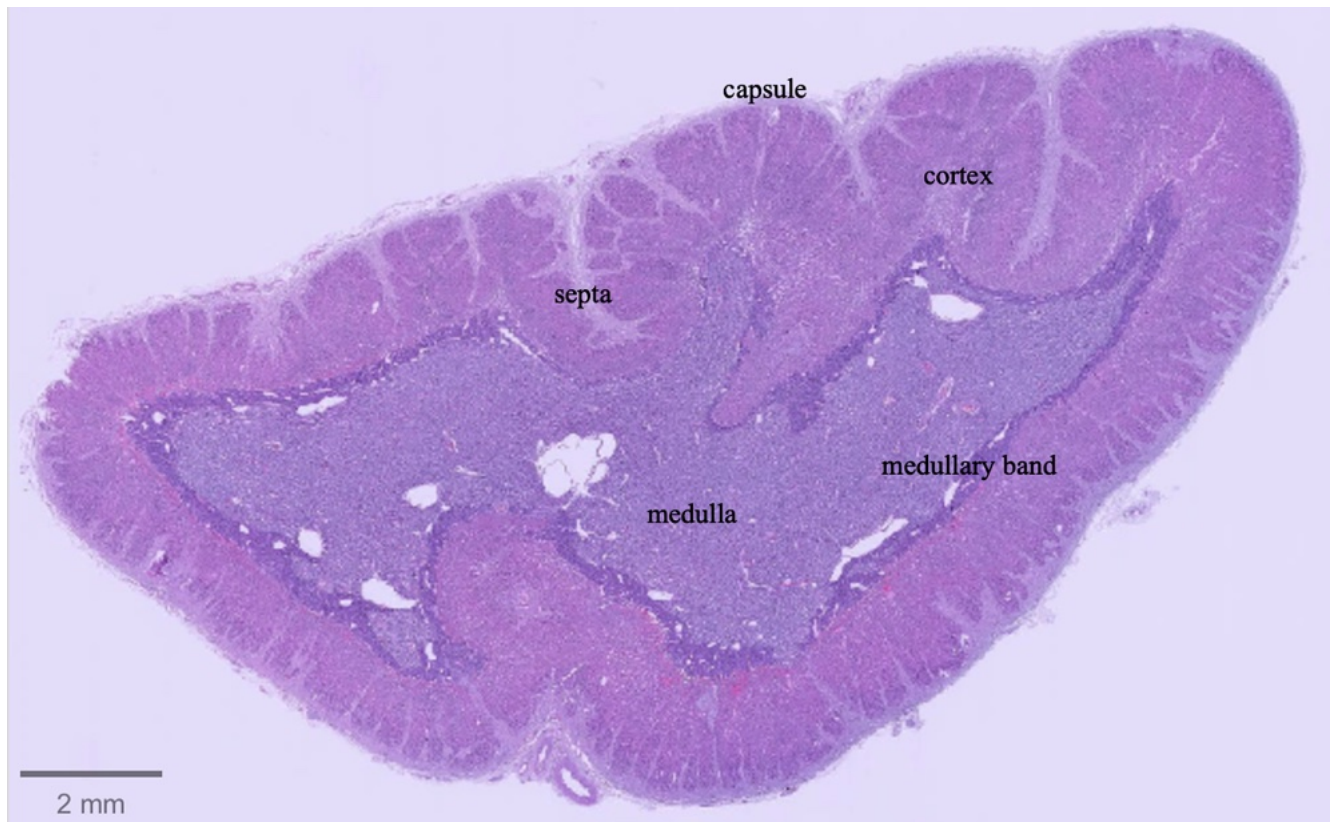


Figure 1.2 Cross-section of an adrenal gland from a common dolphin (*Delphinus delphis*, KS25-08Dd L); stain, H&E; scale bar, 2 mm.

1.2.3.1 Capsule

The adrenal capsule is composed of dense connective tissue and provides structural integrity to the gland. In striped dolphins (*Stenella coeruleoalba*) and bottlenose dolphins, the capsule is comparatively thick relative to other cetacean species (Vuković et al., 2010). A rich network of blood vessels and nerve fibres surrounds and penetrates the capsule, supporting both vascular supply and neural integration of adrenal function. Internally, connective tissue septa (also termed trabeculae; Vuković et al., 2010) composed of collagen and elastic fibres, extend from the capsule into the cortex and toward the corticomedullary junction, contributing to the pseudolobular appearance that is characteristic of many cetacean adrenal glands (Figure 1.2; Zhongjie, 1988; Clark et al., 2005, 2008; Vuković et al., 2010).

Septal organisation varies among species, with minimal lobulation observed in spotted dolphins (*S. attenuata*), pronounced lobulation in spinner dolphins (*S. longirostris*), and intermediate patterns in bottlenose dolphins (Clark et al., 2008; Vuković et al., 2010). Notably, in Adriatic bottlenose dolphins, the cortical cells display a distinct periseptal arrangement compared to regions lacking septal organisation (Vuković et al., 2010). Additional complexity arises from cortical invaginations into the medulla and medullary protrusions extending toward the capsule (Vuković et al., 2010), features that may reflect continuous adrenal growth following attainment of adult body length (Cowan, 1966).

1.2.3.2 Cortex

The cortex is divided into three layers, the *zona glomerulosa*, *zona fasciculata*, and *zona reticularis* (Figure 1.3). Each layer, or *zona*, is responsible for the secretion of specific classes of steroid hormones, including mineralocorticoid, glucocorticoids, and androgens (Clark et al., 2008; Cozzi et al., 2017; Galligan et al., 2023; Narayan et al., 2024). These cortical layers are highly vascularised, with sinusoidal capillary networks that facilitate efficient hormone exchange and distribution.

In contrast, the medulla, synthesises catecholamines and associated neurotransmitters, notably epinephrine and norepinephrine, reflecting its neuroectodermal origin and close functional association with the sympathetic nervous system (Clark et al., 2008; Cozzi et al., 2017; Galligan et al., 2023; Narayan et al., 2024).

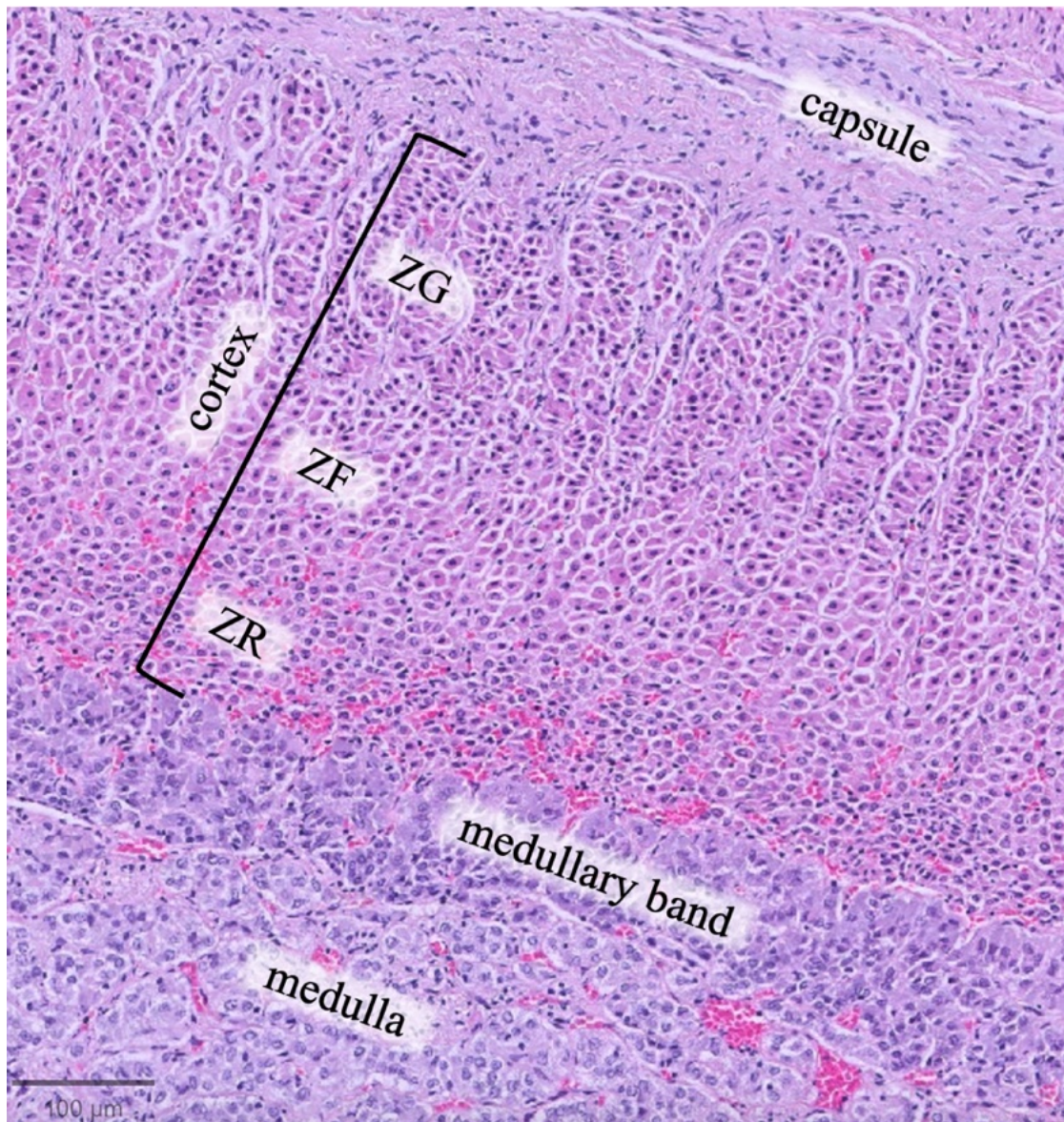


Figure 1.3 Histological section of a common dolphin (*Delphinus delphis*) adrenal gland showing the capsule, three cortical zones (ZG, zona glomerulosa; ZF, zona fasciculata; ZR, zona reticularis), medullary band, and central medulla. Stain, H&E; scale bar, 100 μm (KS23-58Dd R).

1.2.3.2.1 *Zona glomerulosa*

The *zona glomerulosa* is the outermost cortical layer, located immediately beneath the adrenal capsule (Figure 1.3). This zone has been identified in humans, cattle, and several marine mammal species examined to date (Simpson & Gardner, 1972; Zhongjie, 1988; Bragulla et al., 2004). However, in striped dolphins, *zona glomerulosa* cells are arranged as clustered polygonal cells (Vuković et al., 2010), whereas in Atlantic bottlenose, spotted, and spinner dolphins, cells are predominantly cuboidal to columnar in morphology (Clark et al., 2005, 2006, 2008).

In some marine mammals, including Adriatic bottlenose dolphins and in young common seals (*Phoca vitulina*), a *zona arcuata* has been described in place of the *zona glomerulosa* (Banks, 1993; Dellmann, 1993; Bragulla et al., 2004; Vuković et al., 2010). Cells within the *zona arcuata* are arranged in columnar arches with concavities oriented toward the medulla (Figure 1.4). One hypothesis proposes that the *zona arcuata* represents a transitional developmental stage that converts to a *zona glomerulosa* as a result of stress changes with aging (Bragulla et al., 2004). However, Vuković et al. (2010) found no evidence of *zona arcuata* to *zona glomerulosa* transformation in bottlenose dolphins across a broad range of ages, suggesting that the *zona arcuata* represents a persistent cortical organisation rather than a transient stress- or age-related state. The occurrence of a *zona arcuata* as a stable outer cortical zone has also been described in several domestic mammals, including horses, donkeys, and pigs (Banks, 1993; Dellmann, 1993).

Functionally, both the *zona glomerulosa* and *zona arcuata* synthesise mineralocorticoids, primarily aldosterone (Banks, 1993; Clark et al., 2005; Dellmann, 1993; Vuković et al., 2010). These regions are characterised by a dense network of sinusoidal capillaries, facilitating rapid hormone secretion into circulation. Aldosterone plays a critical role in maintaining sodium and potassium balance, which is essential for cardiovascular, renal, and osmotic homeostasis (Louden, 2012; Crocker, 2018; Fountain et al., 2024). In cetaceans, extended fasting, diving physiology, and exposure to variable salinity environments impose additional osmoregulatory demands (Hart et al., 2015; Crocker, 2018; McClain et al., 2020). Several delphinid species are regularly observed in brackish or freshwater systems for prolonged periods, necessitating precise regulation of electrolyte and fluid balance (Ewing et al., 2017; McClain et al., 2020).

Vuković et al. (2010) also describes a potential intermediate zone in the Adriatic bottlenose dolphin between the *zona arcuata* and the *zona fasciculata*, consisting of small cells with

irregularly shaped, darkly stained nuclei. A comparable region has been described in domestic mammals and pinnipeds (Bragulla et al., 2004), though its functional significance in cetaceans remains unclear.

Collectively, these findings indicate that the structural organisation of the aldosterone-producing region in cetacean adrenal glands is variable and not yet fully characterised, both within and between species. Further investigation incorporating larger sample sizes and life-history variables (e.g., age, body size, and environmental exposure) is required to determine whether these differences in *zona arcuata*/ *zona glomerulosa* architecture reflect stable anatomical specialisations or plastic responses to physiological and environmental demands.

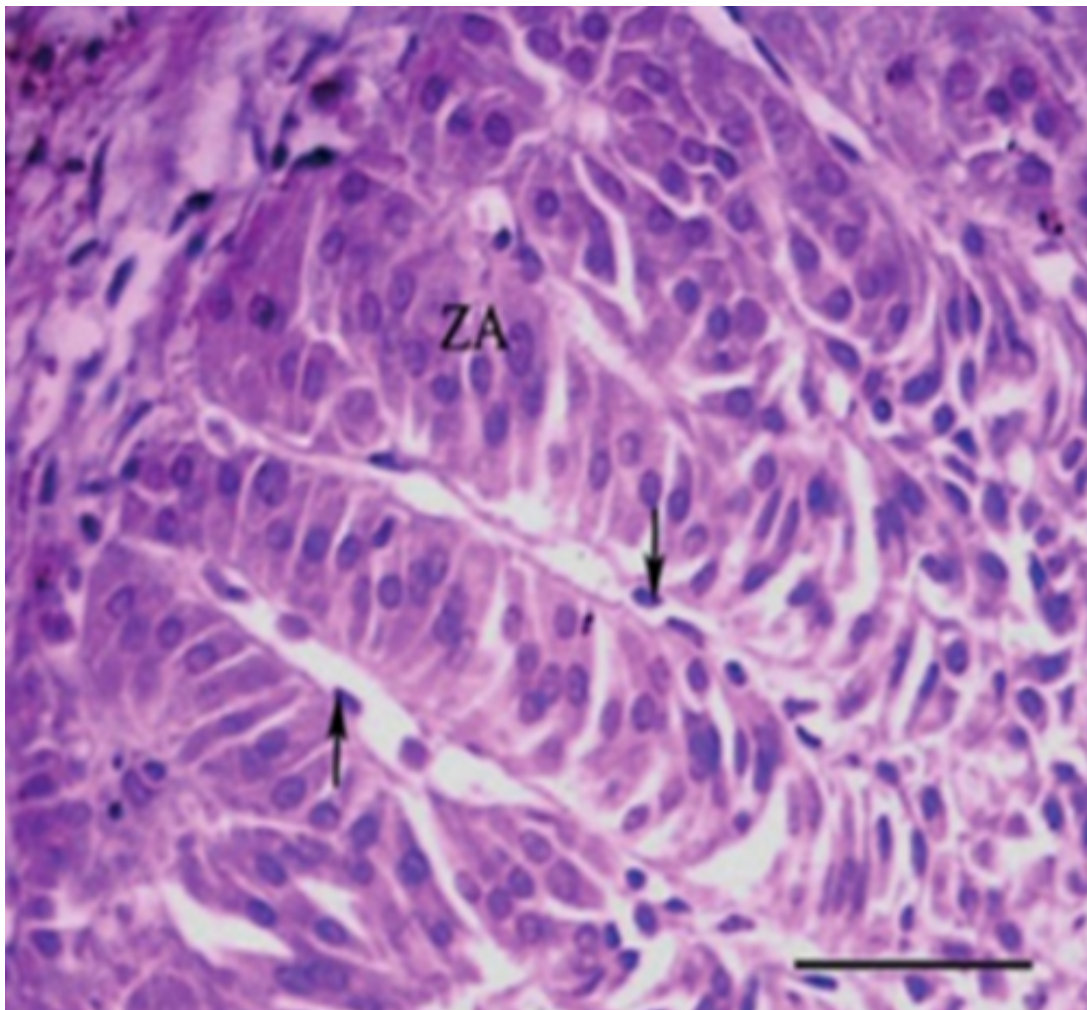


Figure 1.4 Bottlenose dolphin adrenal gland within the *zona arcuata*. Columnar cells are arranged in arches. Endothelial cells of blood capillaries are found within and between arches (arrows); stain, H&E; scale bar, 50 μ m (courtesy Vuković et al., 2010).

1.2.3.2.2 *Zona fasciculata*

The *zona fasciculata* is situated beneath the *zona glomerulosa* (or *zona arcuata*) (Figure 1.3). In humans, *zona fasciculata* cells are typically arranged in parallel cords and exhibit a vacuolated appearance due to the dissolution of lipid droplets during histological processing (Hammersen & Sobotta, 1985). In delphinids, cellular organisation of the *zona fasciculata* is variable. In Adriatic bottlenose dolphins and striped dolphins, cells are often polygonal and arranged in radially oriented cords composed of one or two rows of large, lightly staining cells with central, often binucleate nuclei (Vuković et al., 2010). In Atlantic bottlenose, spotted, and spinner dolphins, *zona fasciculata* cells are more frequently columnar and grouped into cords separated by abundant capillaries (Clark et al., 2005, 2008).

The *zona fasciculata* synthesises glucocorticoids, a class of steroid hormones derived from cholesterol, under regulation of the HPA axis (Dong & Cidlowski, 2003). This zone represents the primary effector of the HPA axis, translating central neuroendocrine signals into systemic hormonal responses. In marine mammals, cortisol is the dominant circulating glucocorticoid, although corticosterone can also be used as a biomarker of *zona fasciculata* function and stress physiology (St. Aubin et al., 1996; Ortiz & Worthy, 2000; Katsu & Baker, 2021).

1.2.3.2.3 *Zona reticularis*

The *zona reticularis* is the innermost cortical layer and lies adjacent to the medulla (Figure 1.3). In bottlenose dolphins, the *zona reticularis* is composed of irregular, branching cords of polygonal or cuboidal cells with darkly staining nuclei and the highest capillary density of all cortical zones, imparting a woven appearance (Clark et al., 2005; Vuković et al., 2010). The boundary between the *zona fasciculata* and *zona reticularis* is often not well defined, with intermixing of cells from both zones.

The *zona reticularis* produces adrenal androgens, primarily dehydroepiandrosterone (DHEA) and androstenedione, which serve as precursors for peripheral sex steroid synthesis, including testosterone and oestrogen (Clark et al., 2008; Vuković et al., 2010). Although the gonads are the primary source of sex steroids, adrenal-derived androgens, together with peripheral tissues, such as adipose tissue, contribute to the regulation of reproductive and metabolic processes (Kellar et al., 2009; Kershaw et al., 2018). In addition, increasing evidence suggests that adrenal androgens

may play a modulatory role in stress physiology, although their functional significance in cetaceans remains poorly understood (Gabai et al., 2020; Maia et al., 2025).

1.2.3.3 Medulla

The medulla, located within the inner portion of the adrenal gland, is primarily composed of cuboidal chromaffin cells, as observed in bottlenose dolphins, pan-tropical spotted dolphins, and spinner dolphins (Clark et al., 2005, 2008). In some species, the medulla is divided into a central medulla and a medullary band or outer medulla, which is distinguishable histologically (Figure 1.2, 1.3; Clark et al., 2008; Vuković et al., 2010). Chromaffin cells within these regions differ in morphology and function. Cells in the central region typically exhibit clear cytoplasm and dark nuclei and predominantly secrete norepinephrine, whereas chromaffin cells of the medullary band exhibit darker cytoplasm and nuclei and primarily secrete epinephrine (Vuković et al., 2010). The relative proportion and spatial distribution of epinephrine- and norepinephrine-secreting cells and the thickness of the medullary band vary among species (Clark et al., 2008; Vuković et al., 2010).

In contrast to the steroidogenic cortex, the medulla secretes catecholamines, primarily epinephrine and norepinephrine, which act as both hormones and neurotransmitters. These catecholamines play a central role in the acute stress response system ("fight-or-flight"), mediating rapid physiological adjustments, such as increased heart rate, vasoconstriction, modulation of oxygen stores, thermoregulation, and mobilisation of energy reserves in the form of glucose (Cowan & Curry, 2002; St. Aubin, 2002; Clark et al., 2005; Cozzi et al., 2017). In diving mammals, these responses are tightly integrated with cardiovascular and respiratory adaptations, highlighting the importance of medullary function in coping with repeated hypoxic and environmental challenges (Clark et al., 2008; Cozzi et al., 2017; Vuković et al., 2010).

1.2.4 Corticomedullary proportions

The cortex-to-medulla (C:M) ratio quantifies the relative cross-sectional area of cortical and medullary tissue and serves as a primary morphometric descriptor of adrenal gland composition (Clark et al., 2005; Clark et al., 2006; Santana et al., 2025; Alonso-Almorox et al., 2026). This

ratio reflects the structural balance between steroidogenic and catecholaminergic compartments and provides a basis for comparing adrenal architecture across species, demographic groups, and health states.

Among delphinids, C:M ratios vary considerably across species. In Atlantic bottlenose dolphins, the mean C:M ratio is approximately 1.54 ± 0.17 , with the cortex comprising approximately 48% of the cross-sectional area, the medulla 41%, and other tissue including capsule and vasculature approximately 11% (Clark et al., 2005). In pan-tropical spotted dolphins, the mean C:M ratio is higher at 2.34 ± 0.79 (range 1.46-3.71), with cortex comprising 64.4% of tissue area and medulla 29.4% (Clark et al., 2008). Spinner dolphins exhibit sex-specific differences in C:M ratios: females have a mean C:M ratio of 2.46 ± 0.18 (range 1.17-3.19; composition approximately 65% cortex, 27.3% medulla), while males have a higher mean of 3.21 ± 0.24 (range 2.85–3.89; composition approximately 71% cortex, 27.3% medulla) (Clark et al., 2008). In common dolphins from the Irish coast, acutely stressed individuals had a mean C:M ratio of 1.96 ± 0.23 (approximately 66% cortex, 31% medulla), while chronically stressed individuals exhibited a significantly higher ratio of 2.89 ± 0.19 (approximately 71% cortex, 25% medulla) (Santana et al., 2025).

Beyond delphinids, the limited data available suggest broader interspecific variation. In the Chinese river dolphin (*Lipotes vexillifer*), a notably high C:M ratio of 6.59 has been reported, reflecting a cortex-dominant gland (Zhongjie, 1988). Among mysticetes, sei whales (*Balaenoptera borealis*) have a mean C:M ratio of 2.65 (range 1.09-10.1), with sexual differences observed in some age classes (Miyazawa & Usui, 1983). In two odontocete species examined by Carballeira and colleagues (1987), the pygmy sperm whale (*Kogia breviceps*) and Gervais' beaked whale (*Mesoplodon europaeus*), cortical tissue comprised greater than 80% of the gland cross-section, yielding C:M ratios of at least 4:1.

Collectively, these data indicate substantial interspecific variation in adrenal composition among cetaceans. Cortical dominance appears to be a consistent feature across species, though the degree varies widely, from near-equal proportions in bottlenose dolphins to cortex-dominant architectures in river dolphins and beaked whales. Species-specific differences in body size, diving physiology, metabolic demands, and life-history may contribute to this variation, though

the functional significance of interspecific differences in C:M ratios remain poorly understood. Intraspecific variation associated with sex, age, stress exposure, and pathology has also been described in subsequent sections (Sections 1.3.3 and 1.4).

1.3 Functional organisation and stress physiology

1.3.1 Physiologic stress response

The adrenal glands play a central role in coordinating physiological responses to internal and external stressors through tightly integrated neuroendocrine pathways (Atkinson et al., 2015; Herman et al., 2016). Their functional organisation reflects the interaction between neural signalling, endocrine hormone synthesis, and downstream systemic effects. In vertebrates, stress physiology is primarily mediated through two complementary systems: the SAM axis and the HPA axis (Figure 1.5). Together, these systems regulate acute and chronic stress responses, energy mobilisation, cardiovascular function, immune modulation, and homeostatic balance.

Under unstressed conditions, glucocorticoids are secreted in cyclical patterns from the adrenal cortex into the bloodstream (Timmermans et al., 2019). These baseline rhythms reflect circadian, seasonal, and life-history-related variation (St. Aubin et al., 1996; Atkinson et al., 2015; Hart et al., 2015). A stress response is initiated when physical or psychological stimuli disrupt this equilibrium, resulting in increased secretion of corticotropin-releasing hormone (CRH), adrenocorticotrophic hormone (ACTH), and adrenal hormones, such as cortisol and catecholamines. The cumulative effect of stress-induced hormonal elevation, layered upon natural cyclic secretion, can drive physiological and cellular changes and increase susceptibility to morbidity and mortality events (Cowan & Curry, 2002; Clark et al., 2005; Venn-Watson et al., 2015; Guo et al., 2022).

Stress in cetaceans is commonly categorised as acute or chronic, although definitions vary substantially across studies (Atkinson et al., 2009; Clark et al., 2006). Acute stress is typically associated with rapid-onset events, such as vessel strike, net entanglement, intraspecific aggression, or acute infection, whereas chronic stress is linked to prolonged disease, starvation, or sustained environmental pressures (Clark et al., 2006). Atkinson et al. (2009) further describe acute stress as anxiety-related behavioural deviation and chronic stress as frequent, intermittent,

or prolonged exposure that overwhelms adaptive capacity in living animals. While acute stress responses are generally adaptive and essential for survival, chronic stress is maladaptive and may exhaust endocrine and immune systems through prolonged activation of adrenal pathways.

Distinguishing acute from chronic stress in stranded or deceased animals is challenging due to retrospective analysis and limited contextual data. As a result, definitions applied to post-mortem studies often differ from those used in live-animal behavioural assessments. In this thesis, stress classification aligns with Clark et al. (2006), reflecting applicability to stranded and necropsied individuals.

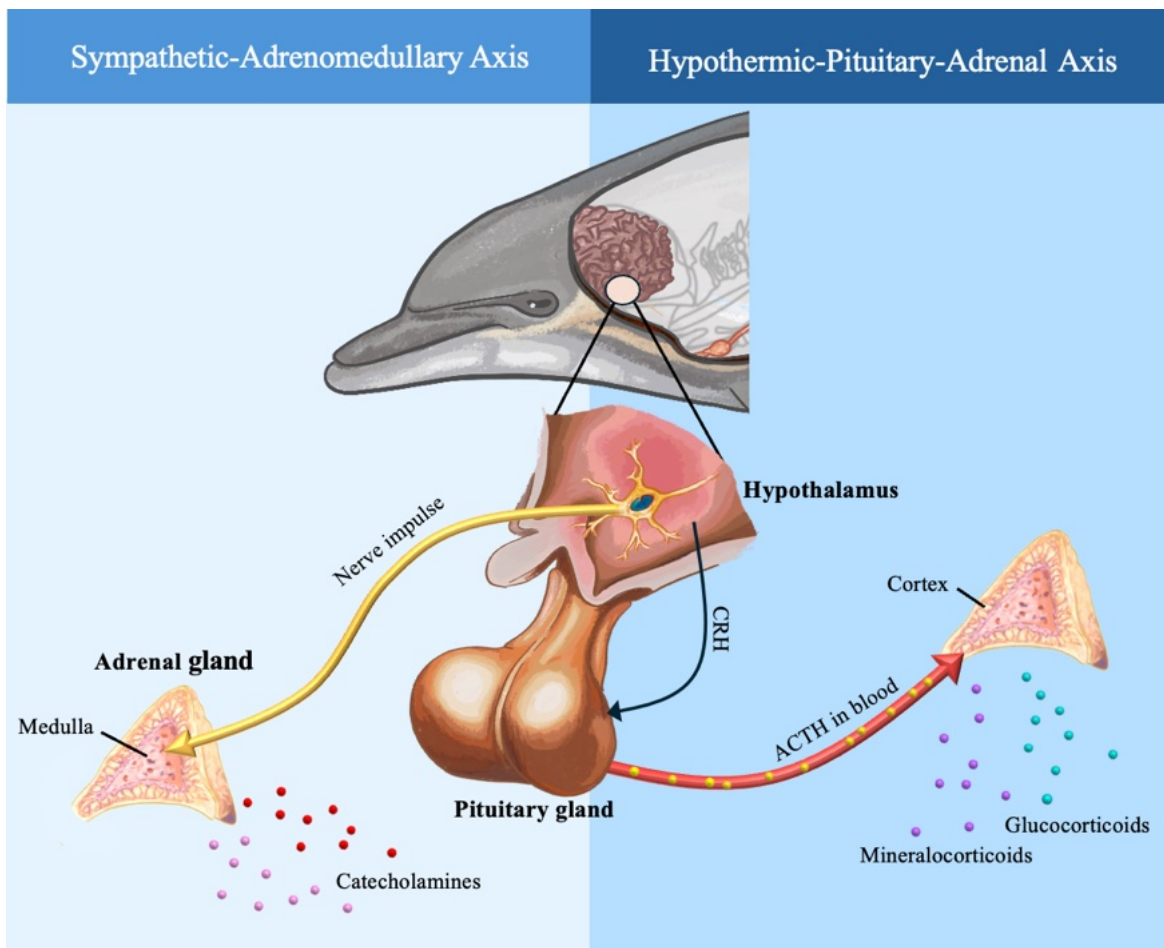


Figure 1.5 Schematic of the stress response pathways. The sympathetic-adrenomedullary axis stimulating the release of catecholamines and the hypothalamic-pituitary-adrenal axis stimulating the release of glucocorticoids and mineralocorticoids. CRH, corticotropin-releasing hormone produced by the hypothalamus; ACTH, adrenocorticotropic hormone produced by the pituitary gland (illustrated by Micaelyn Mann).

Anthropogenic stressors, including acoustic disturbance, vessel traffic, fisheries interaction, pollution, and oil and gas activity, have been widely documented to affect cetacean physiology, reproduction, and immune function (Fair & Becker, 2000; Clark et al., 2006; Bossart, 2011; Atkinson et al., 2015; Venn-Watson et al., 2015). Natural stressors such as climate-driven ecosystem change, harmful algal blooms, predation attempts, and social disruption further compound stress burden in delphinid populations (Fire et al., 2007; Gulland & Hall, 2007; Williams et al., 2025).

1.3.2 The sympathetic-adrenomedullary axis

The sympathetic-adrenomedullary (SAM) axis mediates rapid physiological responses to acute stress through direct activation of the sympathetic nervous system via preganglionic innervation of the adrenal medulla (Figure 1.5). In cetaceans, SAM activation occurs in response to a real or perceived threat, including during the pursuit, capture, and handling of wild or captive animals, predation, and exposure to noise (Atkinson & Dierauf, 2018). Neural stimulation of the adrenal medulla triggers the release of catecholamines, primarily epinephrine and norepinephrine, which facilitate the “fight or flight” response.

Elevated catecholamine concentrations increase heart rate, blood pressure, vasoconstriction, thermogenesis, and glucose mobilisation, while suppressing non-essential functions, such as reproduction, immune response, and digestion (Cowan & Curry, 2002; St. Aubin, 2002; Clark et al., 2005; Cozzi et al., 2017). These effects are essential for short-term survival; however, excessive or extreme SAM activation can be fatal. Extremely high epinephrine concentrations during acute stress have been directly linked to mortality in marine mammals (Clark et al., 2005). Elevated dopamine and skeletal muscle enzyme levels observed in pursued dolphins further indicate muscle damage associated with intense SAM activation (St. Aubin et al., 2013).

Baseline catecholamine concentrations are difficult to establish due to anticipatory hormone release prior to stress exposure, resulting in wide interspecific variability in reported values (Cozzi et al., 2017). Despite this variability, SAM system dysregulation represents a critical pathway through which acute stress can directly impact survival.

1.3.3 The hypothalamic-pituitary-adrenal axis

The hypothalamic-pituitary-adrenal (HPA) axis mediates longer-term endocrine responses to stress and regulates glucocorticoid and mineralocorticoid secretion from the adrenal cortex (Figure 1.5). Activation begins with hypothalamic release of corticotropin-releasing hormone (CRH), stimulating pituitary secretion of adrenocorticotrophic hormone (ACTH) (Figure 1.6). ACTH then drives glucocorticoid synthesis in the adrenal cortex, primarily within the *zona fasciculata*, the main effector of HPA axis activity (Tsigos & Chrousos, 2002; Papadimitriou & Priftis, 2009).

Animals experiencing prolonged stress, such as starvation, disease, or stranding, exhibit elevated cortisol concentrations compared to acutely stressed individuals, with chronically stressed cetaceans showing levels up to sixfold higher (Clark et al., 2006; Kellar et al., 2015; Agusti et al., 2022). However, interpretation of cortisol levels is complicated by seasonal, demographic, and life-history variation, including age, sex, and reproductive status (Fair et al., 2014; Hart et al., 2015; Orlov et al., 1988).

Human interaction and repeated handling can elevate cortisol and aldosterone levels and may lead to desensitisation of stress pathways through altered HPA axis feedback regulation (Houser et al., 2011). Climate- and temperature-related stress has also been shown to increase cortisol concentrations in bottlenose dolphins, with cold exposure eliciting values three times higher than ambient conditions (Houser et al., 2011). Additional anthropogenic disturbances, including vessel noise and traffic, further exacerbate HPA activation across cetacean species (Trana et al., 2016; Champagne et al., 2018; Guo et al., 2022; Pallin et al., 2022).

These sustained endocrine demands are reflected in measurable structural changes within the adrenal gland (Clark et al., 2006). In Atlantic bottlenose dolphins, chronically stressed individuals exhibit significantly higher C:M ratios (1.63 ± 0.13) compared to acutely stressed individuals (1.22 ± 0.06), which is driven primarily by cortical expansion rather than medullary reduction (Clark et al., 2006). A similar pattern has been observed in common dolphins, where chronically stressed individuals have significantly higher C:M ratios (2.89 ± 0.19) than acutely stressed individuals (1.96 ± 0.23), alongside significantly greater cortical mass (4.74 g vs 3.14 g) (Santana et al., 2025). Notably, total adrenal mass did not differ significantly between stress

groups in common dolphins, suggesting that cortex-specific metrics such as the C:M ratio may provide a more sensitive indicator of chronic HPA activation than gross gland mass alone (Santana et al., 2025). Comparable cortical enlargement has been documented in harbour porpoises (*Phocoena phocoena*), where focal cortical hyperplasia was associated with chronic stress pathology and chlorinated hydrocarbon exposure (Kuiken et al., 1993).

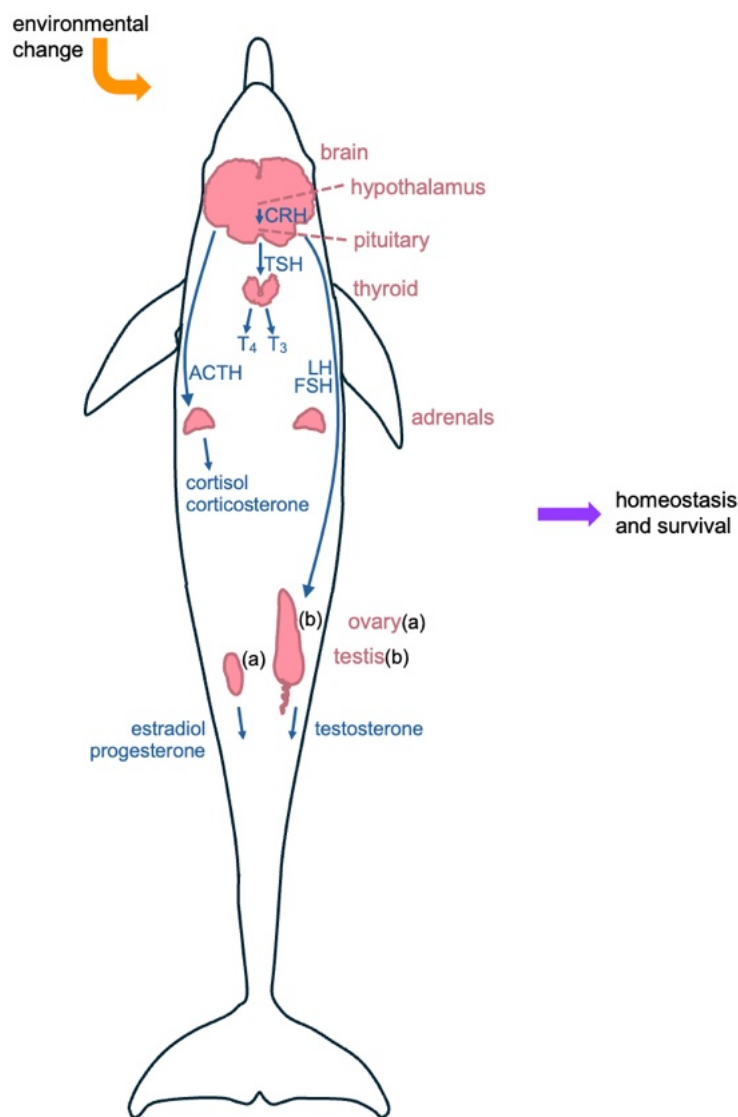


Figure 1.6 The endocrine system of a small odontocete, illustrating endocrine regulation of peripheral organs in response to environmental change. Endocrine organs (pink) and their associated hormones (blue) are shown. CRH, corticotropin-releasing hormone; TSH, thyroid-stimulating hormone; ACTH, adrenocorticotropic hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; T₄, thyroxine; T₃, triiodothyronine (illustrated by Micaelyn Mann).

These findings suggest that prolonged ACTH stimulation drives selective cortical hypertrophy, particularly within glucocorticoid-producing regions, altering the proportional composition of the gland without necessarily affecting medullary tissue.

Sustained glucocorticoid overproduction can suppress immune and reproductive function and can lead to cellular abnormalities, endocrine exhaustion, and death (Sapolsky et al., 2000; Romero & Wingfield, 2016). Conversely, abnormally low glucocorticoid levels are also indicative of extreme stress and adrenal dysfunction (Atkinson & Dierauf, 2018). Following the Deepwater Horizon oil spill, dolphins exposed to oil exhibited hypoadrenocorticism, reflecting adrenal toxicity and impaired stress response capacity (Schwacke et al., 2014; Venn-Watson et al., 2015).

HPA dysregulation compromises immune cell production, including neutrophils, lymphocytes, eosinophils, monocytes, and basophils, increasing susceptibility to infection and disease (Schwacke et al., 2014; Atkinson & Dierauf, 2018). Reproductive suppression through the inhibition of hypothalamic signalling further links chronic stress to reduced fertility and developmental anomalies (Atkinson & Dierauf, 2018). Notably, a mature common dolphin presenting with underdeveloped ovaries and uterus also exhibited bilateral adrenal cysts, highlighting the potential link between adrenal pathology and reproductive dysfunction (Cartee et al., 1995). Associations between endocrine disruption and sterility, reproductive disorders, organ lesions, and cancers have been documented in other mammals (Damstra et al., 2002).

Within the existing literature, acutely stressed delphinids serve as baseline references for adrenal morphology, as animals dying rapidly from acute causes, such as vessel strike or net entanglement, have not undergone prolonged adrenal stimulation (Clark et al., 2006). In contrast, chronic stress is strongly associated with adrenal structural alteration on the cellular and macro-scale, with cascading effects on physiological systems (Clark et al., 2006; Cowan & Curry, 2008). Understanding the functional organisation of stress physiology is therefore essential for interpreting adrenal morphology and pathology in post-mortem assessments.

1.3.4 Structure-function integration

The organisation of the adrenal glands at both gross and histological scales provides a critical framework for interpreting pathological change. Adrenal architecture reflects how the gland accommodates physiological demand and cumulative stress exposure over time (Clark et al., 2006; Lair et al., 1997). Accordingly, evaluation of adrenal cellular organisation may offer insight into chronic stress, disease processes, and environmental disruption, without requiring direct measurements of circulating hormone output levels.

At the tissue and cellular level, the division of the adrenal gland into cortex and medulla reflects distinct but integrated functional domains. The cortex consists of steroidogenic cells arranged into discrete zonal layers, while the medulla is composed of chromaffin cells associated with rapid stress responses (Clark et al., 2005; Vuković et al., 2010). The close anatomical association of these regions supports coordinated adrenal activity and renders the gland susceptible to structural alteration under sustained stimulation and pathological conditions.

Within the cortex, zonal organisation underpins functional specialisation. The *zona glomerulosa* or *zona arcuata* occupies the subcapsular region, while the *zona fasciculata* forms the bulk of the cortex and the *zona reticularis* lies adjacent to the medulla. Differences in cellular arrangement, vascularisation, innervation, and thickness among these zones provide a morphological basis for assessing adrenal variation, structural alterations, and disruption of normal zonation in relation to functional endocrine output (Clark et al., 2005, 2008; Vuković et al., 2010).

Connective tissue elements further shape adrenal architecture. The capsule and septa provide mechanical support, while also serving as conduits for blood vessels and nerve fibres. In cetaceans, prominent septal organisation, cortical pseudolobulation, cortical invagination, and medullary protrusions may influence intraglandular organisation and are therefore relevant features when assessing structural variation and pathology across individuals and species (Clark et al., 2008; Cozzi et al., 2017; Vuković et al., 2010).

In delphinids, adrenal histology reflects cumulative structural responses to physiological stress, disease, and environmental exposure. Assessment of adrenal architecture and pathology, thus, offers an informative approach for evaluating how these factors shape adrenal structure across

life history stages and between individuals and populations, providing a valuable proxy for endocrine activity and stress exposure (Clark et al., 2006; Lair et al., 1997; Venn-Watson et al., 2015).

1.4 Adrenal pathology and environmental disruption

1.4.1 Gross adrenal changes and structural abnormalities

Pathological alterations of the adrenal glands reflect both intrinsic disease processes and extrinsic environmental pressures. In vertebrates, adrenal pathology commonly manifests as changes in gland mass, cellular architecture, and hormone production, with downstream effects on stress physiology, immune competence, and reproduction (Atkinson & Dierauf, 2018). In marine mammals, and particularly delphinids, adrenal pathology offers insight into chronic stress exposure, environmental disruption, and disease, reflecting the integrative role of the adrenal glands in endocrine regulation and overall health, and reinforcing their value as indicators of cumulative physiological stress.

Few studies have investigated deviations in gross adrenal mass in relation to stress within cetacean species (Clark et al., 2006). In Atlantic bottlenose dolphins (*Tursiops truncatus*), chronically-stressed individuals exhibit significantly larger adrenal glands compared to acutely stressed individuals, with mean adrenal masses of approximately 11.01 g and 5.20 g, respectively (Clark et al., 2006). No significant bilateral differences in adrenal mass have been observed within individuals, nor have differences been detected across sex or sexual maturity (Clark et al., 2006). In common dolphins from the Irish coast, however, no significant difference in total adrenal mass have been detected between acute (5.93 ± 0.67 g) and chronic (6.70 ± 0.76 g) stress groups (Santana et al., 2025). The authors attribute this to the confounding effect of nutritional status: chronically-stressed individuals are predominantly in poor body condition, and severe emaciation may have masked stress-related enlargement through catabolic tissue depletion. Despite this, chronically-stressed common dolphins exhibit significantly higher cortical mass and C:M ratios, indicating that cortex-specific metrics can distinguish chronic from acute stress, even when total gland mass does not differ and may therefore represent more sensitive indicators of prolonged HPA axis activation (Santana et al., 2025).

Gross morphological changes in adrenal mass are closely linked to chronic stimulation of the stress response. Increased adrenal mass in chronically stressed bottlenose dolphins is associated with cortical expansion and thickening of the medullary band, suggesting hypertrophy or hyperplasia of hormone-secreting tissues (Clark et al., 2006). In common dolphins, lower cortical cell density in chronically stressed individuals, despite thicker cortices, is consistent with cellular hypertrophy, rather than hyperplasia (Santana et al., 2025). These findings collectively suggest prolonged endocrine activation produces selective cortical remodelling, particularly within glucocorticoid-producing regions of the cortex, the detection of which may require histological assessment beyond gross mass measurement alone.

1.4.2 Adrenal lesions and degenerative changes

Adrenal lesions are broadly classified as benign or malignant and as functioning or non-functioning, depending on their hormone-producing capacity (Bracci et al., 2022). In humans, adrenal adenomas represent the most common benign adrenal lesion, with frequency increasing with age (Romeo et al., 2018; Bracci et al., 2022; Altay et al., 2023). Approximately 20% of adrenal adenomas in humans are bilateral and associated with cortisol overproduction.

In cetaceans, the prevalence and distribution of adrenal lesion types remain poorly characterised (Newman & Smith, 2006). Hyperplastic and degenerative changes have been reported in several species, including bottlenose dolphins, harbour porpoises (*Phocoena phocoena*), and beluga whales (*Delphinapterus leucas*; Kuiken et al., 1993; Lair et al., 1997; Clark et al., 2006). Three primary forms of adrenal hyperplasia have been described: (1) cortical hyperplasia, (2) cortical and medullary nodular hyperplasia, and (3) serous cyst formation (Kuiken et al., 1993; Lair et al., 1997; Clark et al., 2006; Cowan & Curry, 2008; Atkinson & Dierauf, 2018). Adrenal neoplasia has also been reported sporadically in odontocetes, including adrenal cortical adenomas in bottlenose dolphins and a medullary lesion suggestive of pheochromocytoma in a spotted dolphin (Estep et al., 2005; Newman & Smith, 2006), though the prevalence and biological significance of such lesions in free-ranging populations remains largely unknown.

In Atlantic bottlenose dolphins, chronic stress is associated with increased C:M ratios, indicating cortical hypertrophy or hyperplasia (Clark et al., 2006). Chronically-stressed individuals exhibit

an average C:M ratio of approximately 1.61, compared to 1.22 in acutely-stressed individuals. This difference reflects a greater proportional increase in cortical tissue, particularly within the *zona glomerulosa* and *zona fasciculata*, consistent with prolonged ACTH stimulation, and sustained activation of the HPA axis (Clark et al., 2006).

Medullary hyperplasia has also been documented, with chronically-stressed dolphins demonstrating increased medullary band thickness and a higher abundance of epinephrine-producing chromaffin cells (Clark et al., 2006). These findings suggest sustained activation of catecholaminergic pathways in response to prolonged stress exposure and highlight the involvement of the SAM axis alongside cortical responses. Necropsies of mass-stranded beluga whales in the St. Lawrence Estuary and animals harvested in Hudson Bay, Canada revealed adrenal lesions in 71% of individuals, ranging from mild to severe (Lair et al., 1997). Cortical hyperplasia was present in approximately 80% of affected animals. Lesion severity increased with age, suggesting cumulative exposure to stressors or environmental toxicants over the lifespan (Lair et al., 1997).

Neoplasms represent abnormal proliferations of tissue that may be benign or malignant (Ridgway, 1972; Bryden & Harrison, 1986; Cartee et al., 1995; Newman & Smith, 2006; Cowan & Curry, 2008; Atkinson & Dierauf, 2018). Dolphins exhibit the highest reported frequency of neoplasia among marine mammals, underscoring their value as sentinel species for environmental health (Newman & Smith, 2006). Reported neoplastic varieties include adenocarcinomas, squamous cell carcinomas, lymphomas, fibromas, teratomas, and adrenal adenomas across multiple delphinid species (Newman & Smith, 2006; Tarin, 2023).

Adrenal cysts have been reported in several delphinids, including a mature common dolphin (*Delphinus delphis*) with bilateral medullary cysts and associated reproductive underdevelopment (Cartee et al., 1995). Similar cystic lesions have been described in Atlantic white-sided dolphins (*Leucopleurus acutus*), with notable sex bias, as females exhibited significantly higher cyst prevalence than males (Geraci & St. Aubin, 1979). Cortical cysts have also been documented in beluga whales, with bilateral cortical-filled cysts observed in 19 of 35 St. Lawrence Estuary individuals, with lesion severity increasing with age (Lair et al., 1997).

These findings suggest potential links between adrenal pathology, reproductive demand, and endocrine disruption.

1.4.3 Endocrine contaminants and environmental disruption

Environmental contaminants are pervasive in marine ecosystems, with an estimated 220 billion tons of chemical pollutants released globally each year (Hall et al., 2023). These contaminants include anthropogenic sources, such as pesticides, industrial effluents, agricultural runoff, and pharmaceuticals, as well as naturally-derived inputs, including volcanic activity and storm-driven sediment mobilisation (Vos et al., 2000; Damstra et al., 2002; Conrad et al., 2020; El-Sharkawy et al., 2025).

Many environmental contaminants function as endocrine-disrupting compounds by mimicking, blocking, or altering hormone receptor activity (Delgado-Suarez et al., 2023; Jepson et al., 2005; Williams et al., 2025). Marine mammals are particularly vulnerable due to their high trophic position, extensive lipid stores, and long lifespan, which facilitate bioaccumulation and biomagnification of contaminants over extended temporal scales (Stockin et al., 2010; Page-Karjian et al., 2020; Stockin et al., 2021).

Endocrine-disrupting contaminants impair immune and reproductive function and increase susceptibility to infection, parasitism, and mass mortality events (Vos et al., 2000; Damstra et al., 2002; Newman & Smith, 2006; Balali-Mood et al., 2021; Lozano-Bilbao et al., 2021; Naccari et al., 2024). Maternal offloading of contaminants during gestation and lactation exposes offspring to elevated toxicant burdens, potentially compromising endocrine development from *in utero* exposure onward (Jepson et al., 2005; Kooyomjian et al., 2022; Nehring & Staniszewska, 2023).

Heavy metals and trace elements represent a significant class of endocrine-disrupting contaminants. Non-essential metals such as arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) are toxic at low concentrations and exhibit long biological half-lives, due to limited excretion (Sabath & Robles-Osorio, 2012; Hall et al., 2023; Naccari et al., 2024). In marine mammals, elevated metal concentrations are associated with immunosuppression, reproductive impairment, premature birth, and increased neonatal mortality (Martin et al., 1976).

Metal exposure influences adrenal hormone pathways directly, altering aldosterone, glucocorticoid, and catecholamine production and disrupting feedback regulation (Joshi et al., 2024). Hepatic and renal tissues are commonly used to assess metal burden due to their central role in detoxification and metal storage, with tissue concentrations serving as proxies for endocrine exposure, including adrenal tissues (Frodello et al., 2000; Page et al., 2024).

Collectively, environmental contaminants and heavy metals represent persistent stressors that may influence adrenal structure and pathology. Understanding these interactions is essential for interpreting adrenal pathology in delphinids, assessing long-term population health in increasingly impacted marine ecosystems, and providing a critical context for linking environmental exposure with endocrine and structural adrenal alterations.

1.5 Knowledge gaps in delphinid adrenal research

Despite increasing recognition of the adrenal glands as central regulators of stress physiology, metabolism, immune function, and reproduction, substantial knowledge gaps remain in the understanding of adrenal structure, function, and pathology in delphinid species. Much of the current knowledge on adrenal morphology and physiology is derived from terrestrial mammalian models or a limited number of cetacean papers and case studies, many of which have contrasting findings across species, life-history stages, and ecological contexts.

One major limitation in existing studies is the restricted taxonomic scope. Investigations of adrenal gland structure and pathology have predominantly focused on a small subset of odontocete species, including bottlenose dolphins, spotted dolphins, and spinner dolphins, often with small sample sizes and skewed demographic representation. As a result, cross-species comparisons are constrained, and the extent of interspecific variation in adrenal gland architecture, mass, corticomedullary ratios, and zonal organisation remains poorly understood.

In addition to limited species coverage, inconsistencies in anatomical terminology and histological descriptions present challenges for comparative synthesis. Differences in nomenclature, such as the use of medullary band versus outer medulla, or trabeculae versus connective tissue bands or septa, complicate interpretation across studies, even when structures are functionally analogous. Similarly, discrepancies in descriptions of cortical zonation,

including the presence or absence of a *zona arcuata* or intermediate zones, raise questions regarding true biological variability versus methodological or interpretive differences.

Another critical gap lies in the integration of adrenal structure with functional and physiological outcomes. While associations between chronic stress and adrenal hypertrophy, cortical hyperplasia, or medullary expansion have been documented, interpretation of these histological patterns remains limited, particularly in post-mortem studies. This is further complicated by variability in definitions of stress, which differ across studies and are often influenced by whether assessments are conducted in live animals or derived retrospectively from stranded individuals.

Life-history factors such as age, sex, sexual maturity, reproductive status, and mortality event are frequently underrepresented in adrenal studies. Yet evidence from both cetaceans and terrestrial mammals suggests that these variables can significantly influence adrenal mass, cellular composition, hormone secretion, and susceptibility to pathology. Without systematic incorporation of life-history data, it remains difficult to disentangle normal ontogenetic variation from disease- or stress-related alterations.

Environmental context represents another major area of uncertainty. While marine mammals are known to accumulate heavy metals and other endocrine-disrupting contaminants, the specific pathways through which these toxicants influence adrenal gland structure and function are not well defined. Most contaminant studies rely on hepatic or renal tissues as proxies for exposure, and direct assessments of adrenal tissue remain rare. Consequently, the relationships among contaminant burden, adrenal histopathology, hormone dysregulation, and downstream health effects are poorly understood.

Overall, existing studies provide valuable but incomplete insight into delphinid adrenal biology. Limitations in species coverage, anatomical consistency, life-history integration, and environmental context restrict the interpretation of adrenal findings, highlighting the need for more systematic and comparable assessments of adrenal structure and pathology.

1.6 Thesis rationale and objectives

Delphinids, including the common dolphin, are increasingly exposed to a combination of natural and anthropogenic stressors, including climate change, environmental contamination, fisheries interactions, vessel traffic, and habitat alteration. These stressors have the potential to drive histological and physiological changes in adrenal tissue, as well as developmental-, reproductive-, and immuno-suppression. Despite the critical role of the adrenal glands in endocrine regulation, stress response, and maintaining physiological homeostasis, their gross morphology, cellular architecture, and pathological variation remain poorly characterised in delphinid species.

Current understanding of delphinid adrenal biology is limited by small sample sizes, species representation, life-history demography, pathological, and environmental data, leading to high variability and discrepancies in previous studies (Clark et al., 2006; Clark et al., 2008; Santana et al., 2025; Alonso-Almorox et al., 2026; Table A1). Existing studies have reported associations between chronic stress and adrenal hypertrophy, cortical hyperplasia, medullary expansion, and lesion development. However, these findings are often derived from case studies or restricted datasets and lack comprehensive demographic context.

Histological and pathological investigation of adrenal tissue may provide invaluable means of linking physiological stress, environmental exposure, and health outcomes in marine mammals. Structural changes within specific adrenal regions, including alterations in corticomedullary ratios, zonal proportions, and cellular composition, may serve as indicators of chronic stress, disease processes, or contaminant-related disruption. Investigating the role of ontogeny will provide a baseline for common dolphins across age, sex, and reproductive status, as well as help build a framework to broaden our understanding of inter-specific comparative analyses.

This thesis aims to assess adrenal gland anatomy and pathology in the context of life history and trace element burdens in common dolphins assessed post-mortem. Specifically, the following objectives are addressed:

(a) to quantify gross adrenal morphology, including weight, length, width, and depth, and assess bilateral asymmetry between left and right glands in relation to life history variables, including age, sex, sexual maturity, and gland lateralisation;

- (b) to characterise the histological composition of adrenal glands, including the relative proportions of capsule, cortex, and medulla and the C:M ratio, and determine how these vary with age, sex, sexual maturity, prominent medullary band, presence of pathological features, disease classification, and stress chronicity;
- (c) to describe the prevalence of adrenal pathological lesions, classify individuals by stress chronicity, and assess associations between pathology presence and life history variables;
- (d) to assess the influence of kidney and liver trace element indices on adrenal gland structure in the context of life history and pathological status (pathological presence, disease classification, and stress type); and
- (e) to investigate associations between cumulative element burden and adrenal gland architecture, including C:M ratio and zonal proportions, using correlation screening and linear mixed-effects modelling.

1.7 Thesis structure

This thesis follows a traditional thesis format as follows:

Chapter 1 – Introduction

Contextualises current literature on adrenal gland anatomy, stress physiology, and contaminant effects on adrenal structure, identifying knowledge gaps in delphinid research and establishing study objectives.

Chapter 2 – Materials and Methods

Describes sample collection, histological preparation, digital morphometric annotation, pathological assessment, trace element analysis, and statistical approaches.

Chapter 3 – Results

Presents findings on adrenal morphometrics, zonal composition, pathology prevalence and disease classification, and trace element burdens and their associations with adrenal architecture.

Chapter 4 – Discussion

Interprets findings in the context of cetacean adrenal physiology, stress biology, and contaminant exposure, with implications for health monitoring of common dolphins in New Zealand waters, and outlines directions for future research.

Chapter 2 – Material & Methods

2.1 Post-mortem sampling and biological data

2.1.1 Sample Collection

All sampling was conducted under research permits 39239-MAR, RNW/22/2003/182, and RNW/HO/2008/03 issued by the New Zealand Department of Conservation Te Papa Atawhai. Iwi approval was given for all post-mortem examinations, sample collection, and scientific data acquisition of carcasses around Aotearoa New Zealand.

Post-mortem sampling was conducted from 103 common dolphins (*Delphinus delphis*; female = 58; male = 45) between 2008 and 2025, following IJsseldijk et al. (2019). Samples were derived from cadavers recovered following live stranding events, beach cast incidents, or as submitted incidental fisheries bycatch. Post-mortem sampling across multiple tissues enabled comprehensive biological and pathological assessment. Age was determined from dentine growth layer groups (GLG); sex and reproductive status were evaluated through gross and histological examination of the gonads; adrenal glands were collected for morphometric and histopathological assessment; hepatic and renal tissues were collected for heavy metal and trace element concentrations; mixed tissue samples were used for gross histopathology; morphometric measurements, evaluation of body condition, decomposition state, and blubber thickness were recorded as metrics for animal health status. All sampling and assessments followed the standardised procedures outlined in IJsseldijk et al. (2019).

2.1.2 Biological Data

2.1.2.1 Age estimation

Age estimation was conducted using dentine, following Murphy et al. (2014). Decalcified sections of dentine were stained and examined using microscopy under 10-40x magnification to determine age by counting annual growth layer groups ($n = 102$; Myrick et al., 1983; Lockyer, 1995). Tooth sections were initially read blind with no prior knowledge of biological life history traits by a minimum of two, often three, experienced readers. Neonates were identified from the absence or early formation of the neonatal line in the dentine. Discrepancies were resolved by the assessment of additional teeth until a final age estimate was determined (Murphy et al., 2014). In

cases with high tooth wear or damage and age was unable to be determined, individuals were excluded from further analysis (Palmer et al., 2022). As such, one individual (KS19-23Dd) was not assigned an age due to lack of reader consensus and excluded from age related analyses (Table A2).

2.1.2.2 Reproductive assessment

Sex was determined anatomically by assessment of reproductive anatomy by experienced examiners during post-mortem dissection. Individuals marked as female had ovaries, uterus, and mammary glands present. Individuals marked as male had testes and a penis. When sex was unable to be determined due to predation or scavenging, genetic analysis via polymerase chain reaction (PCR) amplification was used to establish sex (Bérubé & Palsbøll, 1996; Gilson et al., 1998).

Reproductive stage was determined by the assessment of the testes, penis, ovaries, uteri, and mammary glands, as detailed for males and females in Palmer et al. (2022; 2023), respectively. In summary, male animals were categorised as immature, pubescent, or mature by the presence and abundance of cell types in the seminiferous tubules (Palmer et al., 2023). Females were categorised as immature, pregnant, pregnant and lactating, lactating, and resting mature by the presence of *corpora albicantia* and *corpora lutea* on the ovaries, presence of a foetus *in utero*, and/ or evidence of lactation in the mammary glands (Palmer et al., 2022). For consistency across the data set, this thesis will use reproductive stage (sexually immature and sexually mature; Table A2).

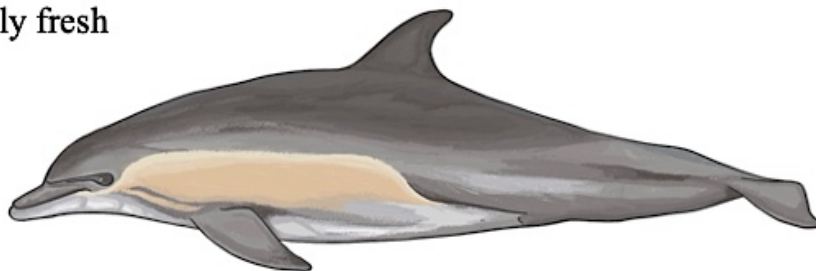
2.1.2.3 Decomposition assessment

Decomposition condition was scored on a five-point scale (IJsseldijk et al., 2019; Figure 2.1; Table 2.1) ranging from code 1, extremely fresh, to code 5, mummified or skeletal remains, and was used to identify and exclude animals with extended decomposition autolysis from analysis (Table A2).

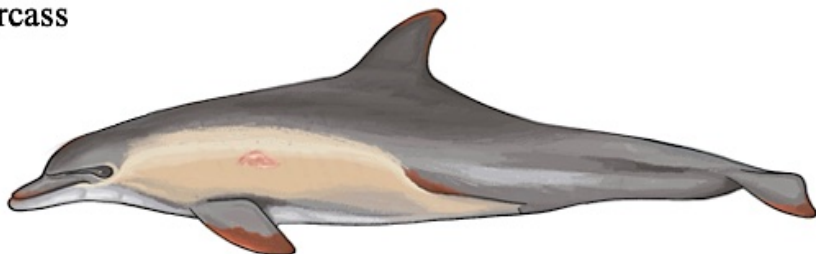
2.1.3 Gross Pathology

Gross and histological assessment of each cadaver was performed following IJsseldijk et al. (2019). Tissues from all major respiratory, cardiovascular, endocrine, lymphatic, reproductive, alimentary organs and glands were fixed in 10% buffered formalin. Fixed tissues were embedded in paraffin wax, sectioned at 5 µm, mounted on slides, and stained with hematoxylin and eosin (H&E), prior to being read by the same two board certified anatomical pathologists (CH, EG). Additionally, vial and/or bacterial swabs were collected when circumstances around the animal's death or putative anomalies in other tissues suggest potential infection (IJsseldijk et al. 2019).

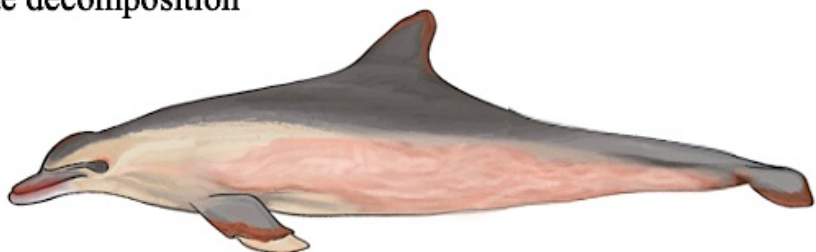
Code 1: extremely fresh



Code 2: fresh carcass



Code 3: moderate decomposition



Code 4: advanced decomposition



Code 5: mummified or skeletal remains



Figure 2.1 Decomposition condition category (illustrated by Micaelyn Mann).

Table 2.1 Characteristics of each decomposition condition category (IJsseldijk et al., 2019)

Score	Definition
Code 1: extremely fresh	Usually live stranded and died/ euthanised cases or those stranded right after death; exhibiting no post-mortem changes (e.g. no bloating or sloughing of skin); fresh smell; clear, glassy eyes; blubber firm and white; muscles firm, dark red, well-defined; viscera intact and well-defined; gastrointestinal tract contains no to little gas (unless pathologic); brain firm with no discolouration, surface features distinct, easily removed intact.
Code 2: fresh carcass	Normal appearance, fresh smell, minimal drying and wrinkling of skin, eyes and mucous membranes; carcass not bloated, tongue and penis not protruded; blubber firm and white, occasionally tinged with blood.
Code 3: moderate decomposition	Bloating evident, with tongue and penis often distended; skin cracked and started sloughing; characteristic (mild) odour can be expected; mucous membranes dry, eyes sunken. Blubber blood-tinged and oily; muscles are softer and poorly defined; gut segments contain gas; brain has soft consistency. Organs are largely intact, still distinguishable and can be easily removed and assessed, although colour is more uniform throughout thoracic and abdominal cavity and consistency, particularly kidneys and pancreas is soft and increasingly friable.
Code 4: advanced decomposition	Carcass may be intact, but collapsed; skin sloughing; epidermis may be largely missing, exposing underlying blubber. Strong odour; blubber soft, often with pockets of gas and pooled oil; muscles nearly liquefied and easily torn, effortless separation from the bones; blood thin and black; viscera often identifiable but friable, easily torn, and difficult to dissect; gut gas-filled; brain liquified, dark red, containing gas pockets, with decreased consistency.
Code 5: mummified or skeletal remains	Skin may be draped over skeletal remains; any remaining tissues are desiccated. Organs partially or totally disappeared, or if present, not completely identifiable.

2.1.4 Stress classification

All cases underwent two classification systems: stress type and cause-of-death (Clark et al., 2006; Santana et al., 2025). Both classifications were assigned following blind quality-assured review, with cross-reader comparison (KA, LS) and final determination by a senior pathologist (KS). Cause-of-death classification demonstrated greater variation between readers, reflecting the inherent challenges of retrospective necropsy datasets where a single proximate cause must be attributed from incomplete or ambiguous evidence. Stress type demonstrated greater inter-reader congruence and was therefore selected as the primary classification system for statistical analyses in this thesis.

Stress type was classified according to the progression of disease processes using a pathology-based framework on distinguishing acute ($n = 28$), chronic ($n = 13$), and acute-on-chronic ($n = 47$) conditions (Table A2). Stress classification relied solely on histopathological descriptors and inflammatory cues (IJsseldijk et al. 2019; Table 2.2), defining stress according to the resulting histopathological process rather than circumstance-derived mortality classifications, which are often undeterminable or difficult to distinguish. This approach demonstrated greater congruence between readers, as classification was based on defined histopathological criteria rather than attribution of a single proximate cause.

Acute disease was defined as a process characterised by rapid onset and short duration, with tissue changes dominated by active injury and immediate inflammatory response.

Histopathological features indicative of acute processes included neutrophilic inflammation, necrosis, and haemorrhage, with minimal evidence of tissue repair. Chronic was defined as a process of long duration, in which injury and repair occur simultaneously, resulting in tissue remodelling. Chronic histopathological cues included fibrosis, encapsulation, granulomatous inflammation, mineralisation, and mononuclear inflammatory infiltrates. Acute-on-chronic disease was defined as superimposed acute injury or inflammation occurring within an established chronic lesion, with histological evidence of both acute and chronic processes present.

Where histopathological descriptors or inflammatory cues were absent or insufficiently described in the original pathology reports, cases were designated low confidence. In total, 15 cases lacked

adequate histopathological information to confidently assign an acute, chronic, or acute-on-chronic designation. These cases were retained for descriptive summaries, but excluded from statistical modelling.

Table 2.2 Histopathological criteria used to classify inflammatory stage

Feature	Acute	Chronic
Process definition	Rapid onset, short duration	Long duration with simultaneous injury and repair
Conceptual timeframe	Hours - days	Weeks - months, or longer
Dominant inflammatory cells	Neutrophils	Macrophages, lymphocytes, plasma cells
Tissue appearance	Swollen, disorganised, fragile	Thickened, scarred, organised
Fibrosis (scar tissue)	No	Yes
Encapsulation	No	Often
Granulomas	No	Often
Mineralisation	Rare	Common
Necrosis	Common, poorly defined	May be present, well defined
Lesion borders	Poorly defined	Well defined
Healing present	No	Yes
Typical causes	Bacteria, toxins, trauma	Parasites, chronic infection, immune disease
Typical pathological context	Active injury, haemorrhage, minimal repair	Tissue remodelling, repair processes ongoing

2.2 Adrenal gland assessment

2.2.1 *In-situ and post-excision*

Adrenal glands ($n = 176$) were visually inspected and photographed *in situ* (Figure 2.2) prior to careful dissection from the body cavity ligaments, using a stainless-steel size 22 scalpel blade. On occasion, only the right or left adrenal was recovered (right adrenal, $n = 96$; left adrenal, $n = 80$) due to use in the wider pathology screening. Post-excision, excess connective tissue was trimmed from each gland using a sterile stainless-steel size 22 scalpel. Each adrenal was weighed (g, 0.01g readability), measured (length, width, and depth; mm), and photographed again in a standardised manner on a green background with scales (cm), and animal identification code. (Figure 2.3, Table A3).



Figure 2.2 *In situ* left adrenal gland, circled in white, positioned cranial to the left kidney, assessed post-mortem (*Delphinus delphis*; KS23-57Dd). Scale, cm.



Figure 2.3 The right and left adrenal glands and kidneys extracted and trimmed (*Delphinus delphis*; KS24-96Dd). Scale, cm.

2.2.2 Adrenal histology preparation

2.2.2.1 Tissue fixation

Each adrenal was placed in 400 mL of 10% neutral buffered formalin for 24 hours before the formalin was changed, following IJsseldijk et al. (2019). After fixation, any remaining connective tissue was trimmed away from each adrenal gland with careful attention to leave the capsule of the adrenal undisturbed. Morphometric data collection and photographs were repeated by a single reader (SSM) for standardisation.

2.2.2.2 Cross-section sampling

The midpoint of each gland was determined by dividing the total length (cranial to caudal) by two (Figure 2.4a,b). A transverse cut was made at the midpoint to obtain a cross-section, with two additional transverse cuts made approximately 0.3–0.5 cm on either side. The mid-region was selected due to its cellular consistency, yielding a representative sample for both structural and pathological assessment (Clark et al., 2005). The section to the right of the midpoint cut was placed into a cassette for histological assessment of gland architecture, and the section to the left was placed into a separate cassette for pathological analysis (denoted with a star symbol; Figure 2.4a-d). Where putative anomalies were observed, additional transverse sections (0.3–0.5 cm) of the affected area were included in the pathological cassette (Figure 2.4b).

2.2.2.3 Slide preparation

Both sets of cassettes (1, architecture; 2, pathology screening) were processed using standard techniques for adrenal histology, following Clark et al. (2005, 2006, 2008) and Vuković et al. (2010). Fixed tissue sections were dehydrated through a graded ethanol series (70%, 95%, and three changes of absolute ethanol), cleared in three changes of xylene, and infiltrated with paraffin wax at 62°C, using an automated tissue processor (Leica ASP 300S). Processed tissues were embedded in paraffin wax blocks (Trajan Histology Wax Plus; melting point 54–57°C) using a Thermo Histocore Embedder. As the tissue within each cassette was sampled from the mid-region of the gland, where cellular composition is consistent across a narrow (~3-5 mm) interval (Clark et al., 2005), the sectioning face was not specified during embedding. Blocks were sectioned at 5 µm using a Leica RM2235 manual rotary microtome or Leica RM2245 semi-automatic rotary microtome fitted with EpreDia MX35 Ultra Microtome blades. Sections were floated on a warm water bath (Electrothermal Paraffin Section Mounting Bath; 50°C) and mounted onto EpreDia Superfrost glass microscope slides. Mounted sections were dried in an oven at 60°C, deparaffinised in two changes of xylene, rehydrated through a descending ethanol series (absolute, 70%), and stained with Gill's (II) haematoxylin and eosin/phloxine using a Leica Autostainer XL. Stained sections were dehydrated through an ascending ethanol series (70%, 95%, absolute), cleared in xylene, and coverslipped wet using Entellan® Rapid Mounting Medium (Leica CV5030 Coverslipper). All slide preparation was performed by histology technicians (PD and EL) at the School of Veterinary Science, Massey University.



Figure 2.4 (a, b) Orientation of adrenals and placement of transverse cuts for cross-section sampling, pathology (left) and architecture (right) for KS23-47Dd; (b) Additional cross-section samples with putative anomaly present on left shown for KS17-03Dd; (c) Cross-section on their sides prior to placement in respective cassettes for KS23-47Dd; (d) Cross-sections of adrenal gland in their respective cassettes for pathology (left) and architecture (right) assessment for KS14-42Dd. Scale, cm.

2.2.3 Slide digitisation

Histological slides were digitised to enable quantitative assessment of cellular differentiation between the capsule, cortex, and medulla. A total of ($n = 164$) architecture slides were selected for scanning using a Phillips Ultra Fast scanner at $40\times$ resolution. Digitised whole slide images

were downloaded using the Philips Image Management System Selection and stored in .tiff format for downstream analysis.

Selection criteria for slide quality assurance and control included cross-section integrity, staining adherence, and quality of slide preparation. Cross-section integrity was assessed by the completeness and preservation of the tissue mounted on the slide, ranging from a full, intact cross-section encompassing all anatomical regions to an incomplete or torn fragment containing only part of the gland. Cross-sections with missing tissue or those cut into two or more pieces were excluded from morphometric analysis ($n = 14$), as regional proportion calculations would be compromised. Cross-sections containing a cut through the tissue without missing tissue were scanned and assessed via software visualisation, with outliers excluded during analysis ($n = 21$). A further 28 glands showed marked autolysis and were excluded due to lack of cellular detail. All H&E staining adhered to the tissues adequately. The final morphometric dataset comprised 113 adrenal glands from 75 individuals.

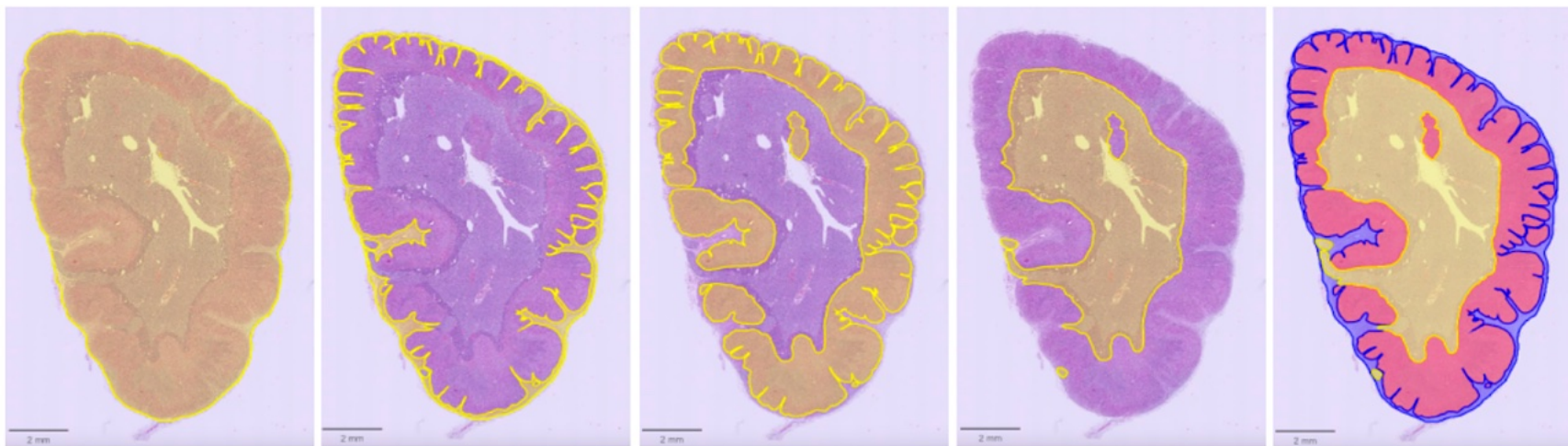
2.2.4 Digital morphometric annotation

2.2.4.1 Annotation approach

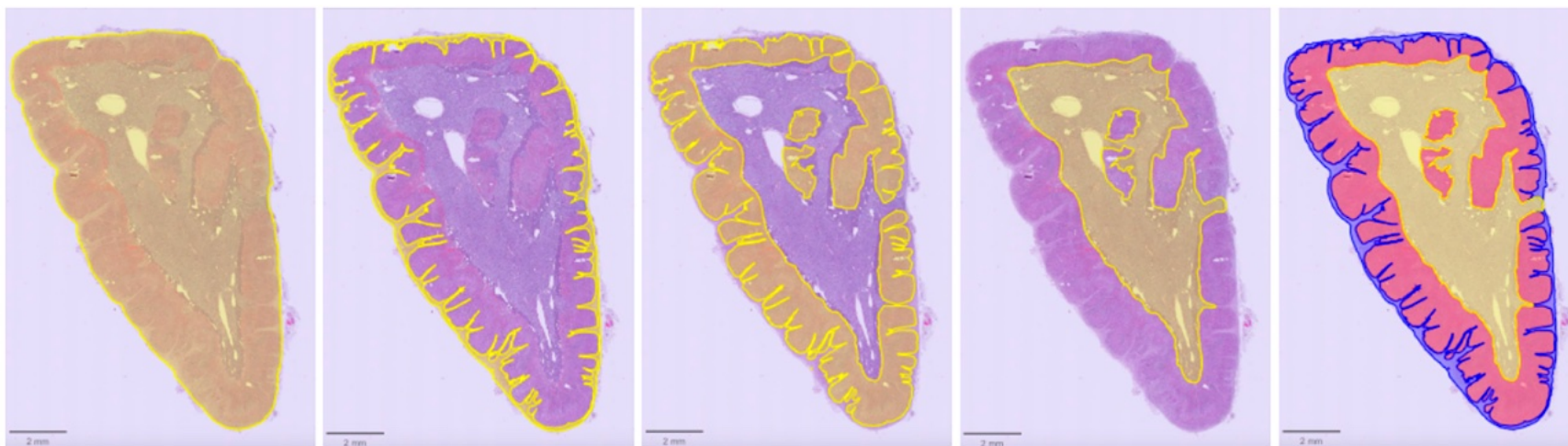
Quantification of adrenal tissue compartments was performed by manual digital planimetry on whole slide images using QuPath (version 0.5.1-x64 [2024]; Bankhead et al., 2017), an open-source software platform for digital pathology and bioimage analysis. For each adrenal gland, the boundaries of the capsule, cortex, and medulla were individually delineated by tracing the perimeter of each tissue compartment, using the region of interest (ROI) annotation tools in QuPath (Figure 2.5). The corticomedullary junction was identified based on the transition from radially arranged cortical cell cords to the more irregular chromaffin cell clusters of the medulla, while the capsule-cortex boundary was delineated at the interface between the dense connective tissue capsule and the outermost cortical cells (Table 2.3).

Annotations were performed at working magnifications of approximately $0.5\ \mu\text{m} - 5\ \text{mm}$ field of view, where at the former magnitude, individual cells were visible and the latter capturing most glands within a single screen. QuPath automatically calculated the enclosed area (μm^2) of each annotation object based on the coordinate geometry of the traced boundary. The total cross-

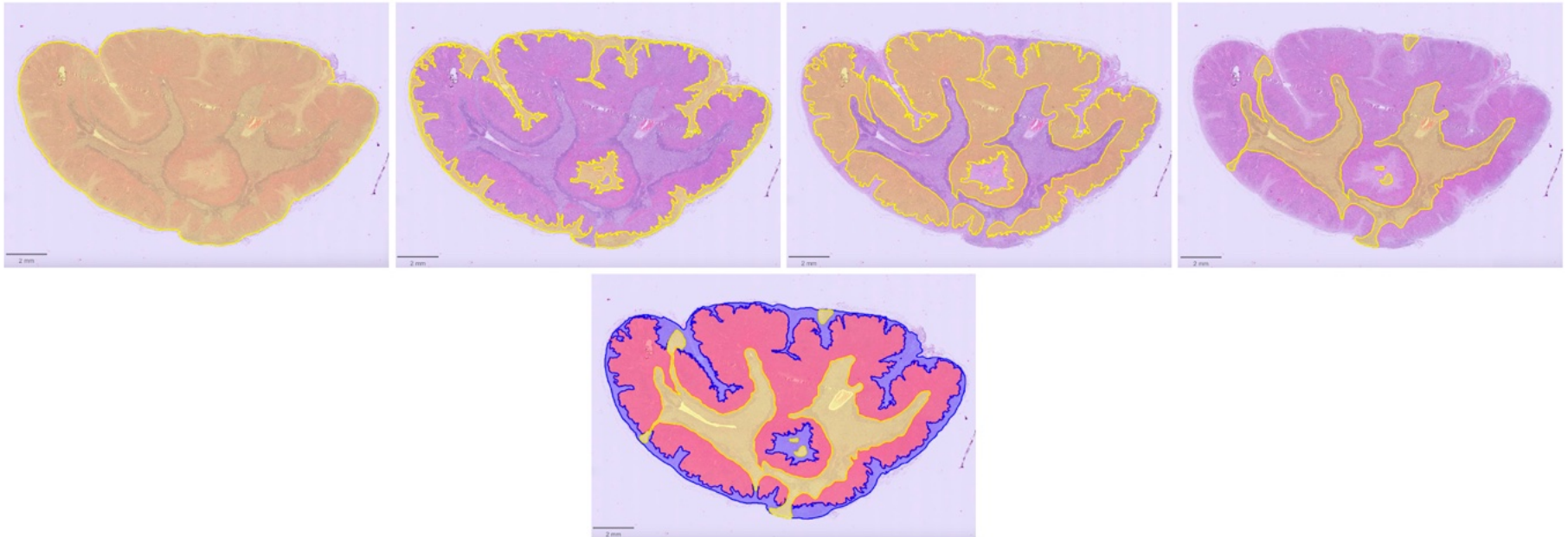
sectional area was measured in a single annotation and confirmed with the sum of capsule, cortex, and medulla areas. Proportions were calculated as the percentage of total cross-sectional area occupied by each compartment. The C:M ratio was derived by dividing the cortex area by the medulla area. Areas occupied by vasculature, processing artefact, or other pathological features were attributed to the surrounding parenchymal or stromal region, in which they were embedded.



KS24-86Dd (L)



KS24-86Dd (R)



KS23-46Dd (L)

Figure 2.5 Sequential digital planimetric annotation of adrenal gland cross-sections in QuPath (v0.5.1), demonstrated for two individuals: KS24-86Dd (left and right adrenal glands; rows 1 and 2) and KS23-46Dd (left adrenal gland; row 3). Each row shows the progressive annotation of tissue regions from left to right: (1) cross-sectional annotation; (2) capsule annotation; (3) cortex annotation; (4) medulla annotation; (5) composite overlay of all annotated regions (blue = capsule, pink = cortex, yellow = medulla). Areas occupied by major vasculature or processing artefact were assigned to the anatomical region (capsule, cortex, or medulla) in which they were situated. Stain, H&E. Scale bars = 2 mm for all whole slide images pictured.

2.2.4.2 Methodological rationale

The digital planimetric approach used here differs from the stereological point-counting method described by Weibel (1963, 1979) and applied to cetacean adrenal glands by Clark et al. (2005, 2006, 2008) and adapted by Santana et al. (2025) to include a greater number of points. In point-counting, a lattice reticle or digital grid of test points was superimposed over the histological section and the proportion of points falling on each tissue type was used to estimate area fractions, which served as unbiased estimators of volume fractions under the Delesse principle (Weibel, 1979). This approach was efficient when performed with a conventional microscope, as it eliminated the need to trace boundaries manually. However, point-counting provided a sample-based estimate of the true area, with precision dependent on the number of grid points counted, and introduced a source of sampling variance independent of the observer's ability to classify tissue boundaries.

Digital planimetric annotation, by contrast, traced the full boundary of each tissue region on a digitised whole slide image, yielding a direct measurement of the enclosed area, rather than a sample-based estimate. This eliminated the sampling variance inherent to point-counting, while retaining the requirement for the observer to make subjective judgments regarding tissue boundaries. Both approaches were therefore subject to inter-observer variability in boundary identification (Acer et al., 2008). However, planimetric annotation removed the additional source of variance introduced by point-grid sampling and was better suited to the high-resolution digital images produced by modern slide scanners.

Digital planimetry on whole slide images in QuPath was a well-established approach in quantitative histopathology across a range of tissues and organ systems (Farris et al., 2016; Montezuma et al., 2023; Tratwal et al., 2020). The present study extended this digital planimetric approach to cetacean adrenal gland morphometry, representing the first application of annotation-based area quantification to delphinid adrenal histology.

2.2.4.3 Additional architecture metrics

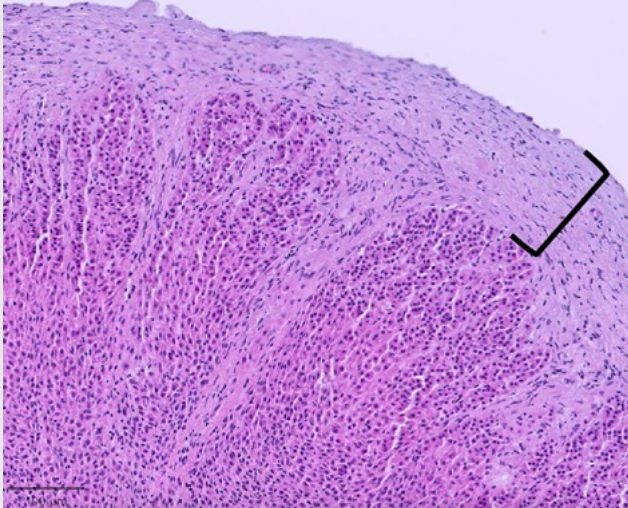
In addition to regional area measurements, the presence of a prominent medullary band was recorded as a binary variable (present/absent) for each cross-section (Clark et al., 2005, Vuković et al 2010). A prominent medullary band was defined as a clearly visible, continuous extension

of medullary tissue along the corticomedullary junction that stained darker than the surrounding medulla with H&E and was readily distinguishable at low magnification. The presence of medullary protrusions and cortical invaginations was additionally noted for each cross-section and reported descriptively as prevalence counts.

2.2.4.4 Data export and observer consistency

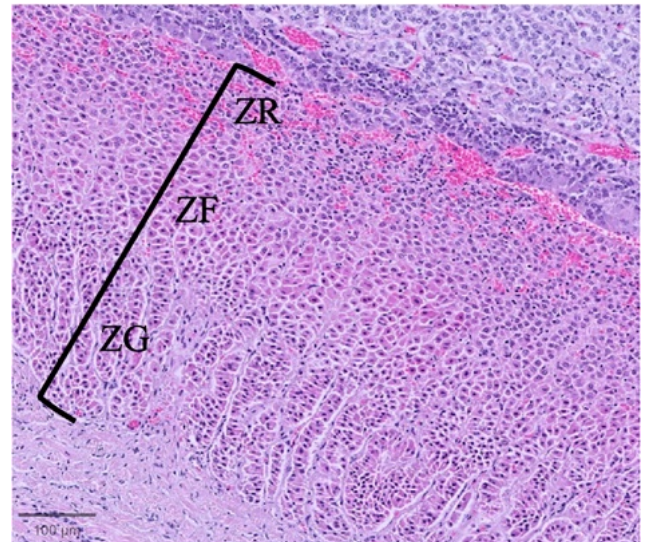
All annotation and measurement data were exported from QuPath as comma-separated value (CSV) files via the built-in measurement export function and imported into R version 4.5.2 (R Core Team 2025) for subsequent statistical analysis. All annotations were performed by a single observer (SSM) to eliminate inter-observer variability.

Table 2.3 Characteristics defining adrenal gland structure

Feature	Definition	Images
Capsule	A thin, dense layer of connective tissue surrounding the adrenal gland. KS23-08Dd R; stain, H&E; scale = 200 μm.	 A histological image of an adrenal gland cross-section stained with H&E. The image shows a dense, pink-stained layer of connective tissue (the capsule) surrounding the adrenal gland. A black bracket on the right side of the image indicates the capsule. A scale bar is visible in the bottom left corner.

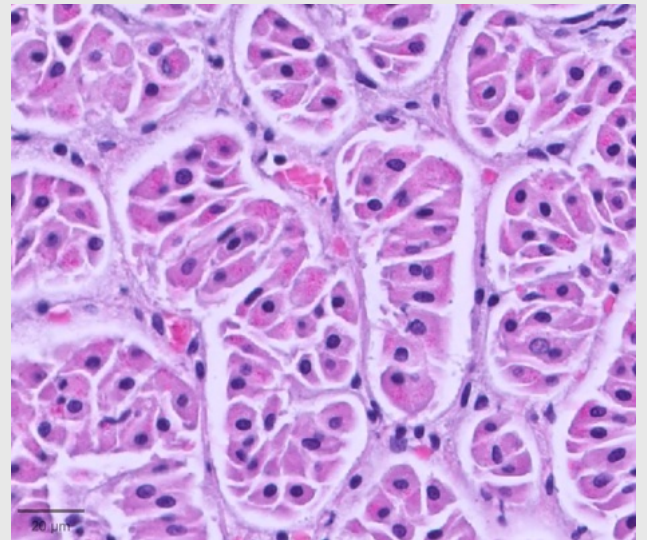
Cortex

The outer region of the adrenal gland responsible for steroid hormone synthesis; histologically divided into three subregions: *zona glomerulosa*, ZG; *zona fasciculata*, ZF; *zona reticularis*, ZR.
KS23-58Dd R; stain, H&E;
scale = 100 μm .



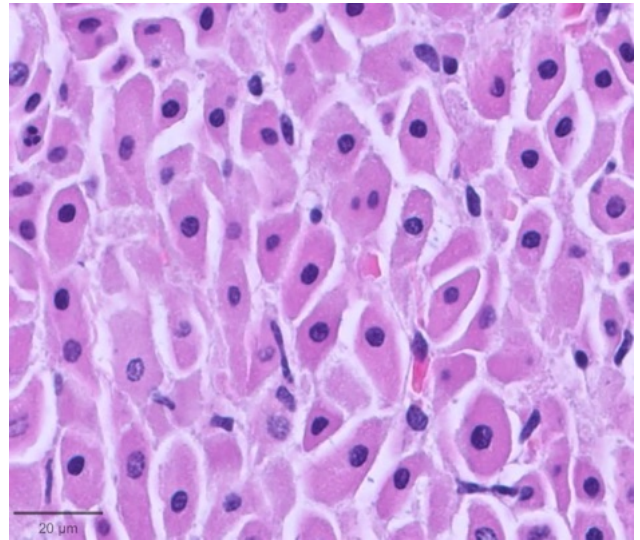
Zona glomerulosa

The sub-capsular cortical layer containing densely packed, rounded clusters of cells that primarily produce mineralocorticoids.
KS23-58Dd R; stain, H&E;
scale = 20 μm .



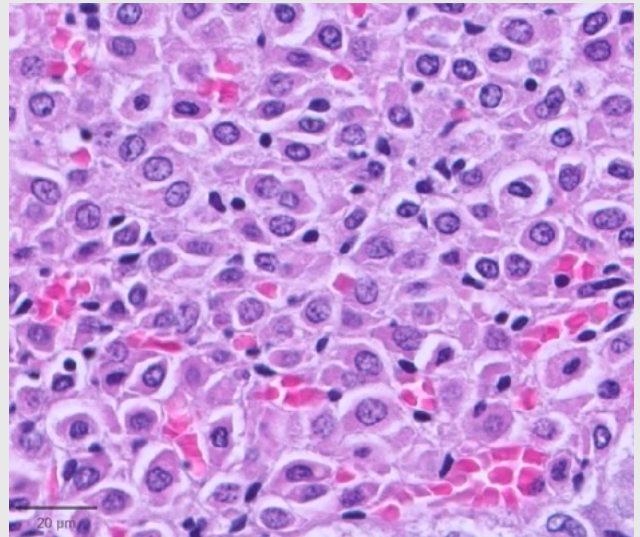
Zona fasciculata

The middle cortical layer containing cords of large, lipid-rich cells, radially arranged that produce glucocorticoids.
KS23-58Dd R; stain, H&E;
scale = 20 μ m.



Zona reticularis

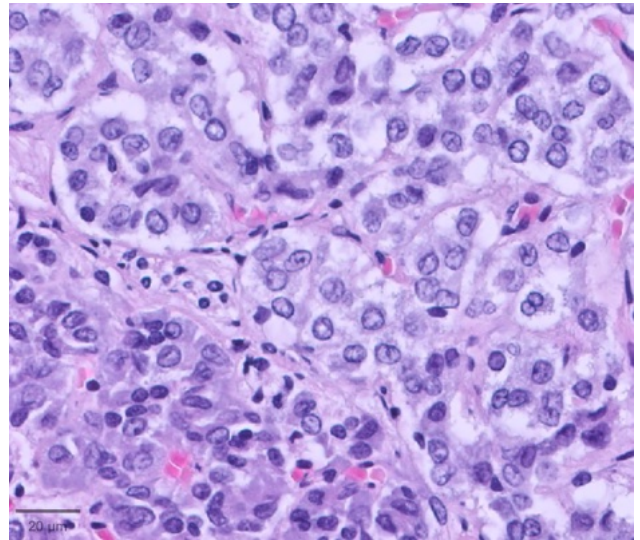
The innermost cortical layer containing cords of smaller cells arranged in a network that primarily produces androgens and some glucocorticoids.
KS23-58Dd R; stain, H&E;
scale = 20 μ m.



Medulla

The central region of the adrenal gland is composed of ganglion cells and chromaffin, that synthesise and secrete catecholamines; medullary band present in bottom left corner.

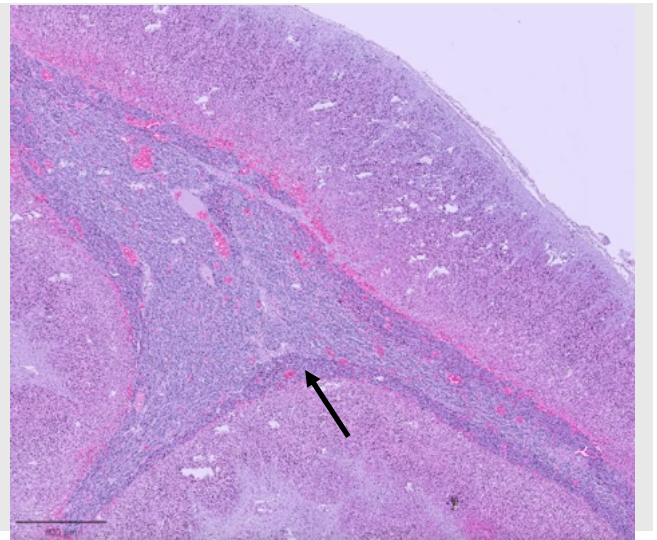
KS23-58Dd R; stain, H&E;
scale = 20 μm .



Medullary band

The peripheral medullary tissue along the corticomedullary junction; stains darkly with H&E.

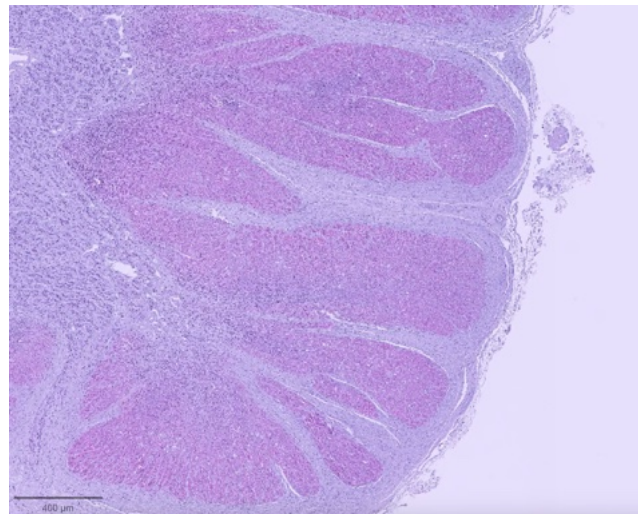
KS25-61Dd L; stain, H&E;
scale = 800 μm .



Septa

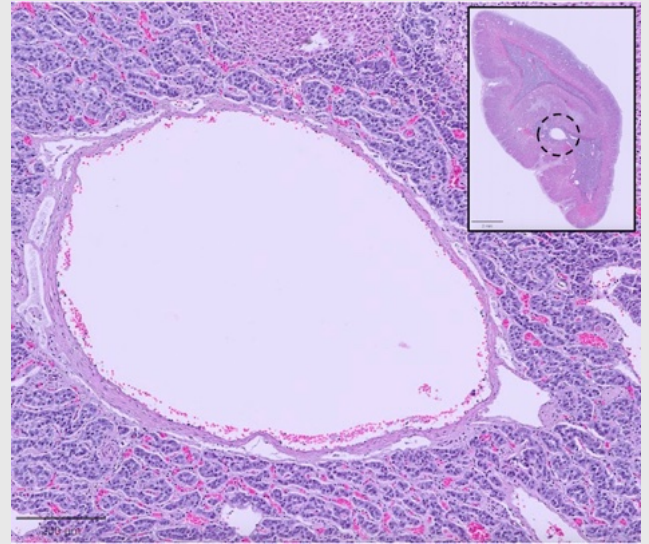
Connective tissue projections from the capsule that extend inward through the cortex.

KS13-12Dd R; stain, H&E;
scale = 400 μm .



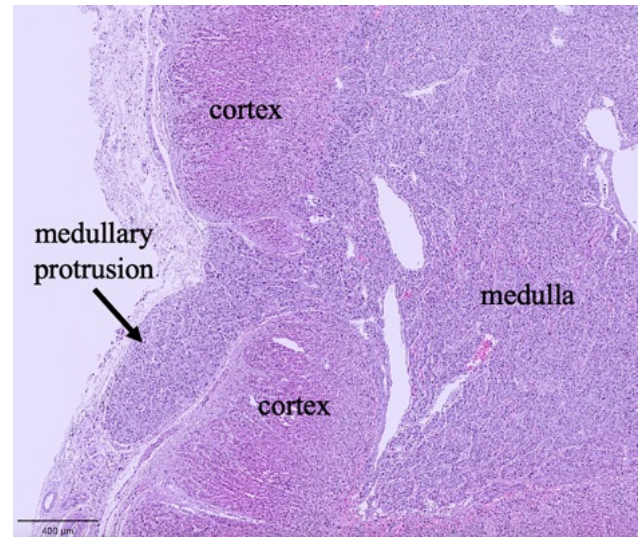
Vascularisation

Veins, arteries, or other vessels; sometimes clear space, containing blood cells, lined with endothelial cells. KS25-61Dd L; stain, H&E; scale = 200 μ m and 2 mm.



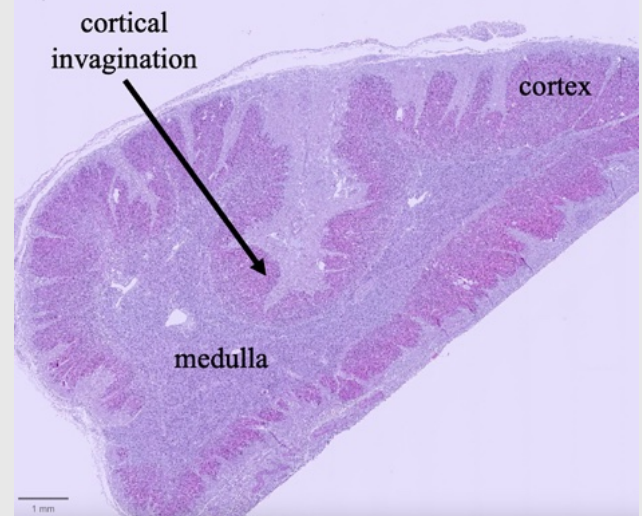
Medullary protrusion

Medullary tissue extending outward into the cortex or capsule. KS12-17Dd R; stain, H&E; scale = 400 μ m.



Cortical
invagination

Cortical tissue projecting
inward into the medulla.
KS12-13Dd R; stain, H&E;
scale = 1 mm.



2.2.4.5 Pathological assessment of the adrenal glands

Adrenal slides underwent independent pathological assessment for evidence of disease by a third board-certified veterinary anatomical pathologist (ASP). Adrenal sections stained with H&E were viewed using Leica DM500 microscope. Initial assessment of the overall structure of the glands, presence of autolysis, and lesions was completed at a magnification of 4x, followed by 10x and 40x to assess abnormalities in the architecture, presence of inflammation, pathogens, and additional cell features.

For the purposes of binary pathology assessment, glands were classified as pathology present, if any histological finding was recorded by the pathologist, regardless of clinical significance or disease score, and pathology absent, if no findings were noted. This binary classification was used in group comparisons of pathology prevalence across life history variables and as a predictor variable in linear mixed-effects models examining associations between pathology presence and adrenal zonal composition, as histological findings such as congestion, fibrosis, and mineralisation represent the contribution of non-parenchymal tissue to gland structure and may independently influence morphometric and compositional measurements.

A disease score was produced for each adrenal gland (D0-3) based on predetermined criteria (Stockin et al., 2010; Table 2.4) A score of D0, indicated non-diseased; D1, diseased present but unlikely significant to the animal; D2, disease likely to have had a clinical effect on the animal; D3, disease caused or contributed to mortality; and unknown (UK), due to autolysis with marked

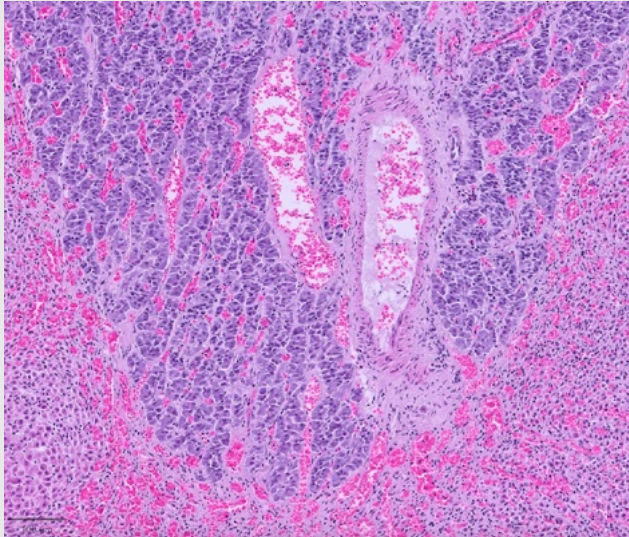
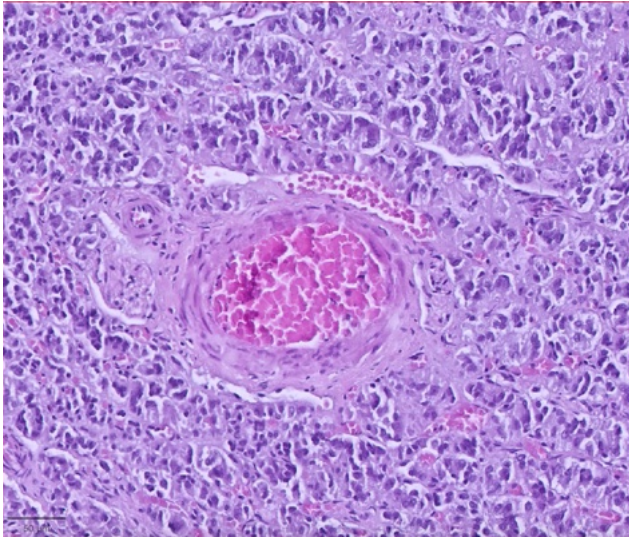
loss of cellular detail (Table A2). In cases where pathology was reported at any level, a description was recorded. Any additional anomalies present within the tissue were also noted, even if these findings were unlikely to have contributed to disease, including haemorrhaging of the cortical or medullary tissue, congestion, hyperplasia or neoplasia, mineralisation, inflammation infiltration, lipid infiltration, vessel changes (dilation, constriction, or ischemia), fibrosis, and necrosis (Table 2.5).

The disease classification system is distinct from the binary pathology presence/absence assessment described above, which captures any histological finding regardless of clinical significance. Histological findings such as vascular congestion, fibrosis, and mineralisation may not reach the threshold for clinical disease, but nonetheless represent the contribution of non-parenchymal tissue to gland structure and can independently influence morphometric and compositional measurements. Disease classification, by contrast, reflects the clinical relevance of findings; a gland may contain histological changes and still receive a D0 score if those findings were considered incidental or subclinical. These two systems therefore address different biological questions: pathology presence describes the occurrence of any histological alteration and its potential structural contribution, while disease classification describes the severity and likely clinical impact of those alterations.

Table 2.4 Definitions of disease classification

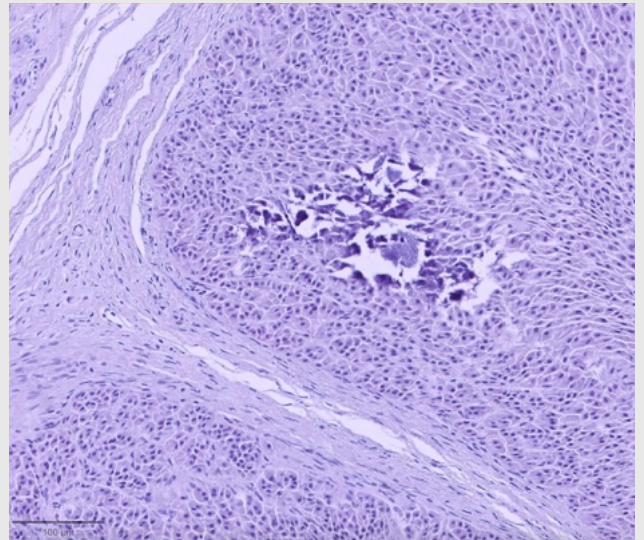
Classification	Definition
Disease score 0 (D0)	Non-diseased
Disease score 1 (D1)	Diseased present but unlikely significant to the animal
Disease score 2 (D2)	Disease likely to have had a clinical effect on the animal
Disease score 3 (D3)	Disease contributed if not caused or contributed to mortality
Unknown (UK)	Marked autolysis and/or damage preventing histological assessment

Table 2.5 Characteristics of putative anomalies observed within the adrenal glands

Feature	Definition	Images
Haemorrhage	The presence of extravasated blood cells within the adrenal tissue. KS25-61Dd L; stain, H&E; scale = 100 µm.	
Congestion/ vasculature changes	The presence of excess blood within the vasculature of the adrenal gland; blood vessels typically appear engorged. KS12-23Dd R stain, H&E; scale = 50 µm.	

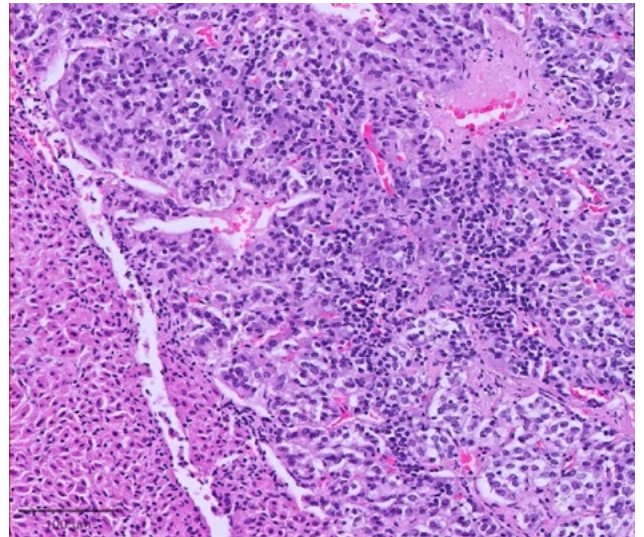
Mineralisation

The abnormal deposition of assumed mineral salts within the adrenal tissue, characterised by deeply basophilic, amorphic material. KS13-07Dd L; stain, H&E; scale = 100 μ m



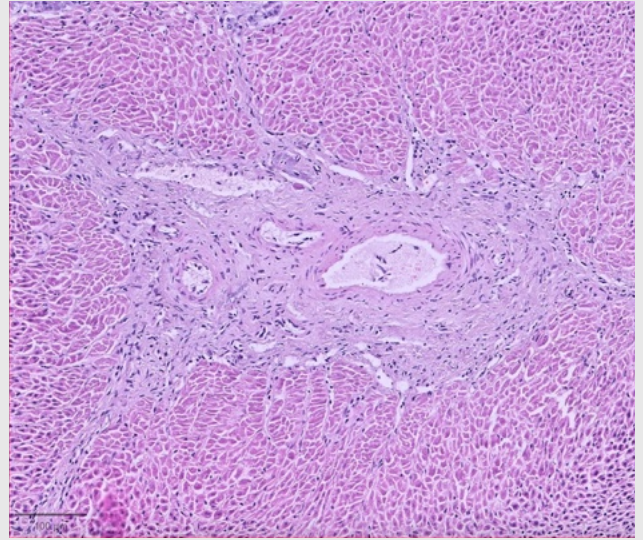
Inflammation infiltration

The presence of immune cells (lymphocytes, macrophages, neutrophils, plasma cells, etc.) within the adrenal tissue. KS23-47Dd R; stain, H&E; scale = 100 μ m.



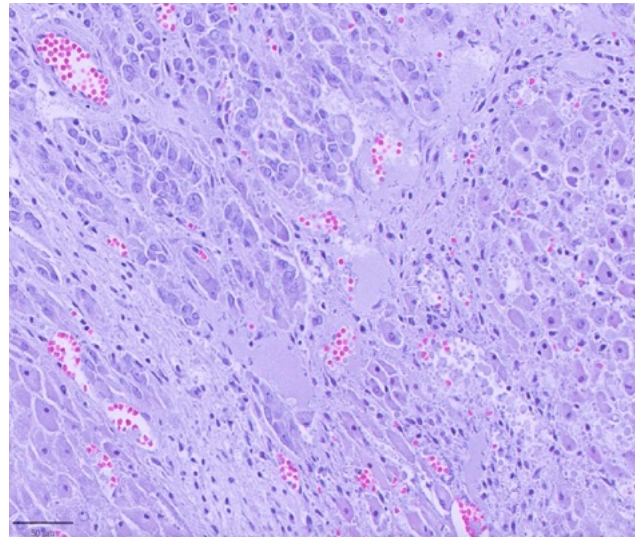
Fibrosis

The replacement of adrenal tissue with fibrous connective tissue, typically following chronic inflammation, injury, or degenerative changes. KS25-61Dd R; stain, H&E; scale = 100 μ m.



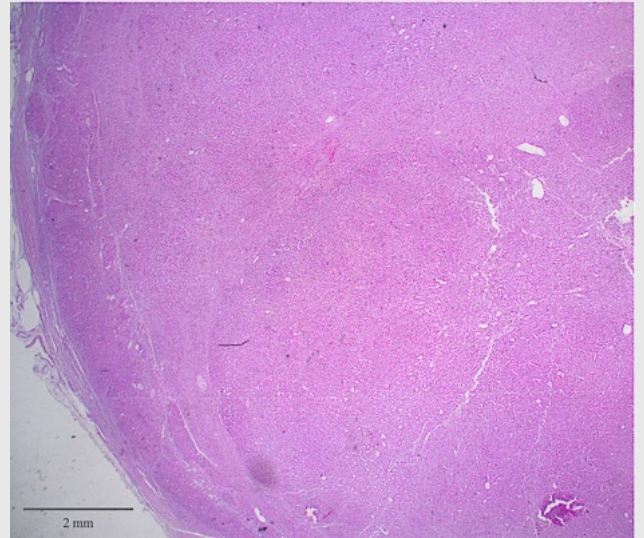
Necrosis

Amorphic eosinophilic material and pyknotic cellular debris, often admixed with inflammatory infiltrate, often due to injury or ischemia. KS11-14Dd L; stain, H&E; scale = 50 μ m.



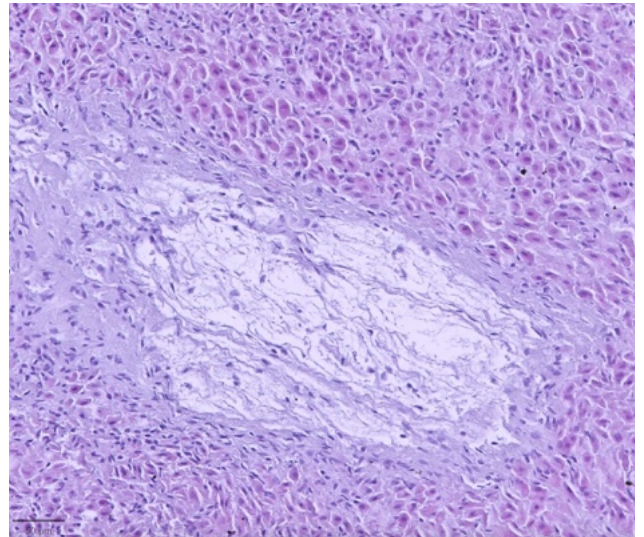
Hyperplasia/
neoplasia

The proliferation of adrenal
cells (hyperplasia) or
abnormal cells (neoplasia).
KS15-16Dd L, neoplasia;
stain, H&E; scale = 2 mm.



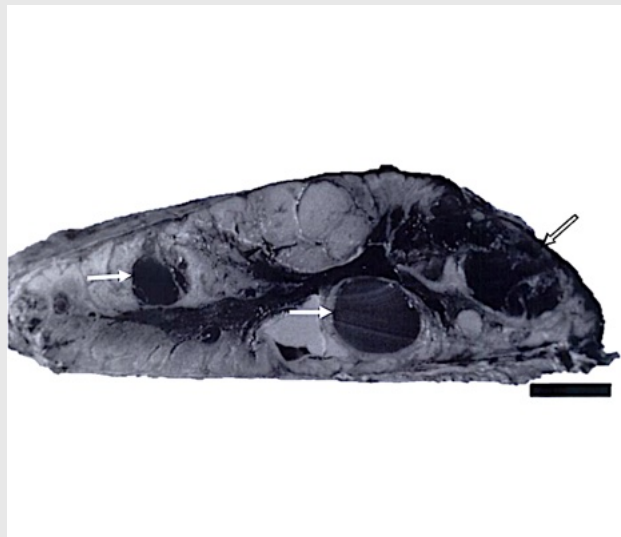
Putative lipid
infiltration

Abnormal accumulation of
lipid droplets within adrenal
cells. KS14-38Dd L, pending
IHC staining; stain, H&E;
scale = 50 μ m.



Cysts

Fluid-filled structures lined by epithelial cells in the cortex or medulla, often compressing adjacent tissue (white arrows; courtesy Lair et al., 1997). Scale, 1 cm.



2.3 Heavy metal and trace element analyses

2.3.1 Tissue collection and preparation

Hepatic and renal tissues were extracted from each cadaver, during necropsy and stored at -20°C for assessment of trace element concentrations (hepatic, $n = 99$; renal, $n = 100$), following Lischka et al. (2021). Approximately 1 g (wet weight, ww) of each tissue was freeze-dried for 77 hours under vacuum at -52°C using a Labconco FreeZone 6 Freeze Dry System (model 77540). Sample moisture loss was recorded to allow for dry weight (dw)/ wet weight (ww) conversion factors (kidney mean dw/ww = 0.2554, $n = 98$; liver mean dw/ww = 0.3088, $n = 98$; one kidney outlier excluded). All trace element concentrations are reported in mg/kg wet weight (ww).

2.3.2 Digestion and analysis

Approximately 100 mg dw of each sample was pre-digested with 3 mL of 69% HNO_3 and 1 mL of H_2O_2 at room temperature, then microwave-digested at 230°C for 25 minutes (Ultrawave Single Reaction Chamber Microwave Digestion Unit). Digested samples were made up to 20 mL with ultrapure water ($<18\text{ M}\Omega$). Trace element concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 8900). Prior to analysis, samples were diluted with 2% HNO_3 , as required. For Hg analyses, samples were diluted with 2% HNO_3 / 0.5% HCl / 0.1% cysteine following the method of Aldridge et al. (2017) to avoid sample carryover.

Rhodium was added online as the internal standard, and He and O₂ were used as collision and reaction gases.

Sixteen elements were targeted: aluminium (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), magnesium (Mg), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se), strontium (Sr), vanadium (V), and zinc (Zn). Instrument analytical limits of detection (LOD) were determined in mg/kg dry weight (dw) and converted to wet weight (ww) equivalents by multiplying by the mean tissue-specific dw/ww ratios (kidney: 0.2554; liver: 0.3088). Wet weight LODs for kidney tissue (mg/kg ww) were: Al (1.79), As (0.02), Cd (0.18), Co (0.02), Cr (0.01), Cu (0.18), Fe (1.79), Hg (0.02), Mg (1.79), Mn (0.02), Ni (0.02), Pb (0.18), Se (0.33), Sr (1.79), V (0.01), and Zn (1.79). Corresponding liver ww LODs were: Al (2.16), As (0.02), Cd (0.22), Co (0.02), Cr (0.01), Cu (0.22), Fe (2.16), Hg (0.02), Mg (2.16), Mn (0.02), Ni (0.02), Pb (0.22), Se (0.40), Sr (2.16), V (0.01), and Zn (2.16). Full instrument dw LODs and derived ww equivalents are provided in Table A4. Quality control measures included procedural blanks, duplicate samples, and certified reference material (DORM-4: Fish protein certified reference material for trace metals). Mean trace element recoveries ranged from 74% to 124% (Table A5).

2.3.3 Data handling and exclusion criteria

Individual measurements below the analytical LOD were substituted with $\text{LOD}/\sqrt{2}$ prior to statistical analysis (Hornung and Reed, 1990). This substitution method is recommended for datasets where the geometric standard deviation (GSD) is less than 3.0; for datasets with GSD approximately 3.0 or greater, $\text{LOD}/2$ is considered more appropriate (Hornung & Reed, 1990). The majority of elements had $\text{GSD} < 3.0$ in both tissues, supporting the use of $\text{LOD}/\sqrt{2}$. Cadmium and chromium exceeded this threshold in both tissues (kidney Cd: $\text{GSD} = 6.04$; liver Cd: $\text{GSD} = 3.95$; kidney Cr: $\text{GSD} = 3.12$; liver Cr: $\text{GSD} = 3.32$); however, given the low proportion of below-LOD values for these elements (Cd: 17.9% kidney, 24.5% liver; Cr: 17.0% kidney, 38.0% liver), the influence of substitution method on derived values was considered unlikely to have materially affected the analytical results. Furthermore, chromium was independently excluded from liver multivariate analyses on the basis that 38% of liver values were below the LOD, exceeding the 30% substitution threshold applied for element selection. As

chromium did not enter the principal component or z-score index models via either tissue, any effect of the substitution method choice for this element on the analytical outcomes was therefore minimal. Wet weight-equivalent LOD/ $\sqrt{2}$ substitutes were derived from the ww LODs in Table A4.

Elements were excluded from multivariate analyses, if more than 30% of measurements in either tissue required LOD/ $\sqrt{2}$ substitution, as a high proportion of substituted constants artificially distorts multivariate structure (Helsel, 2012). Six elements were excluded on this basis: Pb (100% substituted in both tissues), Sr (100% in both tissues), Co (70% kidney, 100% liver), V (50% kidney), Cr (38% liver), and Ni (31% kidney, 11% liver). This resulted in a final panel of 10 elements for multivariate analysis: Al, As, Cd, Cu, Fe, Hg, Mg, Mn, Se, and Zn.

Seventy-one animals had complete concentration data for all 10 elements in both kidney and liver, providing 106 glands for index-based models. All trace element concentrations were log-transformed prior to parametric analyses to reduce positive skew and standardised to zero mean and unit variance within each tissue. Five animals were excluded from all metal analyses due to the absence of tissue samples (KS13-03Dd, KS25-61Dd, KS25-67Dd, KS25-68Dd, W08-28Dd). Sample sizes varied by element due to concentrations below limits of detection (reported in Tables 3.19-3.20).

Wilcoxon signed-rank tests were restricted to the ten elements meeting the $\leq 30\%$ LOD/ $\sqrt{2}$ substitution threshold in both tissues, as cross-tissue comparison is not meaningful where one tissue's values are predominantly analytical substitutes rather than measured concentrations. Vanadium, nickel, and chromium were therefore excluded from tissue comparison analyses, though their concentrations are reported descriptively.

2.3.4 *Hg:Se molar ratio*

To assess the molar relationship between mercury and selenium, Hg:Se molar ratios were calculated for each individual in both kidney and liver tissue using the formula:

$$Hg/Se \text{ molar ratio} = \frac{mmol \text{ Hg } / kg}{mmol \text{ Se } / kg}$$

where Hg and Se are concentrations (mg/kg ww) and 78.96 and 200.59 are the atomic masses (g/mol) of mercury and selenium, respectively. A Hg:Se molar ratio less than 1 indicates a molar excess of selenium relative to mercury, which has been proposed as protective against mercury toxicity through sequestration into inert Hg-Se complexes (Ralston & Raymond, 2010). A ratio exceeding 1 indicates mercury dominance, potentially exceeding the buffering capacity of available selenium. Hg:Se ratios were included as predictors in linear mixed-effects models of adrenal zonal proportions and were compared between pathology and disease groups using Wilcoxon rank-sum tests.

2.4 Data analysis

Statistical analyses were performed in R Studio version 2025.09.2+418 using R version 4.5.2 (R Core Team 2025). Statistical significance was set at $\alpha = 0.05$ for all tests.

2.4.1 Descriptive statistics and group comparisons

Descriptive statistics (mean, median, standard deviation, minimum, maximum) were calculated for all morphometric, compositional, and trace element variables. Where both left and right adrenal glands were available for an individual, weight and length measurements were averaged prior to group comparisons; where only one gland was recovered, the available measurement was used. Averaged values were used for descriptive group comparisons only; inferential modelling was conducted using the full bilateral dataset with gland side included as a fixed effect. For trace elements, statistics were calculated independently for kidney and liver tissues, with elements ranked by mean concentration within each tissue type.

Non-parametric tests were used for group comparisons throughout. Wilcoxon matched-pairs signed-rank tests compared bilateral adrenal measurements and paired kidney-liver metal concentrations. Mann-Whitney U tests compared continuous variables between two independent groups (e.g., adrenal weight by pathology presence). Kruskal-Wallis tests assessed differences across stress type categories. Chi-square tests of independence and Fisher's exact tests evaluated associations between categorical variables (e.g., pathology prevalence by sex, sexual maturity). Spearman rank correlations quantified associations between continuous variables, including

bilateral correspondence and age-morphometric relationships. Logistic regression modelled binary outcomes (e.g., disease presence) as a function of continuous predictors (e.g., age).

2.4.2 Linear mixed-effects models

Linear mixed-effects models were fitted to evaluate the effects of life history variables (age, sex, and gland side) on adrenal morphometrics and zonal proportions, accounting for non-independence of bilateral glands within individuals. Animal identity was included as a random intercept in all models. Response variables were transformed, where required to satisfy model assumptions including, C:M ratio and cross-sectional area, which were log-transformed, while capsule and medulla proportions were modelled untransformed. Sexual maturity was excluded from models where collinearity with age was high. For categorical predictors, females, immature individuals, left adrenal glands, and non-prominent medullary band were used as reference categories throughout. Models were fitted using restricted maximum likelihood (REML) via the lme4 package (Bates et al., 2015), with denominator degrees of freedom and *p*-values estimated using the Satterthwaite approximation in lmerTest (Kuznetsova et al., 2017). Residual versus fitted plots and Q-Q plots were inspected to assess homoscedasticity and normality of residuals. Intraclass correlation coefficients (*ICC*) were calculated to quantify the proportion of total variance attributable to between-individual differences. Marginal and conditional R^2 values were reported for each model.

2.4.3 Trace element indices

To investigate associations between cumulative metal exposure and adrenal architecture, composite *z*-score indices were constructed from log-transformed, standardised metal concentrations in kidney and liver tissue. Metals were grouped into a bioaccumulative index (Se, Cd, Hg) and an essential index (Cu, Zn, Mg). Each index was calculated as the mean of the standardised log-concentrations per animal per tissue. These indices were validated against principal component analysis (PCA) of the full 10-element panels. Validation was confirmed by correlating each *z*-score index with its corresponding principal component score; a strong correlation ($r > 0.85$) was taken as evidence that the index captured the same axis of variation as

the PCA. Separate linear mixed-effects models were then fitted with log(C:M ratio) as the response and each tissue-category z-score index as a single fixed-effect predictor, with animal identity as a random intercept.

Chapter 3 – Results

3.1 Sample Summary

A total of 103 common dolphins (*Delphinus delphis*) were included in this study. Individuals were examined post-mortem following stranding events and represented a range of life history traits, including variation in age, sex, sexual maturity, stress type, and disease status.

Sex was determined anatomically ($n = 102$) or molecularly ($n = 1$) for all 103 individuals, comprising 45 males and 58 females. Sexual maturity status was assigned to 101 individuals as immature ($n = 54$), mature ($n = 47$), or unknown due to scavenging ($n = 2$). Age was calculated via dentine growth layer groups (GLG; Palmer et al 2022; 2023, Massey University, unpublished data) for 102 individuals and treated as a continuous variable. Paired glands were available for 73 individuals.

Decomposition condition category (DCC) was assigned to all individuals ($n = 103$; IJsseldijk et al., 2019; Table A2). Animals were classified by gross stress type via histopathological descriptors and inflammatory cues as either, acute ($n = 28$), chronic ($n = 13$), acute-on-chronic ($n = 47$), or indeterminate ($n = 15$) based on post-mortem. Adrenal glands were assessed by a pathologist for disease and presence of pathology. Adrenals were marked as either, non-diseased (D0; $n = 136$), diseased present but unlikely significant to the animal (D1; $n = 11$), disease likely to have had a clinical effect on the animal (D2; $n = 2$), diseased caused or contributed to mortality (D3; $n = 0$). Pathology presence was marked as either, pathology present where any pathological finding was reported, including incidental or finding insignificant to animal's health ($n = 37$), or pathology absent ($n = 112$). Glands showing marked autolysis ($n = 27$) were excluded from histological assessment.

Fifteen trace elements were quantifiable in kidney tissue and thirteen in liver tissue, with sample sizes varying where individual concentrations fell below limits of detection. A total of 95 individuals with complete life history data and paired kidney-liver tissue samples were included in metal analyses. As a result, sample sizes varied among analyses and were reported within each relevant subsection.

3.2 Adrenal gland structure

In all individuals, adrenal glands were located adjacent to the cranial pole of each kidney. Where both right and left glands were recovered, visual comparisons at the time of extraction found the paired organs varied marginally in shape. On the transverse sectioning, the cortex and medulla could often be seen macroscopically with the corticomedullary junction prominent.

At the histological level, examination revealed a consistent general architecture comprising an outer fibrous capsule, a trilaminar cortex, a central medulla, and in some animals, a medullary band at the corticomedullary junction (Table 2.3). Architectural complexity was common, with cortical and medullary tissue frequently distributed in irregular alternating layers rather than forming a simple concentric pattern. Medullary protrusions, where medullary tissue extended outward into the cortex ($n = 59$ glands, 52.21%), and cortical invaginations, where cortical tissue projected inward into the medulla ($n = 72$ glands, 63.72%), were similarly observed. These features are consistent with architectural variants described in other common dolphins and delphinids and are interpreted as growth-related structural variants rather than pathological changes (Alonso-Almorox et al., 2026; Clark et al., 2005; Vuković et al., 2010).

The capsule was composed of dense connective tissue, staining lightly with H&E. Septa extended from the capsule into the cortex at irregular intervals, narrowing distally with progression into the parenchyma. Where septa were well developed, the cortex was partially compartmentalised into discrete lobule-like regions, referred to as pseudolobulation. The capsule was moderately vascularised and contained small- to medium-diameter blood vessels frequently accompanied by nerve fibres within the pericapsular connective tissue. These neurovascular structures extended inward along connective tissue trabeculae, forming a supporting stromal framework for the gland.

The *zona glomerulosa* formed a narrow subcapsular layer of densely packed, rounded cell clusters immediately beneath the capsule (Table 2.3). The *zona fasciculata*, situated interior to the *zona glomerulosa*, comprised the bulk of the cortical thickness, consisting of large cells with abundant pale, lightly eosinophilic cytoplasm arranged in radial cords. Sinusoidal capillaries were visible between cell cords throughout the cortex, most prominently within the *zona fasciculata*, where they ran longitudinally between the radially arranged cords. The *zona*

reticularis formed the innermost cortical layer, with smaller cells arranged in a network of short cords adjacent to the medulla. The central medulla was composed of neuroendocrine cells arranged around a rich sinusoidal vascular network. Where present, the medullary band was visible as a distinct peripheral region of darkly staining cells at the corticomedullary junction, with more lightly staining cells occupying the central medulla.

3.3 Morphometrics

3.3.1 Symmetry and asymmetry within paired adrenal glands

Bilateral symmetry was assessed in all individuals, for which both glands were available ($n = 73$) to determine whether either side could serve as a representative in downstream analyses.

Bilateral comparisons of adrenal morphology revealed a high degree of symmetry between left and right adrenal glands across all measured metrics. Left and right adrenal measurements were strongly correlated within individuals for weight, length, width, and depth ($\rho = 0.69\text{--}0.93$; all $p < 0.0001$), indicating strong within-individual bilateral correspondence. Despite this, significant directional asymmetries were detected for weight and length. Left adrenal glands were marginally heavier than right glands (left: 3.00 ± 0.31 g, right: 2.71 ± 0.18 g; median difference: 0.08 g; Wilcoxon matched-pairs signed-rank test, $p = 0.0038$; Figure 3.1), while right adrenal glands were longer than left glands (right: 36.44 ± 0.90 mm; left: 34.67 ± 0.95 mm; median difference: 1.33 mm; $p = 0.024$). No significant bilateral differences were detected for adrenal width ($p = 0.292$) or depth ($p = 0.848$). Full descriptive statistics for all gross morphometric variables, including width and depth, are reported by sex and side in Table A3.

Visual inspection of raw data and paired plots identified one individual (KS15-16Dd) with pronounced bilateral asymmetry associated with marked pathology, with the left gland weighing 20.95 g compared to 7.95 g on the right (Figure 3.2). This 26-year-old sexually mature male had a left gland mass approximately 5.3 times the mature male mean (3.92 ± 0.69 g; $n = 23$), exceeding all other individuals in the group. The right gland, while itself approximately 2.0 times the mature male mean, conformed to a typical oblong morphology on visual inspection. Sensitivity analyses, excluding this individual, yielded consistent results (median difference: 0.08 g; Wilcoxon matched-pairs signed-rank test, $p = 0.0061$), indicating that observed bilateral

differences were not driven by this single extreme case (KS15-16Dd L). These findings indicate strong bilateral correspondence, with small but significant directional asymmetries for weight and length.

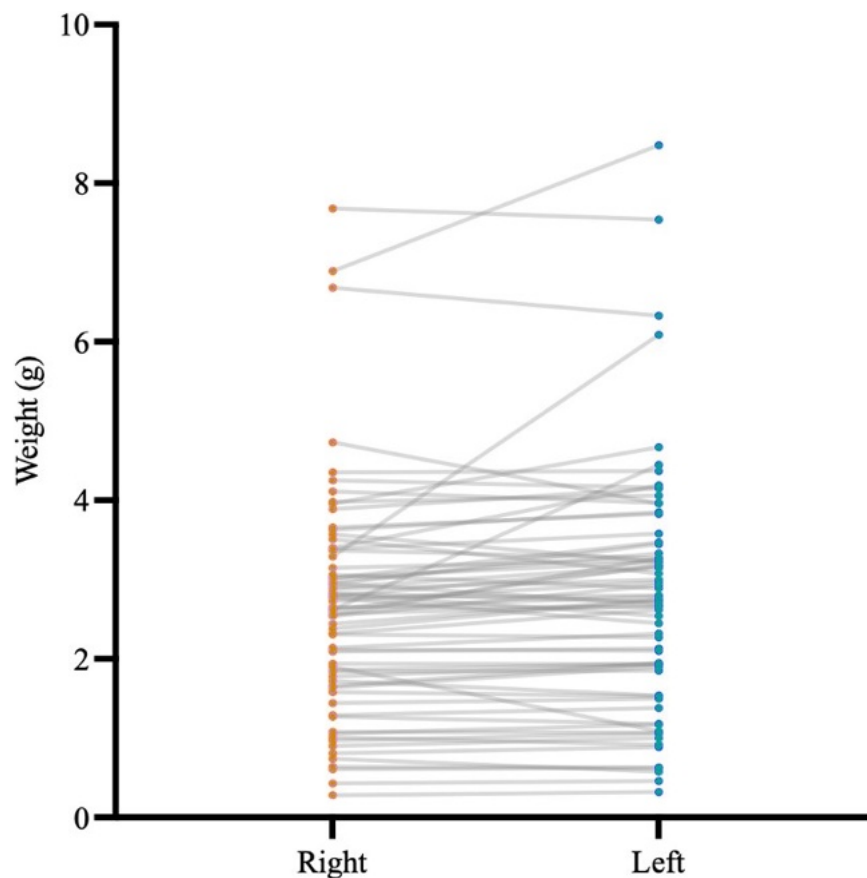


Figure 3.1 Paired comparison of adrenal gland weight (g) between right and left adrenal glands. Each line connects paired measurements from the same individual; points represent individual glands (right, orange; left, blue]. Left adrenal glands were significantly heavier than right adrenal glands (Wilcoxon matched-pairs signed-rank test, $p = 0.0038$). One individual (KS15-16Dd; excluded from plot) exhibited a pronounced left-right difference due to marked pathology; however, this observation did not drive the overall result (sensitivity analysis, excluding this individual: $p = 0.0061$).



Figure 3.2 Gross morphology of adrenal glands from KS15-16Dd, a mature male common dolphin (age, 26) exhibiting pronounced unilateral adrenal enlargement. (A) Right adrenal gland (7.95 g) showing typical oblong morphology. (B–D) Left adrenal gland (20.95 g; $\sim 2.6\times$ the mass of the right gland) displaying atypical morphology shown from the top, side and bottom aspect, respectively. (E) Transverse cross-sections on the left, central, and right portion of the left enlarged adrenal gland. Scale bars, cm.

3.3.2 *Adrenal weight and length in relation to life history traits*

To assess the influence of life history on adrenal weight, mean adrenal weight was examined in relation to sex, sexual maturity, and age. Where both glands were available, the mean left and right adrenal weights were calculated. Where only one gland was recovered, the available measurement was used.

No significant difference in mean adrenal weight was detected between sexes (Mann-Whitney U test, $W = 1316.5$, $p = 0.942$; Figure 3.3A). Sexual maturity was significantly associated with adrenal weight, with mature individuals exhibiting heavier glands than immature individuals (Mann-Whitney U test, $W = 184.0$, $p < 0.001$; Figure 3.3B). Adrenal weight increased strongly with age ($\rho = 0.854$, $p < 0.001$; Figure 3.3C). Both the maturity and age effects were robust to the exclusion of KS15-16Dd (maturity: $p < 0.001$; age: $\rho = 0.849$, $p < 0.001$). The outlier, KS15-16Dd, was excluded from all panels for visual clarity; all statistical comparisons were conducted on the full sample and confirmed via sensitivity analysis.

No significant difference in averaged adrenal length was detected between sexes (Mann-Whitney U test, $W = 1472.0$, $p = 0.268$; Figure 3.4A). Sexual maturity was significantly associated with adrenal length, with mature individuals exhibiting longer glands than immature individuals (Mann-Whitney U test, $W = 331.0$, $p < 0.001$; Figure 3.4B). Adrenal length increased strongly with age ($\rho = 0.755$, $p < 0.001$; Figure 3.4C). Both the maturity and age effects were robust to the exclusion of KS15-16Dd (maturity: $p < 0.001$; age: $\rho = 0.748$, $p < 0.001$).

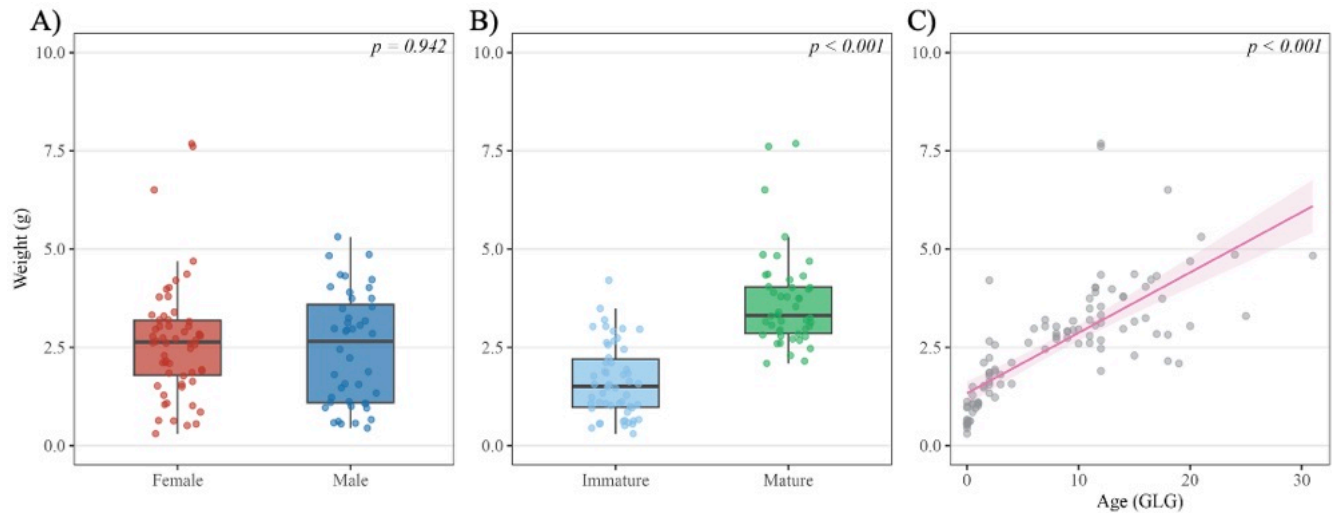


Figure 3.3 Distribution of averaged adrenal gland weight (g) in relation to life history variables. (A) Averaged adrenal weight by sex (female, red; male, blue). (B) Averaged adrenal weight by sexual maturity status (immature, light blue; mature, green). (C) Averaged adrenal weight in relation to age, with a linear regression line and 95% confidence interval. Boxes represent the median and interquartile range (IQR), with whiskers extending to $1.5 \times \text{IQR}$; individual data points are shown as jittered circles. One individual (KS15-16Dd) was excluded from all panels for visual clarity; statistical comparisons were conducted on the full sample. p-values are shown in the upper right corner of each panel.

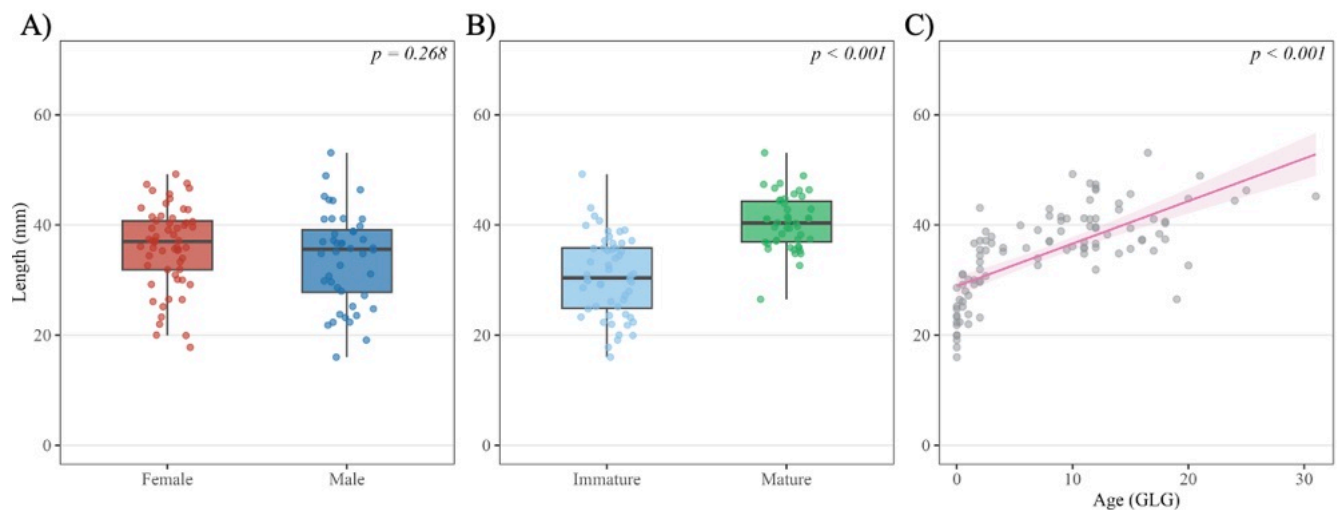


Figure 3.4 Distribution of averaged adrenal gland length (mm) in relation to life history variables. (A) Sex (female, red; male, blue). (B) Sexual maturity status (immature, light blue; mature, green). (C) Age estimated by age, with linear regression line and 95% confidence interval. One individual (KS15-16Dd) was excluded for visual clarity; statistical comparisons were conducted on the full sample. Boxes show median and IQR; whiskers extend to $1.5 \times \text{IQR}$; points represent individual animals. p-values are shown in the upper right corner of each panel.

3.3.3 Adrenal weight and length in relation to pathology, disease and stress type

Averaged adrenal weight differed significantly between individuals with and without reported histopathological lesions (Mann-Whitney U test, $W = 434.0$, $p = 0.013$; Figure 3.5A), with individuals having detectable adrenal pathology exhibiting heavier glands than those with no detectable pathology. When disease was classified by gross disease score (D0 vs D1), a marginal difference was detected (Mann-Whitney U test, $W = 149.0$, $p = 0.048$; Figure 3.5B); however, this result was not robust to the exclusion of KS15-16Dd ($p = 0.142$), and should be interpreted with caution. When classified by whole-body stress category (acute, chronic, acute-on-chronic, or indeterminate), no significant differences in averaged adrenal weight were detected among groups (Kruskal-Wallis test, $H(3) = 6.22$, $p = 0.101$; Figure 3.5C). Together, these findings suggest that adrenal enlargement is associated with the presence of glandular pathology, but did not vary systematically with gross stress classification or disease score, when the influence of marked pathological outliers was accounted for.

No significant difference in averaged adrenal length was detected between individuals with and without histopathological lesions (Mann-Whitney U test, $W = 530.0$, $p = 0.127$; Figure 3.6A), nor between D0 and D1 disease score groups (Mann-Whitney U test, $W = 166.0$, $p = 0.089$; Figure 3.6B). In contrast to adrenal weight, averaged adrenal length differed significantly across whole-body stress categories (Kruskal-Wallis test, $H(3) = 10.16$, $p = 0.017$; Figure 3.6C). Post-hoc pairwise comparisons revealed that indeterminate individuals had significantly shorter glands than both acute-on-chronic ($p = 0.025$) and chronic individuals ($p = 0.029$; Wilcoxon rank sum, Holm-corrected). No significant differences were detected among acute, chronic, and acute-on-chronic groups. This pattern was largely robust to the exclusion of KS15-16Dd, with indeterminate individuals remaining significantly shorter than acute-on-chronic individuals ($p = 0.025$), though the difference with chronic individuals became marginal ($p = 0.052$).

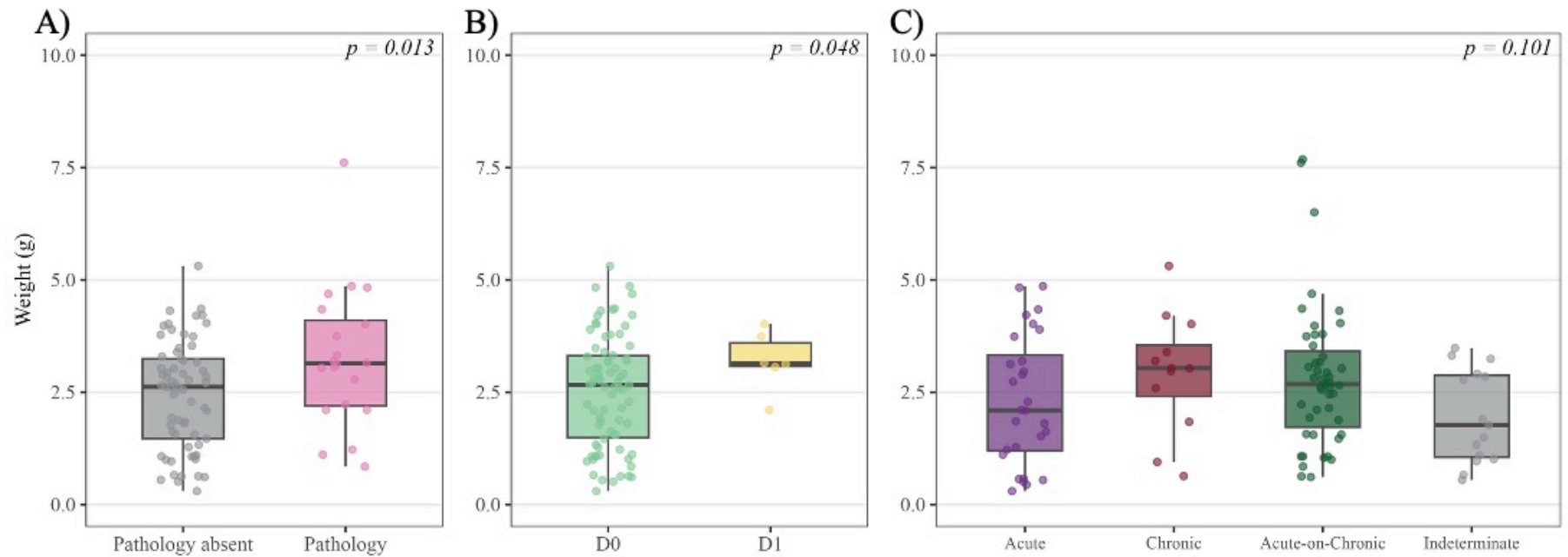


Figure 3.5 Distribution of mean adrenal gland weight (g) in relation to pathology, disease, and stress. (A) Mean adrenal weight by histopathological lesion presence (absent, grey; present, pink). (B) Mean adrenal weight by gross disease score (D0, green; D1, yellow). (C) Mean adrenal weight by whole-body stress classification (acute, purple; chronic, dark red; acute-on-chronic, dark green; indeterminate, grey). Boxes represent the median and interquartile range (IQR), with whiskers extending to $1.5 \times \text{IQR}$; individual data points are shown as jittered circles. One individual (KS15-16Dd) was excluded from all panels for visual clarity; statistical comparisons were conducted on the full sample. *p*-values are shown in the upper right corner of each panel.

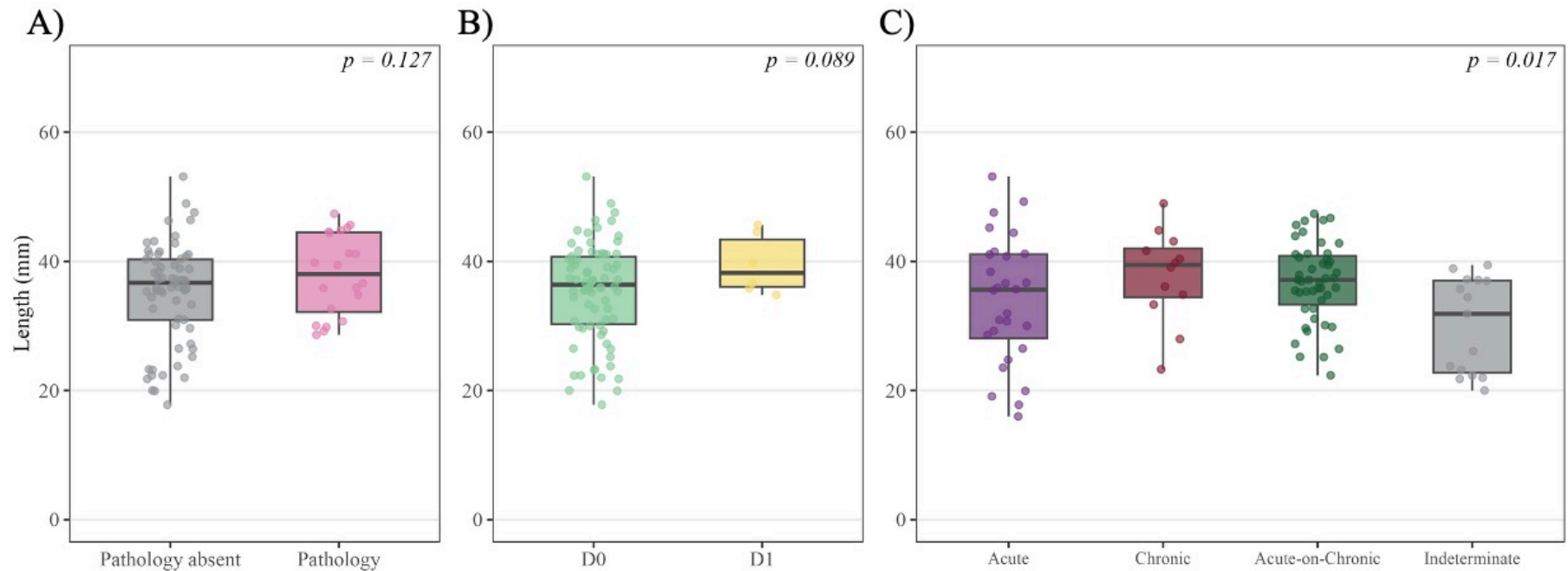


Figure 3.6 Distribution of mean adrenal gland length (mm) in relation to disease and stress variables. (A) Mean adrenal length by histopathological lesion presence (absent, grey; present, pink). (B) Mean adrenal length by gross disease score (D0, green; D1, yellow). (C) Mean adrenal length by whole-body stress classification (acute, purple; chronic, dark red; acute-on-chronic, dark green; indeterminate, grey). Boxes represent the median and interquartile range (IQR), with whiskers extending to $1.5 \times \text{IQR}$; individual data points are shown as jittered circles. One individual (KS15-16Dd) was excluded from all panels for visual clarity; statistical comparisons were conducted on the full sample. *p*-values are shown in the upper right corner of each panel.

3.3.4 Effects of life history variables on weight and length

Linear mixed-effects models identified age as the dominant predictor of both adrenal weight and length, with substantial between-individual variation detected in both models. Age was the strongest predictor of adrenal weight ($\beta = 0.204$ g per GLG year, $SE = 0.036$, $t = 5.73$, $p < 0.001$; 95% CI [0.134, 0.273]; Table 3.1), indicating adrenal glands increased in mass by approximately 0.20 g per year, equivalent to a 7.4% increase relative to the sample mean (2.76 ± 0.15 g) per year of age (Figure 3.7). A significant effect of gland side was detected ($\beta = -0.418$ g, $SE = 0.182$, $t = -2.29$, $p = 0.024$; 95% CI [-0.776, -0.060]), with right adrenal glands lighter than left glands. Thus, left glands were on average 0.42 g (15.2%) heavier than right glands, after accounting for age and individual identity. Sex ($\beta = 0.061$ g, $SE = 0.280$, $p = 0.828$; 95% CI [-0.487, 0.609]; Figure 3.8) and sexual maturity ($\beta = -0.166$ g, $SE = 0.511$, $p = 0.747$; 95% CI [-1.167, 0.836]; Figure 3.8) were not significant predictors of adrenal weight, once age was included.

Age was also a strong predictor of adrenal length ($\beta = 0.800$ mm per GLG year, $SE = 0.140$, $t = 5.70$, $p < 0.001$; 95% CI [0.525, 1.075]; Table 3.2), corresponding to an increase of approximately 0.8 mm per year, or 2.3% of mean length (35.4 ± 0.6 mm) annually (Figure 3.9). Gland side had a significant effect ($\beta = 1.103$ mm, $SE = 0.531$, $t = 2.08$, $p = 0.041$; 95% CI [0.062, 2.144]), with right adrenal glands longer than left glands by approximately 1.10 mm (3.1%) on average. Sex showed a marginal, non-significant trend ($\beta = -2.01$ mm, $SE = 1.113$, $t = -1.81$, $p = 0.074$; 95% CI [-4.191, 0.171]; Figure 3.10), suggesting males may have shorter glands than females. Sexual maturity was not associated with adrenal length ($\beta = 0.636$ mm, $SE = 2.038$, $p = 0.756$; 95% CI [-3.359, 4.630]; Figure 3.10).

For adrenal weight, between-animal variance was 1.122 g^2 and residual variance was 1.240 g^2 , giving a total variance of 2.362 g^2 (Table 3.3). Thus, 47.5% of total variance was attributable to individual identity (ICC = 0.48), indicating moderate within-individual similarity between left and right glands. For adrenal length, between-animal variance (23.71 mm^2) greatly exceeded residual variance (10.10 mm^2), giving a total variance of 33.81 mm^2 (Table 3.4). Therefore, 70% of total variance was explained by individual identity (ICC = 0.70), suggesting adrenal length was more consistent within individuals than adrenal weight.

Table 3.1 Linear mixed-effects model predicting adrenal weight with life history interactions

Model: Adrenal_Weight_g ~ Sex + GLG_Age + Sexual_Maturity + Side + (1 | AnimalID)

Predictor	β (Estimate)	SE	df	t	p	95% CI
Intercept	1.38	0.26	128.91	5.27	<0.001	0.87 to 1.90
Sex (Male)	0.06	0.28	103.28	0.22	0.828	-0.49 to 0.61
Age (GLG)	0.20	0.04	108.23	5.73	<0.001	0.13 to 0.27
Sexual maturity (Mature)	-0.17	0.51	101.96	-0.32	0.747	-1.17 to 0.84
Side (Right)	-0.42	0.18	88.31	-2.29	0.024	-0.78 to -0.06

Table 3.2 Linear mixed-effects model predicting adrenal length with life history interactions

Model: Adrenal_Length_mm ~ Sex + GLG_Age + Sexual_Maturity + Side + (1 | AnimalID)

Predictor	β (Estimate)	SE	df	t	p	95% CI
Intercept	28.93	1.01	112.17	28.69	<0.001	26.95 to 30.90
Sex (Male)	-2.01	1.11	94.82	-1.81	0.074	-4.19 to 0.17
Age (GLG)	0.80	0.14	97.82	5.70	<0.001	0.52 to 1.08
Sexual maturity (Mature)	0.64	2.04	93.92	0.31	0.756	-3.36 to 4.63
Side (Right)	1.10	0.53	72.90	2.08	0.041	0.06 to 2.14

Table 3.3 Variance components for individual ID random effects in linear mixed-effects models of adrenal weight

Random effect	Variance	SD
Individual ID (random intercept)	1.122 g ²	1.059 g
Residual	1.240 g ²	1.114 g

Table 3.4 Variance components for individual ID random effects in linear mixed-effects models of adrenal length

Random effect	Variance	SD
Individual ID (random intercept)	23.71 mm ²	4.87 mm
Residual	10.10 mm ²	3.18 mm

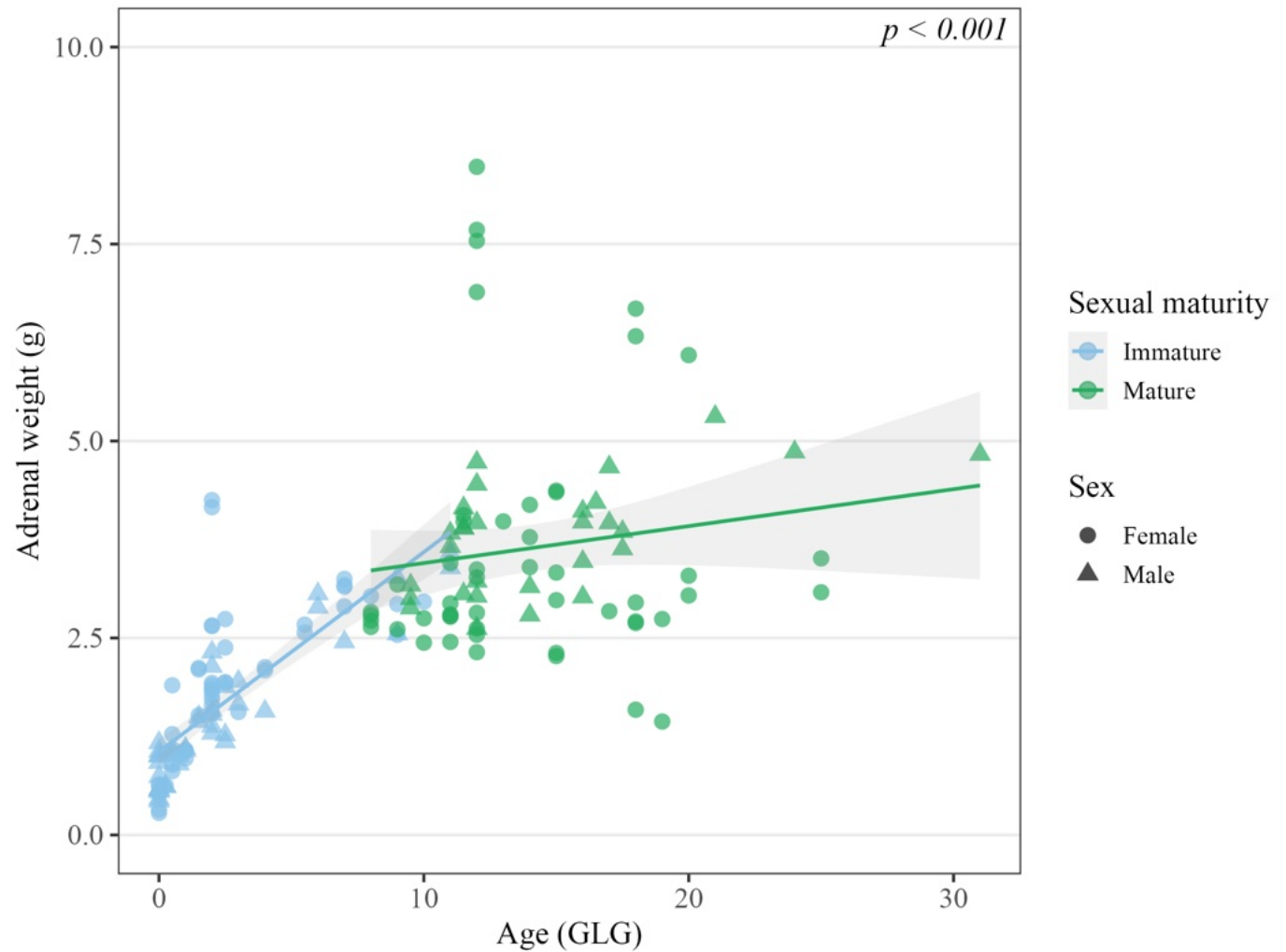


Figure 3.7 Adrenal gland weight (g) against age. Points represent individual glands, with shape indicating sex (circles, females; triangles, males) and colour indicating sexual maturity (light blue, immature; green, mature). Solid lines show fitted linear trends with 95% confidence intervals. Adrenal weight increased significantly with age at approximately 0.20 g per year. One individual (KS15-16Dd) was excluded for visual clarity; statistical models were conducted on the full sample. p -value is shown in the upper right corner of each panel.

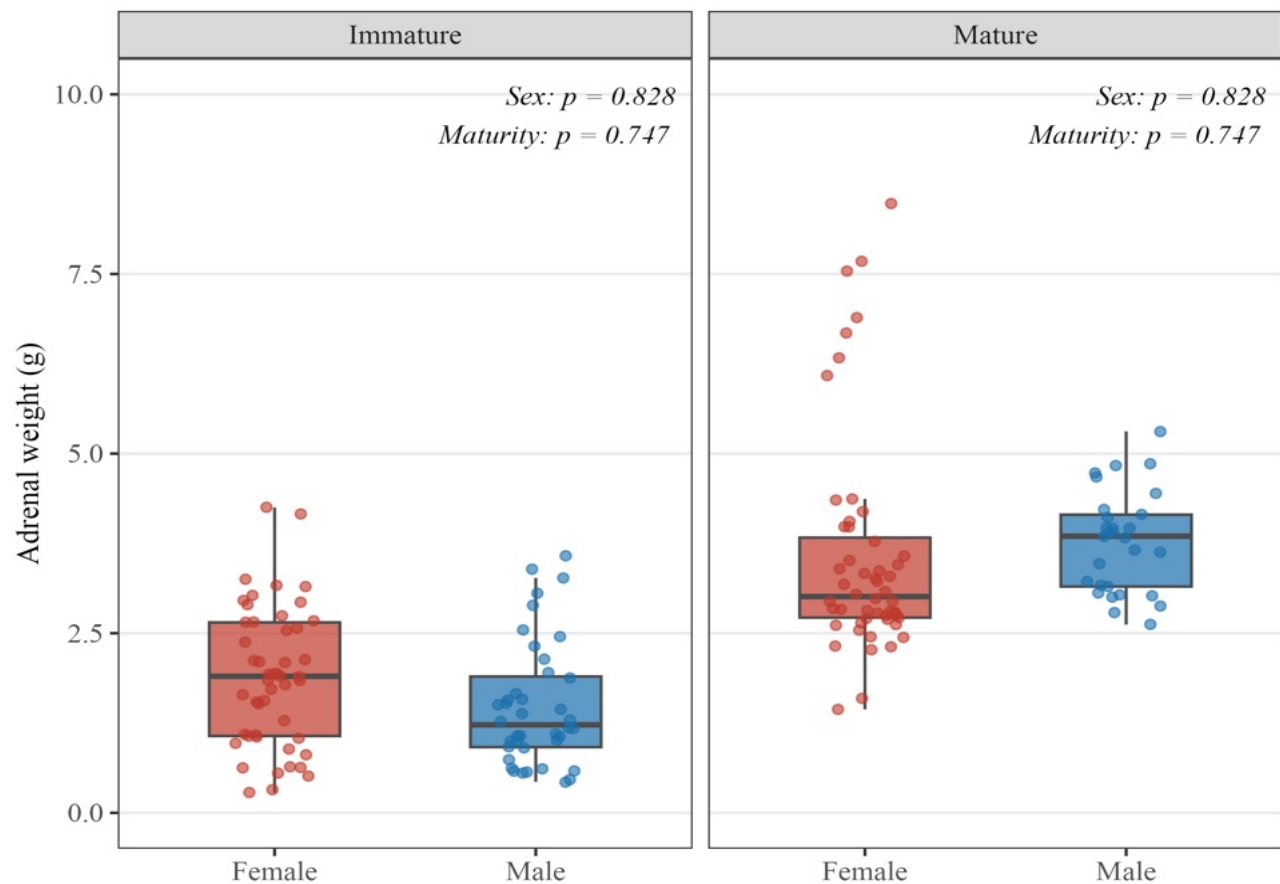


Figure 3.8 Distribution of adrenal gland weight (g) by sex and sexual maturity. Boxplots show median and interquartile range (IQR); whiskers extend to $1.5 \times \text{IQR}$; points represent individual glands. Panels separate immature and mature individuals; red, female; blue, male. Neither sex nor sexual maturity were significant predictors of adrenal weight in mixed-effects models after controlling for age. One individual (KS15-16Dd) was excluded for visual clarity; statistical models were conducted on the full sample. p -values are shown in the upper right corner of each panel.

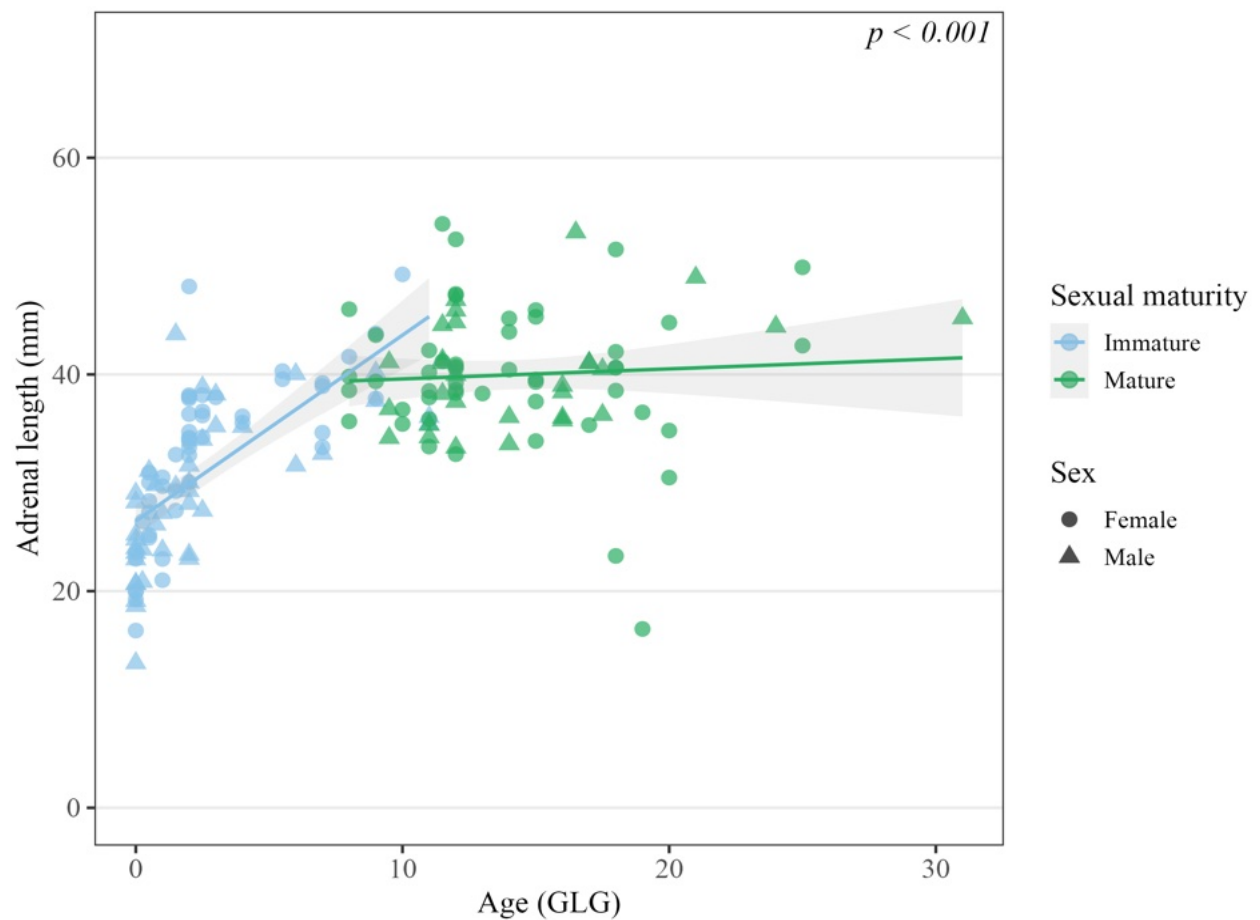


Figure 3.9 Adrenal gland length (mm) against age estimated. Points represent individual glands, with shape indicating sex (circles, females; triangles, males) and colour indicating sexual maturity (light blue, immature; green, mature). Solid lines show fitted linear trends with 95% confidence intervals. Adrenal length increased significantly with age at approximately 0.80 mm per year. One individual (KS15-16Dd) was excluded for visual clarity; statistical models were conducted on the full sample. p -value is shown in the upper right corner of each panel.

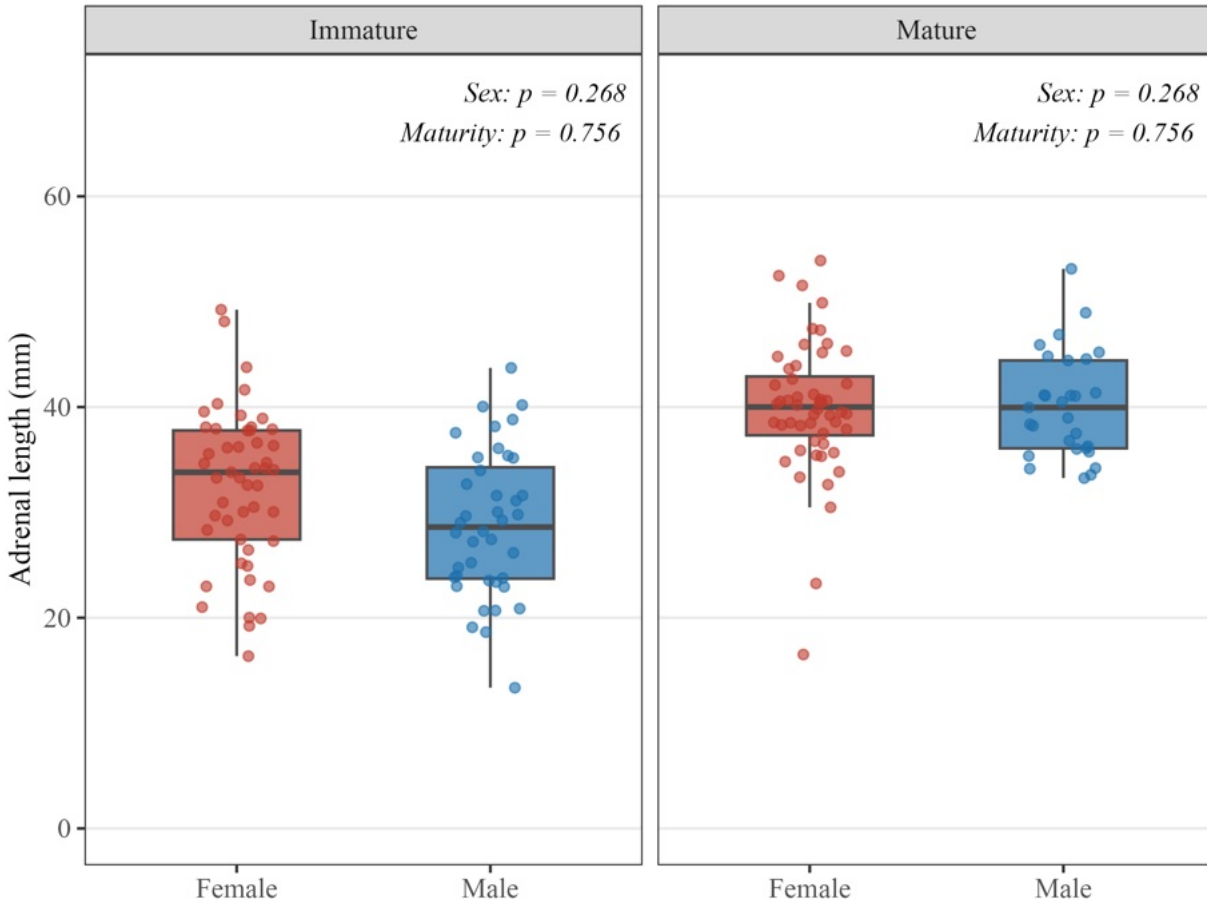


Figure 3.10 Distribution of adrenal gland length (mm) by sex and sexual maturity. Boxplots show median and interquartile range (IQR); whiskers extend to $1.5 \times \text{IQR}$; points represent individual glands. Panels separate immature and mature individuals red, female; blue, male. Neither sexual maturity nor sex were significant predictors of adrenal length in mixed-effects models after controlling for age, though sex showed a marginal non-significant trend toward shorter glands in males. One individual (KS15-16Dd) was excluded for visual clarity; statistical models were conducted on the full sample. *p*-values are shown in the upper right corner of each panel.

3.4 Prevalence of adrenal gland pathology

Of 176 adrenal glands screened for pathology, 15.3% ($n = 27$) displayed marked autolysis. These glands were excluded from analysis, resulting in a sample size of 149 adrenal glands for pathological assessment. Pathology was identified in 37 of 149 adrenal glands, representing 24.8% of assessed samples. More than one distinct pathological finding was noted in 8.7% of the adrenals ($n = 13$), ranging from mild structural alterations to more pronounced lesions, affecting

cortical and/or medullary regions (Table 2.4). Vascular congestion was the most frequently observed pathological feature, occurring in 17.5% ($n = 26$) of adrenals pathologically assessed. Other observed pathologies included focal and multifocal mineralisation (6.0%, $n = 9$), cortical and corticomedullary junction fibrosis (2.7%, $n = 4$), inflammatory infiltrates (2.7%, $n = 4$), vascular dilation (2.0%, $n = 3$), necrotising cortical adrenalitis (1.3%, $n = 2$), putative lipid infiltration (1.3%, $n = 2$; pending IHC staining), lesions suggestive of toxoplasmosis (1.3%, $n = 2$), and neoplastic/ hyperplastic changes (1.3%, $n = 2$). Cortical cysts were not identified in any screened individual. Lesions suggestive of toxoplasmosis, neoplastic/hyperplastic changes are currently undergoing further sectioning and immunohistochemistry (IHC). Putative lipid infiltration is similarly pending IHC staining for confirmation.

Pathological findings were more commonly observed in older individuals, though this difference was not statistically significant (Mann–Whitney $U = 533.5$, $p = 0.065$; Figure 3.11A). The median age of animals with pathological findings was 11.5 years ($n = 20$), compared to 7.0 years ($n = 55$) without. Age was a significant predictor of pathology presence (likelihood ratio test: $\chi^2 = 3.85$, $p = 0.050$), with pathology probability increasing by approximately 7% per additional year of age ($OR = 1.07$, 95% CI: 1.00-1.14; Figure 3.12), suggesting a modest but consistent age-associated increase in pathology risk. However, confidence intervals were wide at older ages, reflecting smaller sample sizes in older age classes, and the lower bound of the odds ratio confidence interval approached 1.00, indicating that the effect, while statistically significant, should be interpreted with caution.

Pathology prevalence did not differ significantly between males and females ($\chi^2(1) = 1.29$, $p = 0.257$; Figure 3.11B), though males exhibited a numerically higher prevalence than females (34.3%, 12/35 vs. 20.0%, 8/40, respectively). In contrast, pathology prevalence was significantly higher in sexually mature than immature individuals ($\chi^2(1) = 4.15$, $p = 0.042$; Figure 3.11C), with mature animals showing approximately 3.4 times higher odds of adrenal pathology than immature animals (38.9%, 14/36 vs. 15.4%, 6/39).

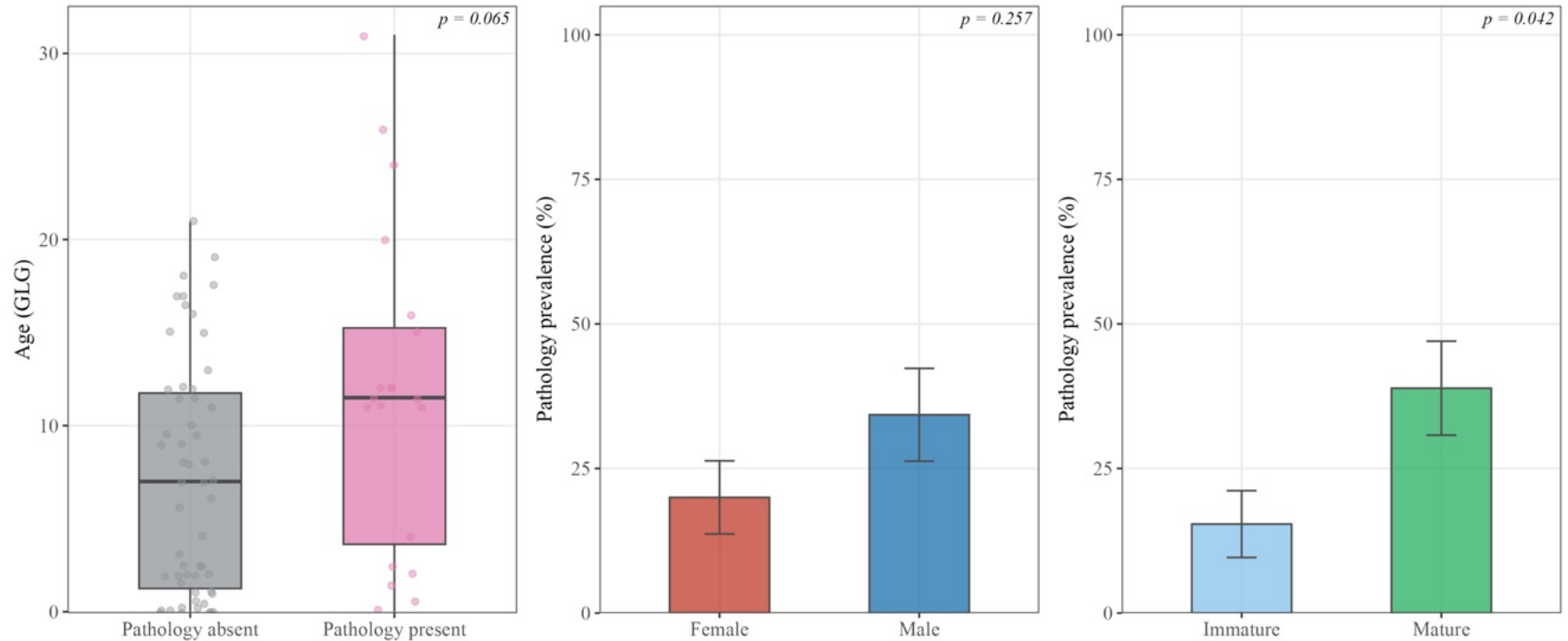


Figure 3.11 Associations between adrenal gland pathology and life history variables. (A) Age of individuals with and without adrenal pathology (absent, grey; present, pink). Boxes represent interquartile ranges with median lines; individual data points are shown. (B) Pathology prevalence (%) by sex (female, red; male, blue). Bars represent the proportion of individuals with pathological findings within each sex; error bars indicate standard errors. (C) Pathology prevalence (%) by sexual maturity class (immature, blue; mature, green). Bars represent the proportion of individuals with pathological findings within each maturity category; error bars indicate standard errors of the mean. p-values are shown in the upper right corner of each panel.

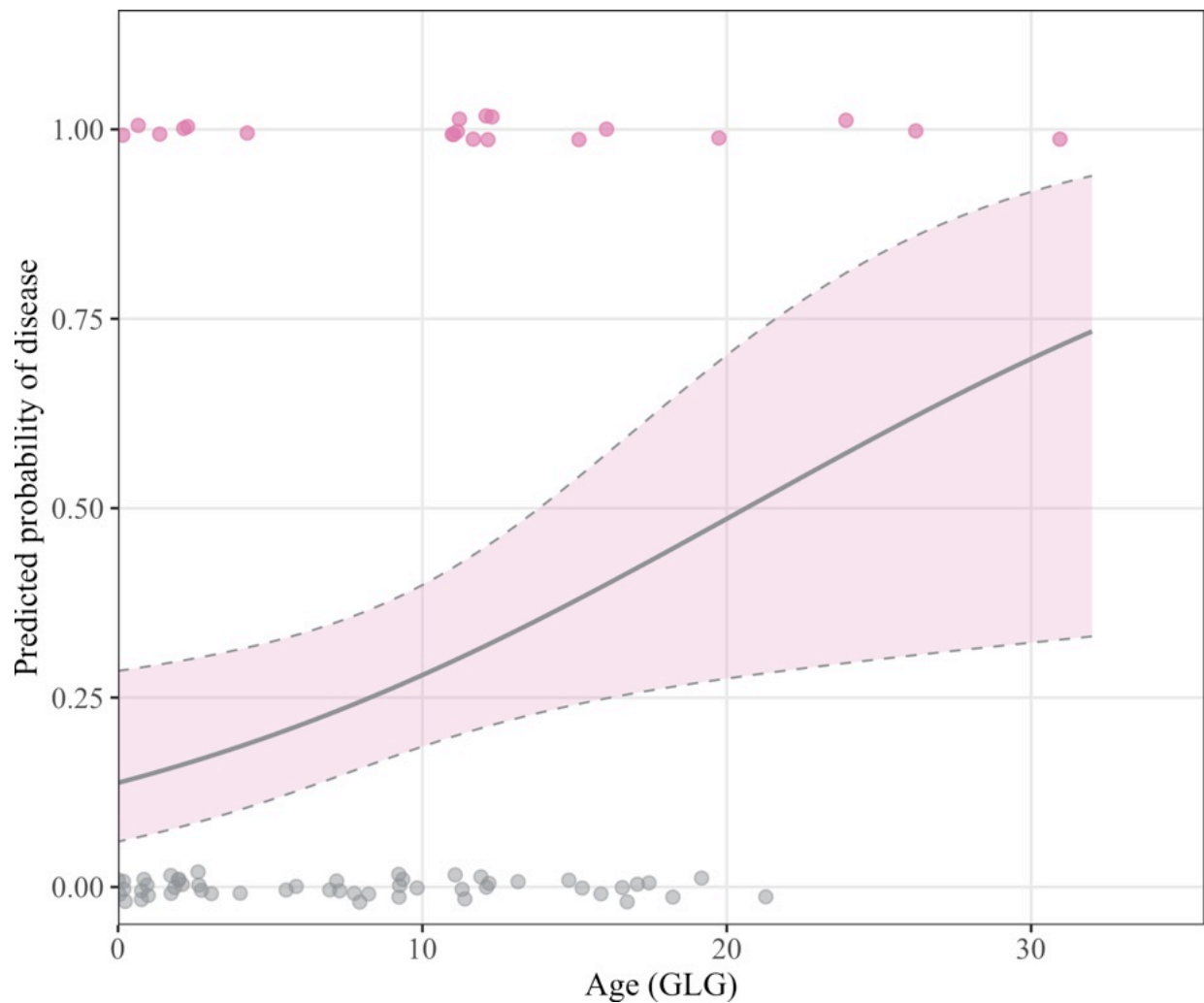


Figure 3.12 Predicted probability of adrenal gland pathology as a function of age. Predicted probabilities were estimated using a simple logistic regression model at the animal level (one observation per individual, $n = 75$) with age as a continuous predictor. The solid line represents the fitted logistic regression curve and the shaded area with dashed boundaries indicates the 95% confidence interval. Pink points represent individuals with pathology present ($n = 20$) and grey points represent individuals with pathology absent ($n = 55$).

Pathological screening of the left adrenal gland from KS15-16Dd, a 26-year-old sexually mature male, identified a neoplastic lesion of the adrenal medulla (Figure 3.2; Figure 3.13). The pathologist's report described a partially encapsulated neoplastic proliferation that was suggestive of a pheochromocytoma. On histologic examination, neoplastic chromaffin cells were arranged in a characteristic nested pattern, separated by thin fibrovascular stroma. Individual cells were polygonal to oval with moderate to abundant pale eosinophilic cytoplasm and indistinct cytoplasmic borders. Nuclei were round to oval and exhibited mild to moderate pleomorphism, with variation in both nuclear size and staining intensity between cells. Occasional cells contained enlarged, hyperchromatic nuclei. Sinusoidal vascular spaces were prominent and irregular, separating chromaffin cell nests of varying size. A significant proliferation of small-calibre blood vessels was present within the lesion. Multifocal small accumulations of lymphocytes were also noted and considered likely incidental. No evidence of local invasion or distant metastasis was identified in available tissue sections. At the periphery of the lesion, residual cortical cells were markedly attenuated, with severe compression of the surrounding cortex.

Both the right and left glands were classified as D1, with mild multifocal cortical mineralisation identified bilaterally. Cross-sectional area and corticomedullary ratio measurements were derived from a standard mid-transverse section not targeted at the region of peak pathology, and therefore, do not fully capture the extent of neoplastic expansion; gland weight (20.95 g) is considered the more representative measure of overall enlargement. Histopathological examination of other organs identified mild myocardial lipofuscinosis and testicular atrophy.

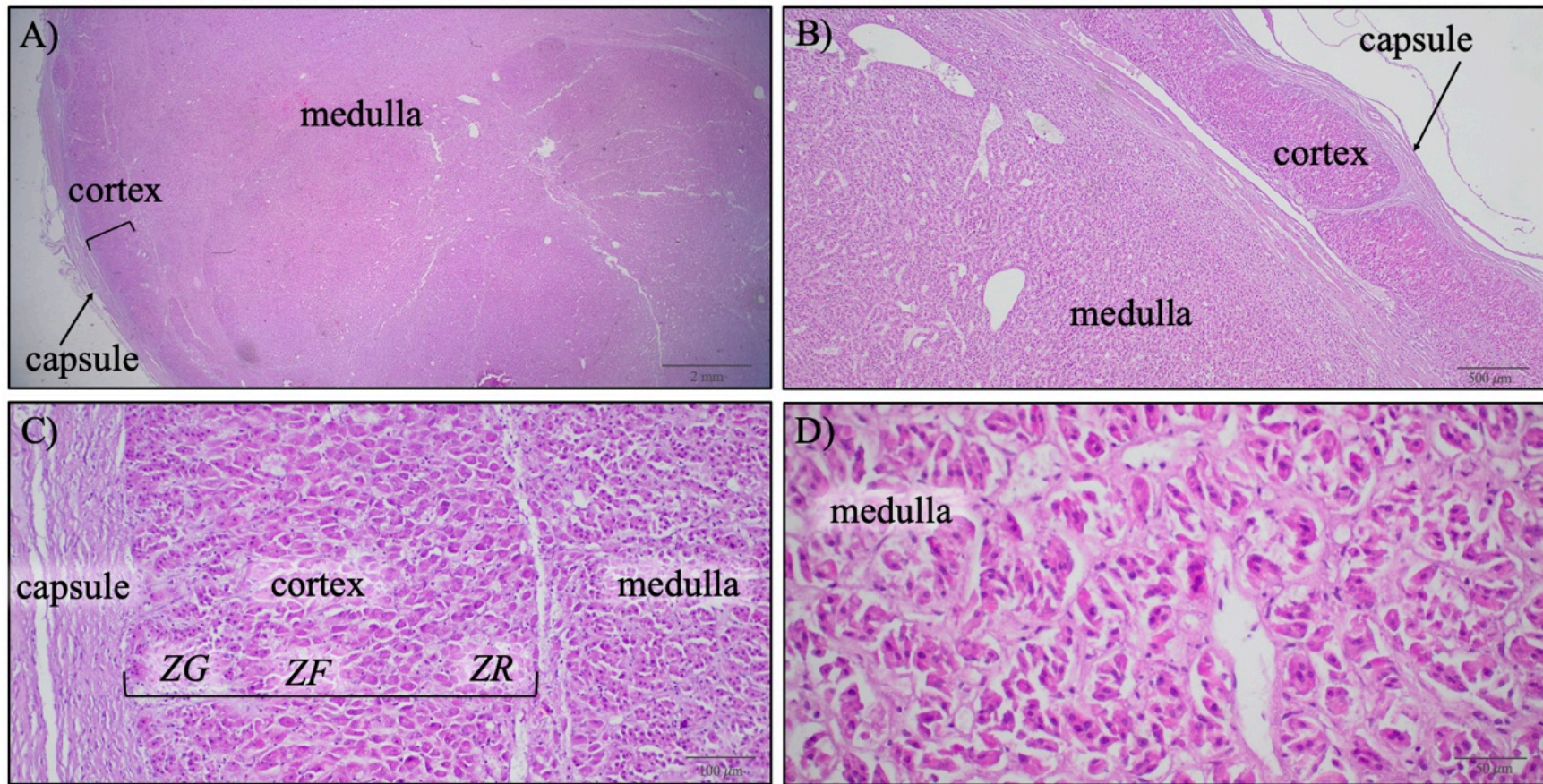


Figure 3.13 Histological sections of the left adrenal gland from KS15-16Dd L demonstrating pheochromocytoma. Stain, H&E for all panels. (A) Marked medullary expansion occupying the majority of the gland cross-section, with the adrenal cortex reduced to a thin peripheral rim. Scale, 2 mm. (B) Corticomedullary junction, with prominent dilated sinusoidal vascular spaces within the neoplastic medullary tissue and compressed residual cortical tissue at the periphery. Scale, 500 μ m. (C) Compressed cortical rim, with residual zonation identifiable: *zona glomerulosa* (ZG), *zona fasciculata* (ZF), and *zona reticularis* (ZR). Scale, 100 μ m. (D) Neoplastic medullary tissue, showing pleomorphic chromaffin cells arranged in irregular nests separated by prominent fibrovascular stroma and dilated sinusoidal spaces. Scale, 50 μ m.

3.5 Adrenal gland composition

3.5.1 Sample summary

A total of 113 adrenal glands from 75 individual dolphins were included in morphometric analysis, comprising 60 right and 53 left glands. Of these, 50.7% ($n = 38$) animals had both right and left glands measured, while 49.3% ($n = 37$) had only one side available. The sample included 40 females (mature: $n = 18$; immature: $n = 22$) and 35 males (mature: $n = 17$; immature: $n = 18$). Age ranged from 0 to 31 (mean = 8.31, $SD = 7.25$, median = 8.0).

Within the 113 glands, most cases were classified as acute-on-chronic stress (46.7%; $n = 35$), followed by 25.3% acute ($n = 19$), 14.7% indeterminate ($n = 11$), and 13.3% chronic ($n = 10$). Most glands were classified as D0: non-diseased (90.3%; $n = 102$), with 8.8% classified as D1: disease present, but unlikely significant ($n = 10$), and a single case marked as D2: disease likely to have had a clinical effect case (0.8%; $n = 1$). No glands were classified as D3: disease caused or contributed to mortality or UK: unknown, in the final dataset. Pathological findings, indicative of disease or incidental, were absent in 72.6% of glands ($n = 82$) and present in 27.4% of glands ($n = 31$).

3.5.2 Morphometry

Cross-sectional area of the adrenal glands ranged from 2.09×10^7 to $3.38 \times 10^8 \mu\text{m}^2$ (mean = 9.85×10^7 , $SD = 4.51 \times 10^7$). Cortex area comprised the largest tissue compartment (mean = 5.64×10^7 , $SD = 2.67 \times 10^7 \mu\text{m}^2$), followed by medulla (mean = 2.85×10^7 , $SD = 1.55 \times 10^7 \mu\text{m}^2$) and capsule (mean = 1.37×10^7 , $SD = 7.40 \times 10^6 \mu\text{m}^2$).

3.5.3 Composition and cortex:medulla ratio

On average, the cortex comprised $57.5 \pm 0.7\%$ ($SD = 7.1$) of total tissue area, followed by the medulla at $28.9 \pm 0.6\%$ ($SD = 6.8$) and the capsule at $13.6 \pm 0.4\%$ ($SD = 4.0$) (Figure 3.14). The C:M ratio ranged from 0.62 to 4.56, with a mean of 2.15 ($SD = 0.74$, median = 2.05), indicating that on average the cortex was approximately twice the area of the medulla.

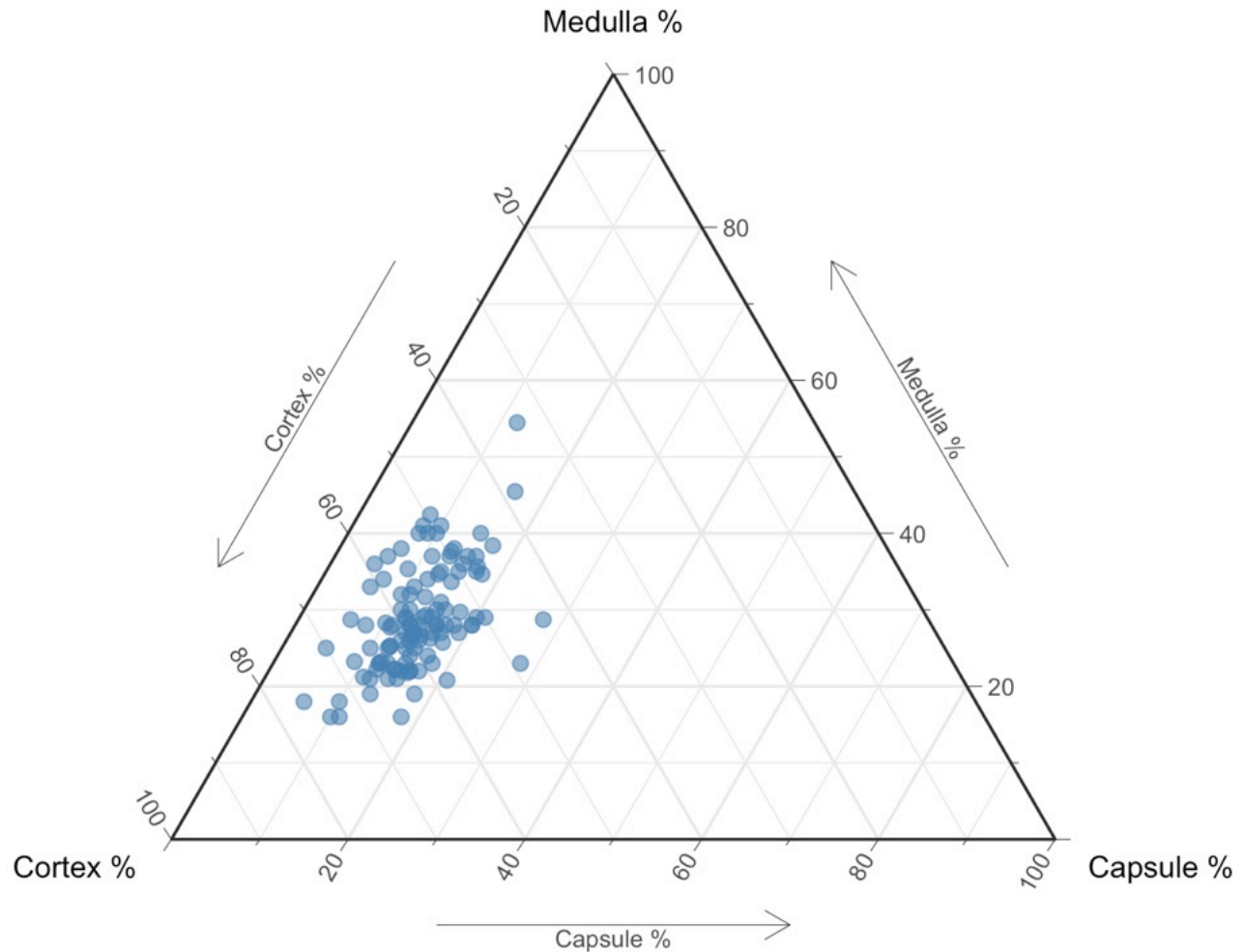


Figure 3.14 Adrenal gland composition. Each point represents an individual gland ($n = 113$), plotted according to the relative proportions of cortex, medulla, and capsule tissue.

3.5.4 Bilateral comparisons

Having established bilateral asymmetry at the gross level (Section 3.3.1), paired comparisons were conducted to determine whether this asymmetry extended to zonal proportions at the histological level. No significant differences were found between right and left glands for C:M ratio (right mean = 2.11 ± 0.11 , left mean = 2.18 ± 0.12 ; Wilcoxon signed-rank $V = 346$, $p = 0.728$; Figure 3.15A), capsule percentage ($p = 0.671$; Figure 3.15B), cortex percentage ($p = 0.591$; Figure 3.15C), or medulla percentage ($p = 0.911$; Figure 3.15D). Paired Wilcoxon signed-rank tests on the absolute areas yielded significant differences for cross-sectional area ($V = 204$, $p = 0.016$) and cortex area ($V = 216.5$, $p = 0.026$), with left glands tending to be slightly larger, though these differences were small relative to overall variability and the corresponding paired t-

tests were non-significant ($p = 0.291$ and $p = 0.095$, respectively). Shapiro-Wilk tests on the paired differences for cross-sectional area ($W = 0.762$, $p < 0.001$) and medulla area ($W = 0.656$, $p < 0.001$) indicated non-normal distributions, suggesting the Wilcoxon results are more appropriate for these variables. No significant bilateral differences were found for the capsule area ($V = 311.5$, $p = 0.396$) or medulla area ($V = 274.5$, $p = 0.166$). Overall, the proportional composition of right and left glands was highly consistent within individuals, indicating that either side may be used in subsequent analyses.

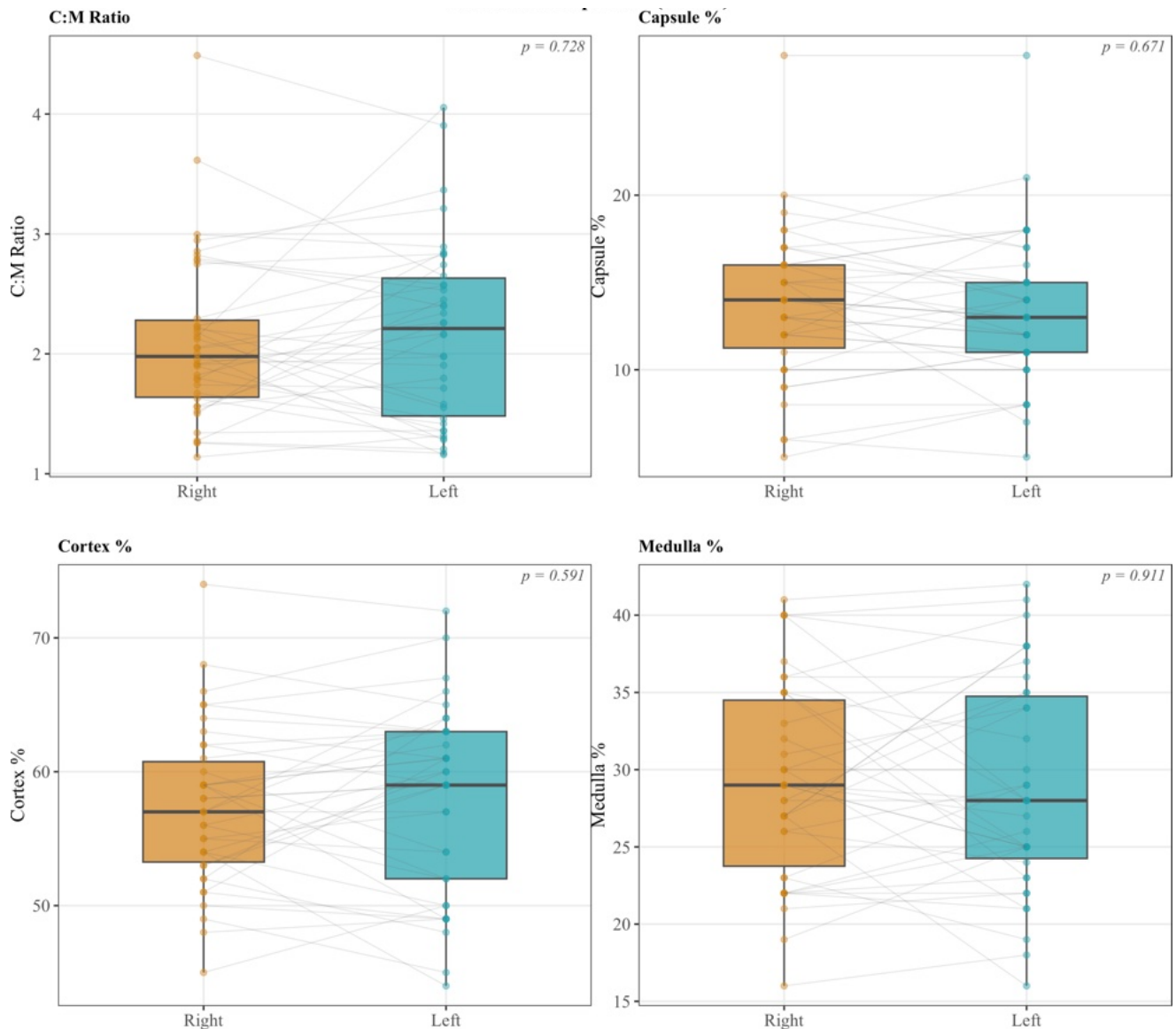


Figure 3.15 Bilateral comparison of right and left adrenal glands. Boxplots with individual data points and paired lines (grey) connecting right (tan) and left (teal) glands from the same animal ($n = 38$ pairs) for (A) C:M ratio, (B) capsule percent, (C) cortex percent, and (D) medulla percent. p -values are shown in the upper right corner of each panel.

3.5.5 Group comparisons

3.5.5.1 Effect of age on zonal proportions

Given that age was the strongest predictor of gross adrenal size (Section 3.3.4), Spearman correlations were calculated to assess whether age-related changes also extended to the proportional composition of cortical and medullary tissue. Spearman correlations between age and morphometric variables revealed that all absolute area measurements were strongly and positively correlated with age (cross-section: $\rho = 0.77$; capsule: $\rho = 0.71$; cortex: $\rho = 0.72$; medulla: $\rho = 0.71$; all $p < 0.001$), reflecting the expected increase in gland size with body growth. In contrast, proportional composition was largely unrelated to age, with no significant correlations between age and the C:M ratio ($\rho = -0.06$, $p = 0.556$; Figure 3.16A), cortex percent ($\rho = -0.13$, $p = 0.157$; Figure 3.16C), and medulla percent ($\rho = -0.01$, $p = 0.903$; Figure 3.16D). The one exception was capsule percent, which showed a modest positive association with age ($\rho = 0.30$, $p = 0.002$; Figure 3.16B), suggesting proportionally greater capsular tissue in older animals. This finding was consistent with the maturity comparison described above.

3.5.5.2 Effect of sex on zonal proportions

Although sex was not a significant predictor of gross adrenal weight or length (Section 3.3.4), sex steroid production within the adrenal cortex may influence zonal proportions, independently of overall gland size. To test for sexual dimorphism in adrenal composition, C:M ratio and zonal percentages were compared between female ($n = 62$) and male ($n = 51$) glands. Females had a higher mean C:M ratio than males (female: 2.32 ± 0.10 , $SD = 0.81$; male: 1.95 ± 0.09 , $SD = 0.61$; Wilcoxon $W = 2016$, $p = 0.012$; Figure 3.17A), driven primarily by higher cortex percent in females ($59.0 \pm 1.0\%$ versus $55.8 \pm 0.9\%$; $p = 0.016$; Figure 3.17C) and lower medulla percent ($27.5 \pm 0.8\%$ vs $30.5 \pm 1.0\%$; $p = 0.015$; Figure 3.17D). Capsule percent did not differ between sexes ($p = 0.700$; Figure 3.17B).

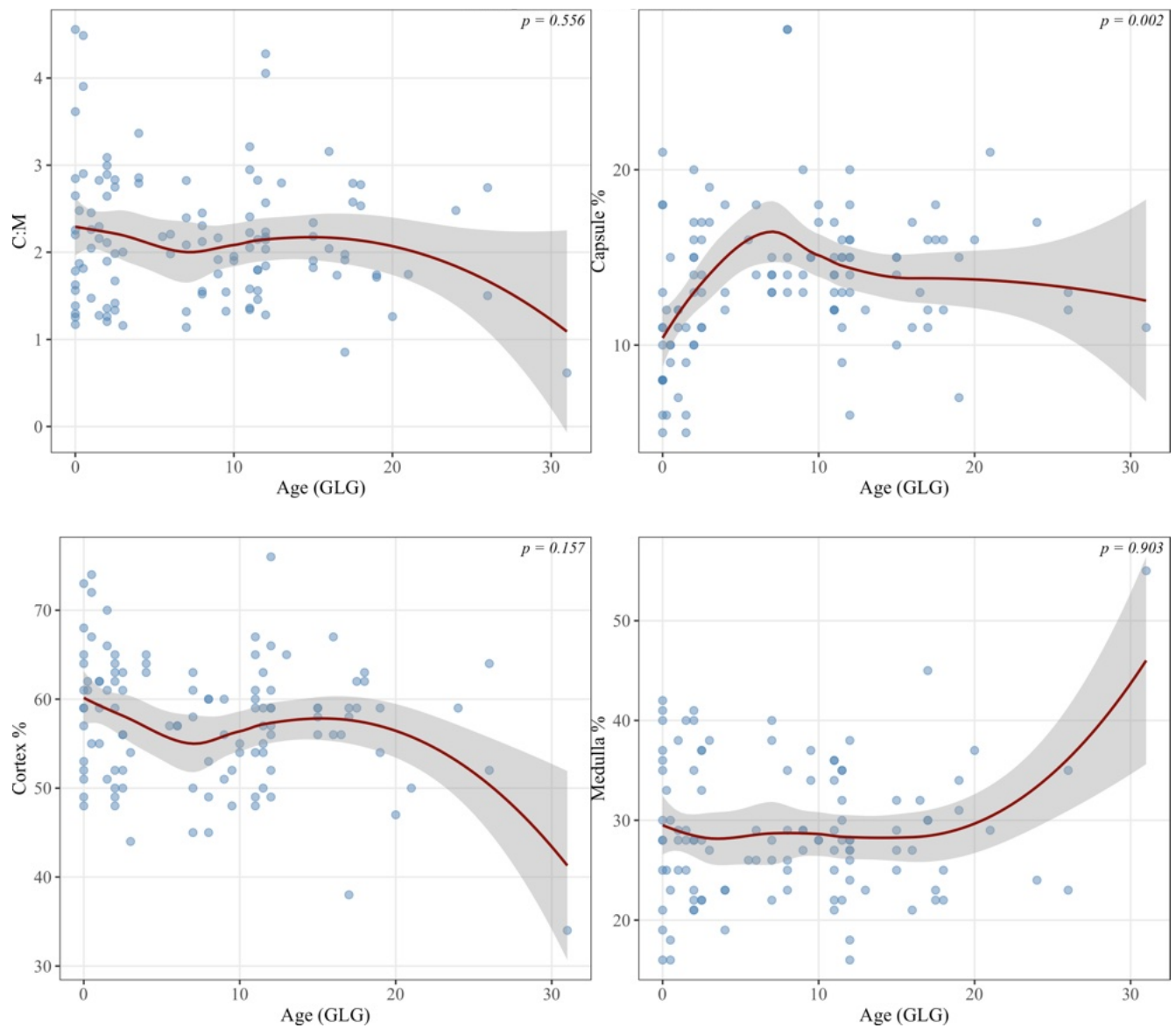


Figure 3.16 Relationship between age (GLG) and adrenal gland composition. Scatter plots with LOESS smoothing curves (brown line) and 95% confidence intervals (shaded area) showing (A) C:M ratio, (B) capsule percent, (C) cortex percent, and (D) medulla percent as a function of age. p -values are shown in the upper right corner of each panel.

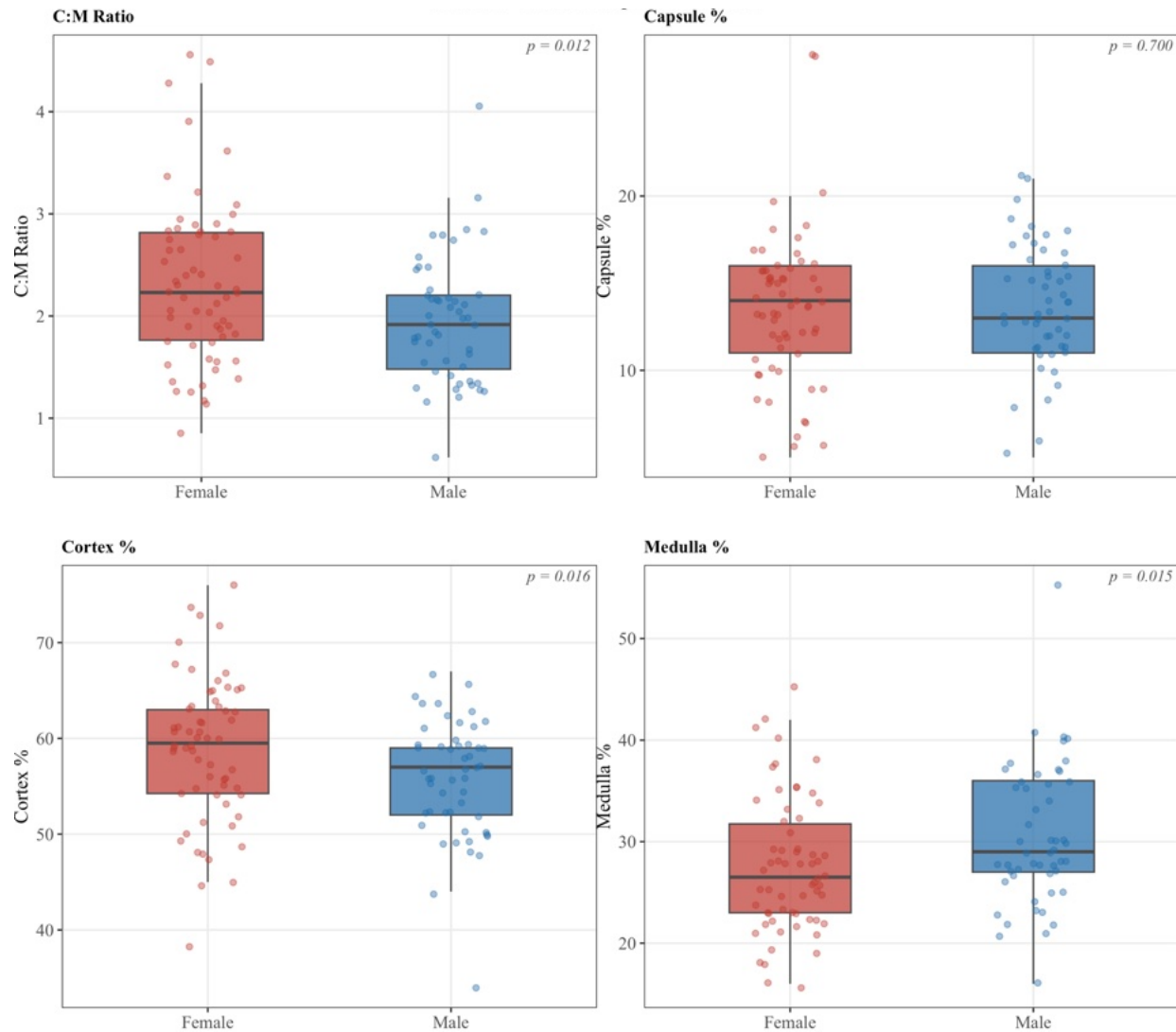


Figure 3.17 Adrenal gland composition by sex. Boxplots with individual data points showing (A) C:M ratio, (B) capsule percent, (C) cortex percent, and (D) medulla percent for female ($n = 62$; red) and male ($n = 51$; blue) glands. p -values are shown in the upper right corner of each panel.

3.5.5.3 Effect of sexual maturity on zonal proportions

Immature and mature animals did not differ significantly in C:M ratio (immature mean = 2.21 ± 0.10 , mature mean = 2.09 ± 0.09 ; $W = 1713.5$, $p = 0.490$; Figure 3.18A), cortex percent ($p = 0.147$; Figure 3.18C), or medulla percent ($p = 0.956$; Figure 3.18D). However, mature animals had significantly higher capsule percent than immature animals ($14.6 \pm 0.5\%$ versus $12.7 \pm 0.5\%$; $W = 1174.5$, $p = 0.016$; Figure 3.18B), consistent with the positive age-capsule correlation described above and suggesting that capsular thickening accompanied maturation.

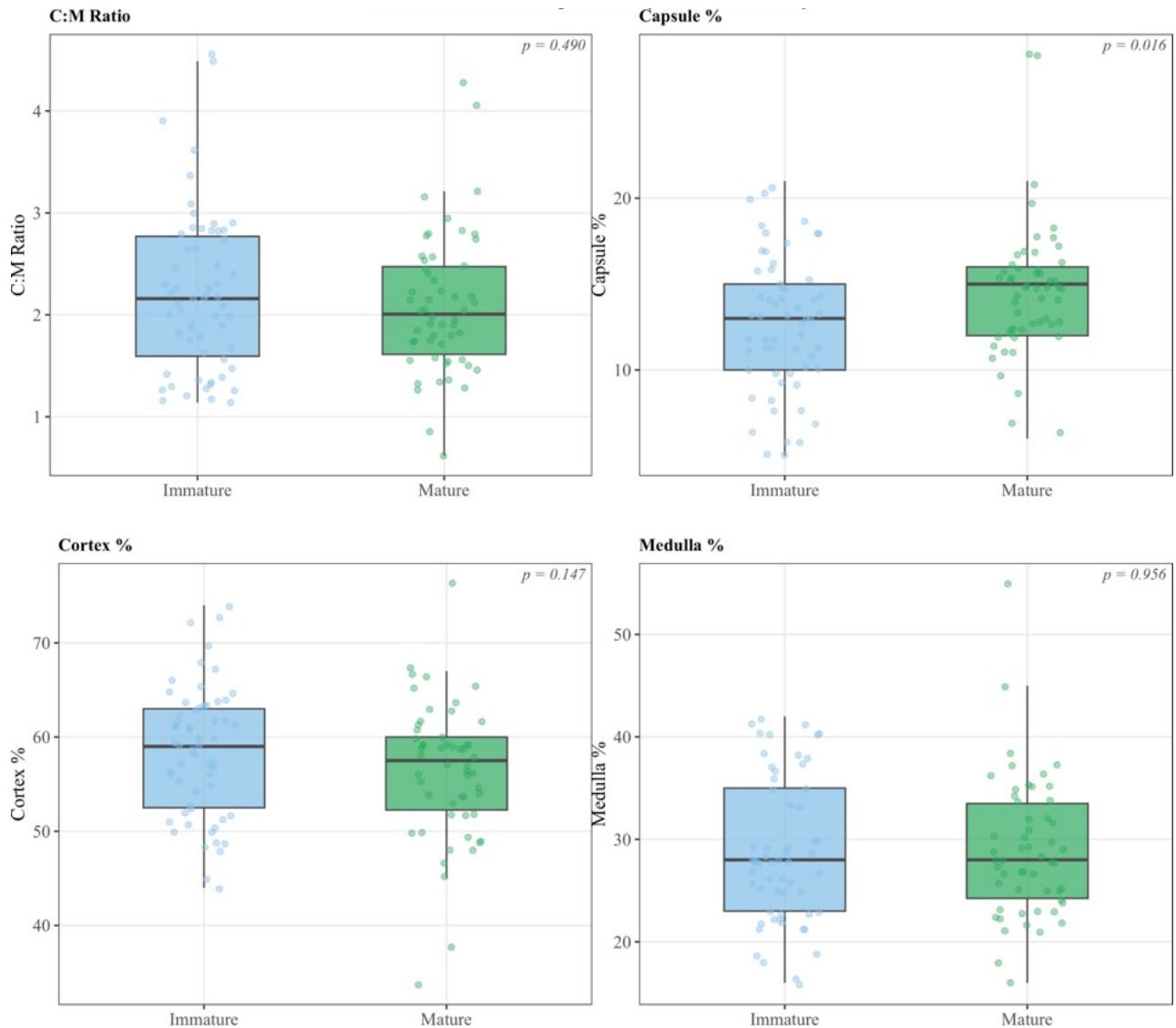


Figure 3.18 Adrenal gland composition by sexual maturity. Boxplots with individual data points showing (A) C:M ratio, (B) capsule percent, (C) cortex percent, and (D) medulla percent for immature ($n = 59$; blue) and mature ($n = 54$; green) glands. p -values are shown in the upper right corner of each panel.

3.5.5.4 Effect of prominent medullary band on zonal proportions

Glands with a prominent medullary band ($n = 59$) had significantly lower C:M ratios than those without ($n = 54$; 2.02 ± 0.10 versus 2.30 ± 0.10 ; $W = 1949$, $p = 0.041$; Figure 3.19A). This was reflected in significantly higher medulla proportion in glands with a prominent band ($30.5 \pm 0.9\%$ versus $27.1 \pm 0.8\%$; $W = 1163.5$, $p = 0.013$; Figure 3.19D). Cortex percent did not differ significantly ($p = 0.263$; Figure 3.19C), and capsule percent showed a trend toward lower values in glands with a prominent band ($12.8 \pm 0.5\%$ versus $14.5 \pm 0.6\%$; $p = 0.065$; Figure 3.19B).

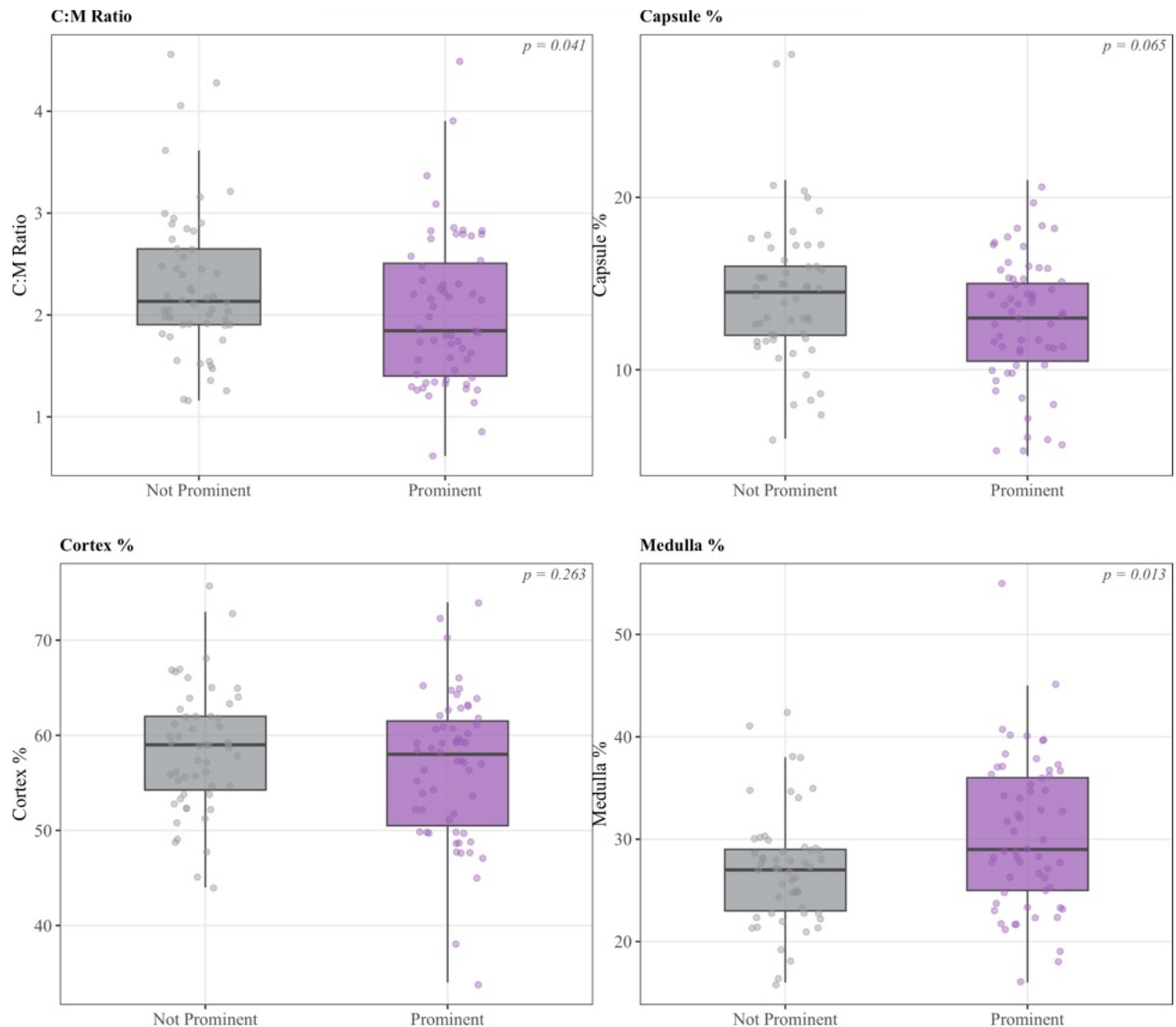


Figure 3.19 Adrenal gland composition by prominent medullary band. Boxplots with individual data points showing (A) C:M ratio, (B) capsule percent, (C) cortex percent, and (D) medulla percent for glands without ($n = 54$; grey) and with ($n = 59$; purple) a prominent medullary band. p -values are shown in the upper right corner of each panel.

3.5.5.5 Effect of pathology presence on zonal proportions

Given that pathology presence was associated with increased adrenal weight (Section 3.3.3), zonal proportions were compared between glands with and without histological pathology to determine whether this enlargement reflected disproportionate expansion of specific tissue compartments. To test this, zonal proportions were compared between glands without pathological findings (pathology absent; $n = 82$) and reported to have one or more pathological

features (present; $n = 31$). No significant differences were found for any compositional measure. C:M ratio (absent mean = 2.11 ± 0.07 , present mean = 2.26 ± 0.17 ; $W = 1198$, $p = 0.641$; Figure 3.20A), cortex percent ($p = 0.464$; Figure 3.20C), medulla percent ($p = 0.777$; Figure 3.20D), and capsule percent ($p = 0.099$; Figure 3.20B) were all non-significant.

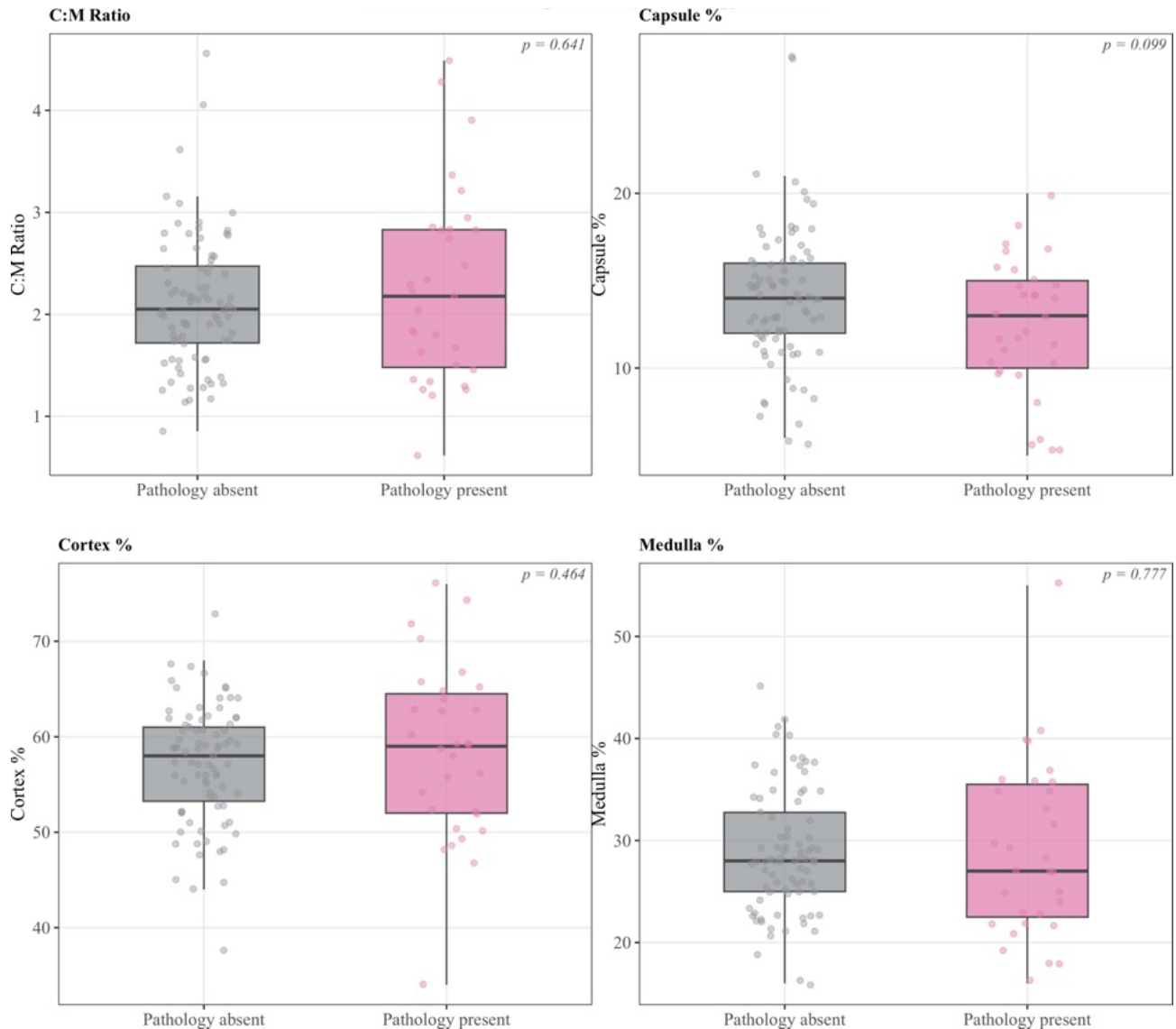


Figure 3.20 Adrenal gland composition by pathology presence. Boxplots with individual data points showing (A) C:M ratio, (B) capsule percent, (C) cortex percent, and (D) medulla percent for glands with pathology absent ($n = 82$; grey) and present ($n = 31$; pink). p -values are shown in the upper right corner of each panel.

3.5.5.6 Effect of disease classification on zonal proportions

Comparisons between D0 (non-diseased, $n = 102$) and D1 (disease present but unlikely significant, $n = 10$) glands revealed no significant differences in C:M ratio (D0 mean = 2.14 ± 0.07 , D1 mean = 2.05 ± 0.19 ; $W = 550$, $p = 0.687$; Figure 3.21A), capsule percent ($p = 0.502$; Figure 3.21B), cortex percent ($p = 0.635$; Figure 3.21C), or medulla percent ($p = 0.826$; Figure 3.21D). The single D2 (disease likely to have had a clinical effect) case was excluded from statistical analysis, but was a notable outlier with a C:M ratio of 4.28, cortex comprising 76% of tissue area, and capsule at 6%.

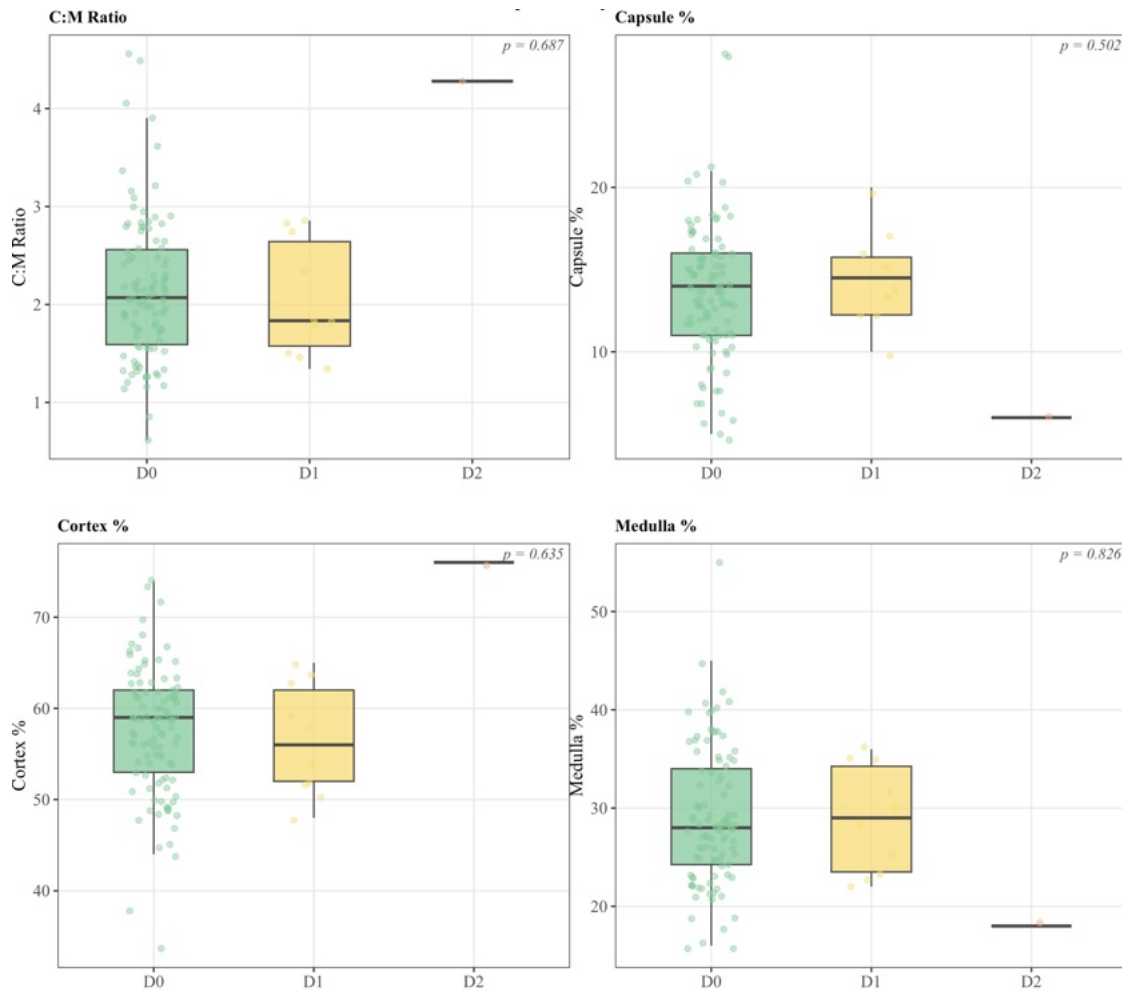


Figure 3.21 Adrenal gland composition by disease classification. Boxplots with individual data points showing (A) C:M ratio, (B) capsule percent, (C) cortex percent, and (D) medulla percent for D0 (non-diseased, $n = 102$; green), D1 (disease present, unlikely significant, $n = 10$; yellow), and D2 (disease likely to have had a clinical effect, $n = 1$) glands. The single D2 case is a notable outlier with a C:M ratio of 4.28 and cortex comprising 76% of tissue area. p -values are shown in the upper right corner of each panel.

3.5.5.7 Effect of stress type on zonal proportions

To assess whether the chronicity of the stress type was reflected in adrenal architecture, zonal proportions were compared among animals classified as acute ($n = 29$), chronic ($n = 17$), acute-on-chronic ($n = 51$), and indeterminate ($n = 16$). No significant differences among the four stress type categories (acute, chronic, acute-on-chronic, indeterminate) for C:M ratio ($\chi^2 = 1.65$, $df = 3$, $p = 0.639$; Figure 3.22A), cortex percent ($\chi^2 = 0.76$, $p = 0.859$; Figure 3.22C), or medulla percent ($\chi^2 = 2.43$, $p = 0.486$; Figure 3.22D). Capsule percent approached significance ($\chi^2 = 7.21$, $p = 0.067$; Figure 3.22B), with chronic stress cases showing the highest mean capsule percent ($15.3 \pm 0.7\%$) and acute cases the lowest ($12.2 \pm 0.8\%$). The lack of significant differences in cortical and medullary proportions across stress types suggested that stress chronicity, as classified post-mortem via histological cues, may not be sensitively reflected in zonal proportions, or that the effect is too subtle to detect at this sample size.

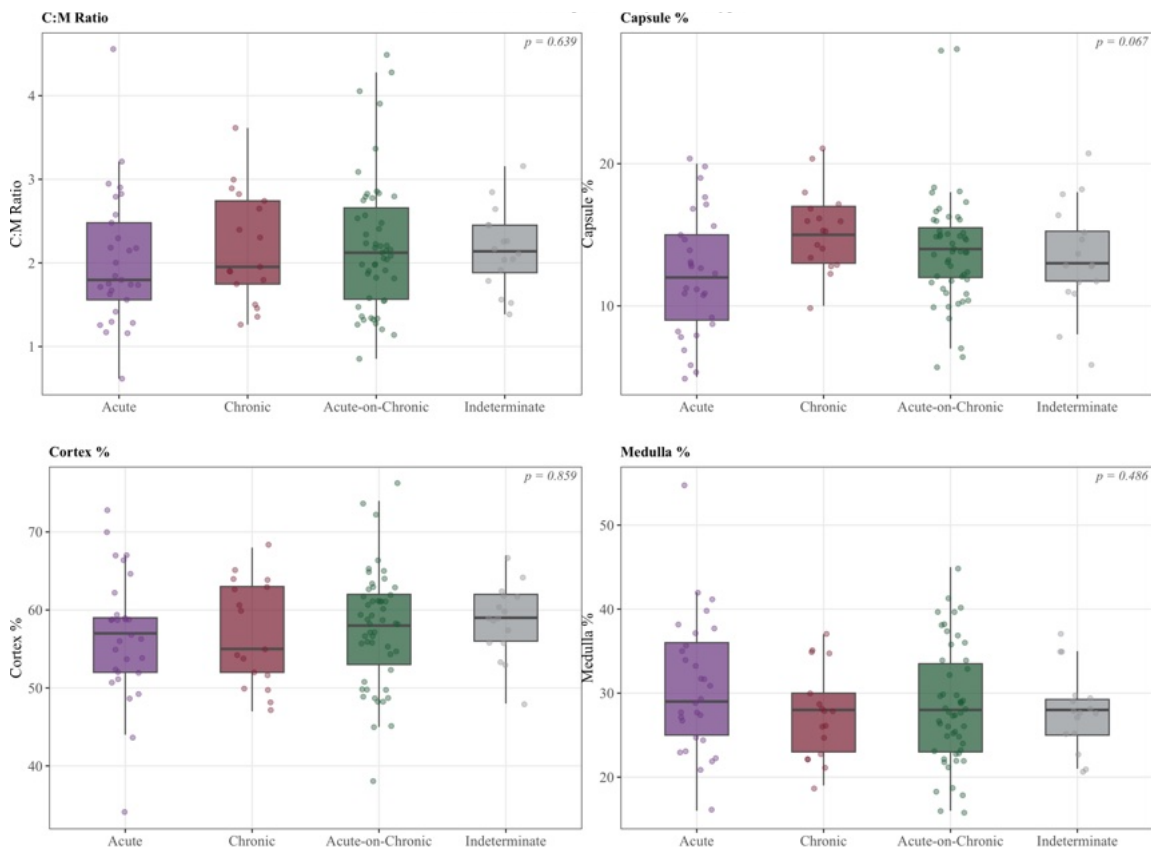


Figure 3.22 Adrenal gland composition by stress type. Boxplots with individual data points showing (A) C:M ratio, (B) capsule percent, (C) cortex percent, and (D) medulla percent across acute ($n = 29$; purple), chronic ($n = 17$; dark red), acute-on-chronic ($n = 51$; dark green), and indeterminate ($n = 16$; grey) stress categories. p -values are shown in the upper right corner of each panel.

3.5.6 Life history predictors of cortex:medulla ratio

A linear mixed-effects model of log(C:M ratio) identified sex as a marginal predictor, with age showing a weak non-significant negative trend (Table 3.5). Maturity status was initially considered as a predictor but was excluded due to high collinearity with age ($r = -0.83$ in the fixed-effects correlation matrix). A likelihood ratio test confirmed that its removal did not significantly reduce model fit ($\chi^2 = 0.74$, $df = 1$, $p = 0.391$; $\Delta AIC = -1.3$).

Substantial between-individual variation was detected. The intraclass correlation coefficient (*ICC*) was 0.650, indicating that 65.0% of the total variance in log(C:M ratio) was attributable to differences among individuals, with the remaining 35.0% reflecting within-individual variation between bilateral glands (Table 3.6).

Table 3.5 Linear mixed-effects model predicting log(C:M ratio) from life history predictors

Model: log(C:M) ~ Sex + GLG + (1 AnimalID)						
Predictor	β (Estimate)	<i>SE</i>	<i>df</i>	<i>t</i>	<i>p</i>	95% CI
Intercept	0.836	0.065	69.3	12.90	<0.001	0.709 to 0.963
Sex (Male)	-0.132	0.076	69.2	-1.73	0.088	-0.282 to 0.018
Age (GLG)	-0.008	0.005	71.6	-1.55	0.126	-0.019 to 0.002

Table 3.6 Variance components for individual ID random effects in the life history model of log(C:M ratio)

Random effect	Variance	<i>SD</i>
Individual ID (random intercept)	0.0768	0.277
Residual	0.0414	0.203

3.5.6.1 Effect of age on cortex:medulla ratio

Age was not a significant predictor of C:M ratio ($p = 0.126$), with a small negative trend of -0.008 log-units per year (Table 3.5). The 95% confidence interval for the age slope included zero (-0.019 to 0.002), indicating that any age-related shift in zonal proportions was modest relative to individual variation.

3.5.6.2 Effect of sex on cortex:medulla ratio

Sex showed a marginal effect on log(C:M ratio) ($p = 0.088$; Table 3.5; Figure 3.23), with males estimated to have approximately 12% lower C:M ratios than females (back-transformed from the log-scale difference of -0.132). The estimated marginal mean log(C:M) was 0.769 (95% CI: 0.666–0.872) for females and 0.637 (95% CI: 0.526–0.749) for males. This trend suggested that females may have relatively more cortical tissue, or less medullary tissue, though the effect did not reach significance.

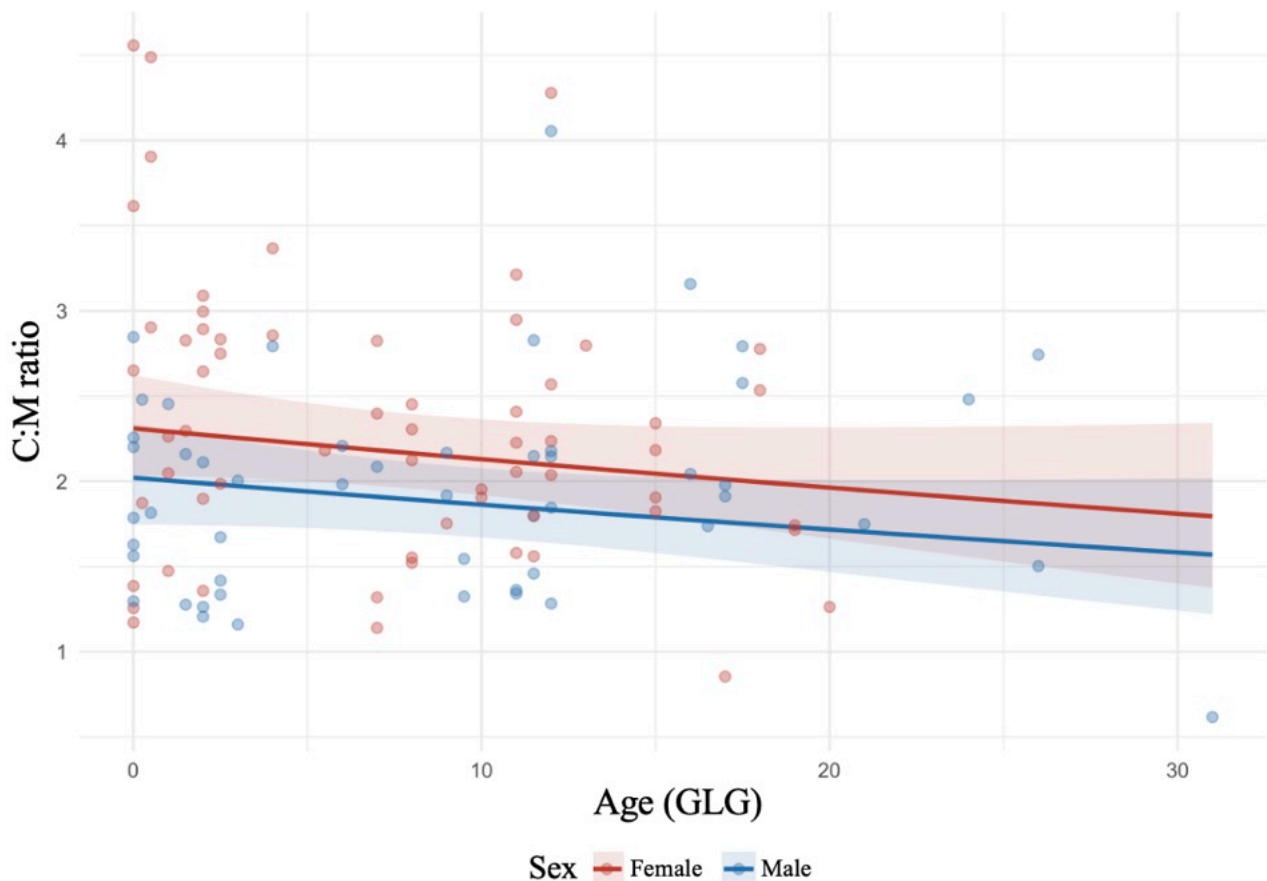


Figure 3.23 Model-predicted C:M ratio as a function of estimated age by sex. Lines show population-level fixed-effect predictions back-transformed to the ratio scale; shaded ribbons represent 95% confidence intervals. Individual gland measurements are shown as points. Females are shown in red, males in blue.

3.5.6.3 Sex-by-age interaction

The interaction term was not significant ($p = 0.290$) and the interaction model was not preferred over the additive model ($\Delta\text{AIC} = -1.0$; Table 3.7), indicating that the relationship between age and C:M ratio did not differ between sexes. The marginal trends for sex ($p = 0.056$) and age ($p = 0.076$) observed in the additive model were preserved in direction, but remained non-significant. Between-individual variance was 0.076 ($SD = 0.277$) and residual variance was 0.042 ($SD = 0.204$), giving an ICC of 0.644, consistent with the life history model and indicating that between-individual variation continued to dominate the variance structure (Table 3.8).

Table 3.7 Linear mixed-effects model predicting log(C:M ratio) with sex-by-age interaction

Model: $\log(\text{C:M}) \sim \text{Sex} \times \text{GLG} + (1 \mid \text{AnimalID})$						
Predictor	β (Estimate)	<i>SE</i>	<i>df</i>	<i>t</i>	<i>p</i>	95% CI
Intercept	0.888	0.081	67.7	10.97	<0.001	0.730 to 1.047
Sex (Male)	-0.226	0.117	68.1	-1.94	0.056	-0.455 to 0.002
Age (GLG)	-0.015	0.008	68.6	-1.81	0.076	-0.032 to 0.001
Sex (Male) $\times$ GLG	0.012	0.011	69.6	1.07	0.290	-0.009 to 0.033

Table 3.8 Variance components for individual ID random effects in the sex-by-age interaction model of log(C:M ratio)

Random effect	Variance	<i>SD</i>
Individual ID (random intercept)	0.076	0.276
Residual	0.042	0.204

3.5.6.4 Effect of prominent medullary band on cortex:medulla ratio

The addition of prominent medullary band (PromBand) improved model fit marginally (LRT: $\chi^2 = 2.99$, $p = 0.084$; $\Delta\text{AIC} = -1.0$). Glands with a prominent medullary band had an estimated 12% lower C:M ratio than those without ($\beta = -0.130$, $SE = 0.077$, $p = 0.094$; Table 3.9). With PromBand included, the sex effect weakened ($p = 0.164$) and age became non-significant ($p = 0.178$), suggesting partial confounding between prominent band presence and these demographic variables. This model had the highest marginal R^2 among the C:M ratio models ($R^2_{\text{m}} = 0.097$).

The *ICC* for this model was 0.644, consistent with the life history model and indicating that between-individual variation continued to dominate the variance structure (Table 3.10).

Table 3.9 Linear mixed-effects model predicting log(C:M ratio) with life history predictors and prominent medullary band

Model: log(C:M) ~ Sex + GLG + PromBand + (1 AnimalID)						
Predictor	β (Estimate)	<i>SE</i>	<i>df</i>	<i>t</i>	<i>p</i>	95% CI
Intercept	0.884	0.070	69.7	12.62	<0.001	0.747 to 1.022
Sex (Male)	-0.108	0.077	68.5	-1.41	0.164	-0.258 to 0.043
Age (GLG)	-0.007	0.005	70.5	-1.36	0.178	-0.018 to 0.003
PromBand (Prominent)	-0.130	0.077	68.0	-1.70	0.094	-0.280 to 0.020

Table 3.10 Variance components for individual ID random effects in the life history + prominent medullary band model of log(C:M ratio)

Random effect	Variance	<i>SD</i>
Individual ID (random intercept)	0.0744	0.273
Residual	0.0412	0.203

3.5.7 Age-related changes in adrenal size and composition

3.5.7.1 Cross-sectional area

Cross-sectional area increased significantly with age ($\beta = 0.048$, $SE = 0.005$, $p < 0.001$; Table 3.11), corresponding to approximately 5% growth per year ($\exp(0.048) = 1.049$). This was the strongest fixed-effect relationship detected, with a marginal R^2 of 0.520 and conditional R^2 of 0.934. Sex had no effect on cross-sectional area ($p = 0.802$). The *ICC* was 0.863, the highest observed, indicating that 86.3% of the total variance in log(cross-sectional area) was attributable to differences among individuals (Table 3.12).

Table 3.11 Linear mixed-effects model predicting log(cross-sectional area) from life history predictors

Model: log(CrossSection) ~ Sex + GLG + (1 AnimalID)						
Predictor	β (Estimate)	SE	df	t	p	95% CI
Intercept	17.896	0.064	72.5	280.74	<0.001	17.771 to 18.021
Sex (Male)	0.019	0.075	72.5	0.25	0.802	−0.128 to 0.166
Age (GLG)	0.048	0.005	73.4	9.29	<0.001	0.038 to 0.059

Table 3.12 Variance components for individual ID random effects in models of adrenal cross-sectional area

Random effect	Variance	SD
Individual ID (random intercept)	0.0923 (log scale)	0.304
Residual	0.0147 (log scale)	0.121

3.5.7.2 Capsule proportion

Capsule proportion was modelled untransformed (normality assumptions satisfied without transformation). Capsule proportion increased significantly with age ($\beta = 0.128$, $SE = 0.061$, $p = 0.040$; Table 3.13), with each GLG year associated with approximately 0.13 percentage point increase. Sex was not significant ($p = 0.893$; Figure 3.24). The ICC was 0.793, indicating that 79.3% of the total variance in capsule proportion was attributable to differences among individuals (Table 3.14).

Table 3.13 Linear mixed-effects model predicting capsule proportion (%) from life history predictors

Model: Capsule_Pct ~ Sex + GLG + (1 AnimalID)						
Predictor	β (Estimate)	SE	df	t	p	95% CI
Intercept	12.554	0.749	73.7	16.76	<0.001	11.086 to 14.023
Sex (Male)	0.119	0.881	73.7	0.14	0.893	−1.609 to 1.847
Age (GLG)	0.128	0.061	75.0	2.09	0.040	0.008 to 0.248

Table 3.14 Variance components for individual ID random effects in models of adrenal capsule proportion

Random effect	Variance	<i>SD</i>
Individual ID (random intercept)	11.955 % ²	3.458 %
Residual	3.126 % ²	1.768 %

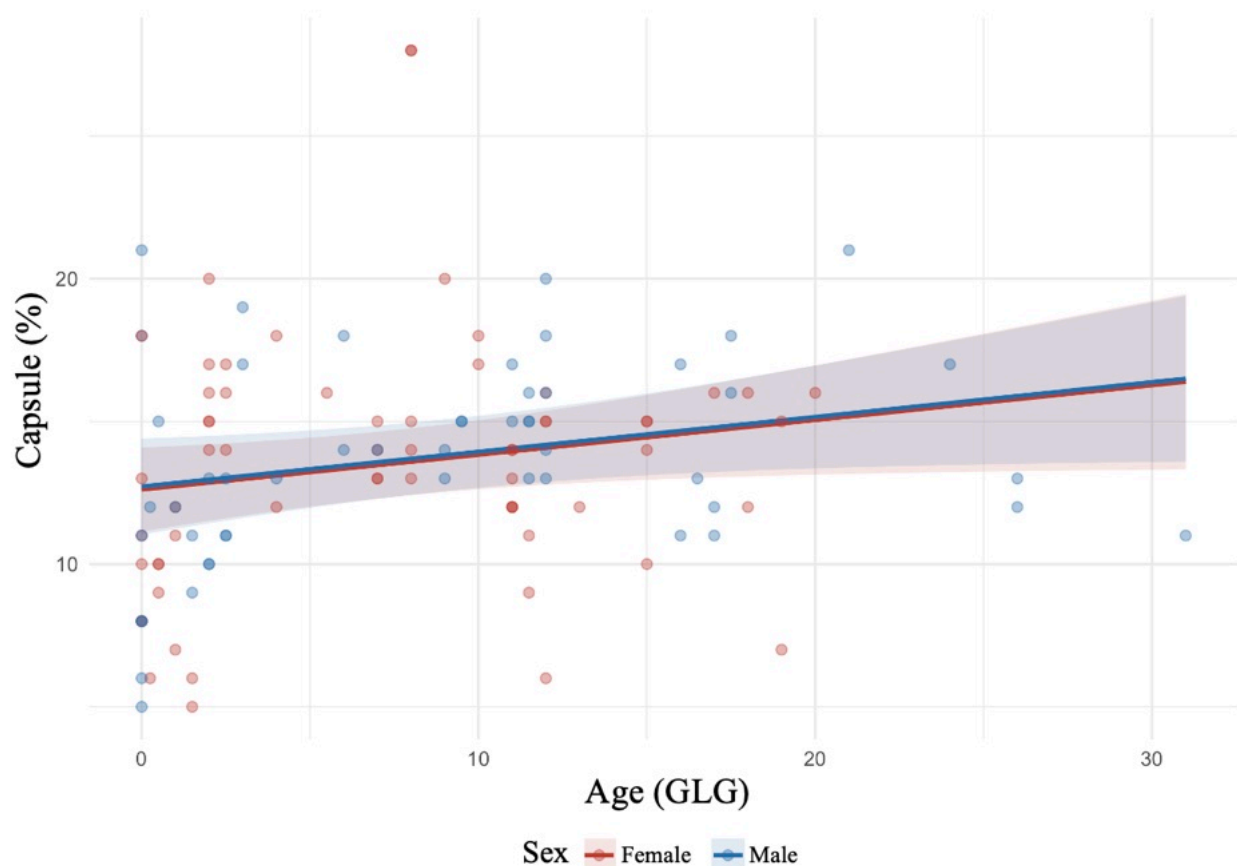


Figure 3.24 Model-predicted capsule proportion as a function of estimated age by sex. Lines show population-level fixed-effect predictions; shaded ribbons represent 95% confidence intervals. Individual gland measurements are shown as points; females, red; males, blue.

3.5.8 Prominent medullary band and medulla proportion

The presence of a prominent medullary band was significantly associated with medulla proportion ($\beta = 3.17$, $SE = 1.50$, $p = 0.037$; Table 3.15). Glands with a prominent band had an estimated mean medulla proportion of 30.5% (95% CI: 28.4–32.5%) compared to 27.3% (95%

CI: 25.1–29.4%) for glands without a prominent band, a difference of approximately 3.2%. Neither sex ($p = 0.210$) nor age ($p = 0.394$) significantly predicted medulla proportion, indicating the histological scoring of the prominent medullary band was reflective of structural variation in medullary tissue volume.

The *ICC* was 0.612, indicating that 61.2% of the total variance in medulla proportion was attributable to differences among individuals, with the remaining 38.8% reflecting within-individual variation between bilateral glands (Table 3.16). This was the lowest *ICC* observed across all models, suggesting greater bilateral variability in medulla proportion compared to other morphometric measures.

Table 3.15 Linear mixed-effects model predicting medulla proportion (%) from life history predictors and prominent medullary band

Model: Medulla_Pct ~ Sex + GLG + PromBand + (1 AnimalID)						
Predictor	β (Estimate)	<i>SE</i>	<i>df</i>	<i>t</i>	<i>p</i>	95% CI
Intercept	25.621	1.367	69.8	18.74	<0.001	22.941 to 28.301
Sex (Male)	1.894	1.496	68.5	1.27	0.210	−1.038 to 4.827
Age (GLG)	0.089	0.103	70.8	0.86	0.394	−0.114 to 0.291
PromBand (Prominent)	3.173	1.495	67.9	2.12	0.037	0.243 to 6.103

Table 3.16 Variance components for individual ID random effects in the medulla proportion model

Random effect	Variance	<i>SD</i>
Individual ID (random intercept)	27.21 % ²	5.217 %
Residual	17.27 % ²	4.156 %

3.5.9 Pathological predictors of cortex:medulla ratio

None of the pathological predictors were significant: pathology presence ($p = 0.286$), disease classification ($p = 0.516$), and stress type (all contrasts $p \geq 0.486$; Table 3.17). The marginal R^2 was 0.016, the lowest of all models examined, indicating that pathological predictors explain virtually none of the variation in adrenal zonal proportions. Estimated marginal means for C:M ratio were similar across pathological categories, with widely overlapping confidence intervals.

Pairwise contrasts between stress types were all non-significant (all $p > 0.76$, Tukey-adjusted). The ICC was 0.736, indicating that 73.6% of the total variance in log(C:M ratio) was attributable to differences among individuals (Table 3.18).

Table 3.17 Linear mixed-effects model predicting log(C:M ratio) from pathological predictors

Model: $\log(C:M) \sim \text{PathPresent} + \text{DiseaseCode} + \text{StressType} + (1 \mid \text{AnimalID})$						
Predictor	β (Estimate)	SE	df	t	p	95% CI
Intercept	0.614	0.088	62.9	7.01	<0.001	0.442 to 0.786
Pathology (Present)	0.095	0.088	84.4	1.07	0.286	−0.078 to 0.267
Disease code (D1)	−0.086	0.132	79.1	−0.65	0.516	−0.345 to 0.173
Stress type (Chronic)	0.094	0.141	56.9	0.67	0.507	−0.181 to 0.369
Stress type (Acute-on-Chronic)	0.073	0.103	57.7	0.70	0.486	−0.130 to 0.275

Table 3.18 Variance components for individual ID random effects in the pathology model of log(C:M ratio)

Random effect	Variance	SD
Individual ID (random intercept)	0.1013	0.318
Residual	0.0364	0.191

3.6 Trace element burden

3.6.1 Population characteristics and tissue-specific accumulation

Mean trace element concentrations differed in both magnitude and rank order between kidney and liver tissues (Tables 3.19–3.20). In kidney tissue, Mg exhibited the highest mean concentration (129.00 ± 34.80 mg/kg ww), followed in descending order by (Mg >) Fe > Zn > Al > Cd > Hg > Se > Cu > Mn > As > Cr. In liver tissue, Fe exhibited the highest mean concentration (200.00 ± 96.60 mg/kg ww), followed by (Fe >) Mg > Zn > Hg > Al > Se > Cu > Mn > Cd > As > Ni > V. Despite broad similarities in the dominant elements (Mg, Fe, Zn) across tissues, notable differences in relative ranking were observed. Cadmium ranked fifth in kidney (7.87 ± 8.10 mg/kg ww) but ninth in liver (2.17 ± 2.68 mg/kg ww), whereas mercury ranked sixth in kidney (6.96 ± 6.38 mg/kg ww) but fourth in liver (45.80 ± 48.10 mg/kg ww), indicating

tissue-specific accumulation patterns consistent with known organ-level partitioning in marine mammals.

Of the ten shared elements, nine showed statistically significant tissue-specific differences (Table 3.21; Figure 3.25). Eight elements were significantly enriched in liver tissue: mercury, manganese, selenium, copper, zinc, iron, magnesium, and arsenic, all at $p < 0.001$ (Table 3.21). Cadmium was the only element significantly elevated in kidney tissue ($p < 0.001$), consistent with its known nephrotoxicity and affinity for renal metallothionein. Aluminium was the only element showing no significant tissue difference ($p = 0.139$), with concentrations broadly similar between tissues. The magnitude of tissue enrichment varied considerably across significant elements, with mercury and manganese showing the greatest liver preference and magnesium and arsenic the most modest (Table 3.21).

Table 3.19 Trace element concentrations (mg/kg ww) in kidney tissue. Elements ranked by mean concentration. Pb, Co, and Sr excluded (*a priori*); Ni and V excluded (>30% LOD/ $\sqrt{2}$ substitution in kidney). LOD, limit of detection (mg/kg ww)

Rank	Element	n	Mean	Median	Min	Max	LOD
1	Mg	71	129.00 $\pm$ 34.8	119.00	80.60	261.00	1.79
2	Fe	71	106.00 $\pm$ 44.1	90.70	43.80	224.00	1.79
3	Zn	71	28.20 $\pm$ 5.37	27.60	18.40	46.50	1.79
4	Al	71	21.30 $\pm$ 20.9	13.80	4.79	140.00	1.79
5	Cd	71	7.87 $\pm$ 8.10	4.53	0.18	29.30	0.18
6	Hg	71	6.96 $\pm$ 6.38	5.96	0.21	26.80	0.02
7	Se	71	6.14 $\pm$ 3.59	5.70	0.66	19.00	0.33
8	Cu	71	4.38 $\pm$ 1.30	4.15	2.28	10.50	0.18
9	Mn	71	0.794 $\pm$ 0.215	0.76	0.37	1.51	0.02
10	As	71	0.434 $\pm$ 0.236	0.37	0.11	1.53	0.02
11	Cr	71	0.056 $\pm$ 0.095	0.02	0.01	0.50	0.01

Table 3.20 Trace element concentrations (mg/kg ww) in liver tissue. Elements ranked by mean concentration. Pb, Co, and Sr excluded (*a priori*); Cr excluded (>30% LOD/ $\sqrt{2}$ substitution in liver). LOD, limit of detection (mg/kg ww)

Rank	Element	n	Mean	Median	Min	Max	LOD
1	Fe	71	200.00 $\pm$ 96.60	179.00	65.80	567.00	2.16
2	Mg	71	153.00 $\pm$ 29.90	148.00	88.60	235.00	2.16
3	Zn	71	59.30 $\pm$ 33.80	48.10	28.20	243.00	2.16
4	Hg	71	45.80 $\pm$ 48.10	24.60	0.65	185.00	0.02
5	Al	71	24.10 $\pm$ 21.30	18.60	8.44	147.00	2.16
6	Se	71	18.80 $\pm$ 18.70	12.40	1.33	79.80	0.40
7	Cu	71	11.50 $\pm$ 8.94	9.56	4.57	61.70	0.22
8	Mn	71	3.88 $\pm$ 1.52	3.43	0.88	7.63	0.02
9	Cd	71	2.17 $\pm$ 2.68	1.14	0.22	15.00	0.22
10	As	71	0.53 $\pm$ 0.23	0.52	0.14	1.31	0.02
11	Ni	71	0.05 $\pm$ 0.15	0.03	0.02	1.23	0.02
12	V	71	0.05 $\pm$ 0.05	0.04	0.01	0.18	0.01

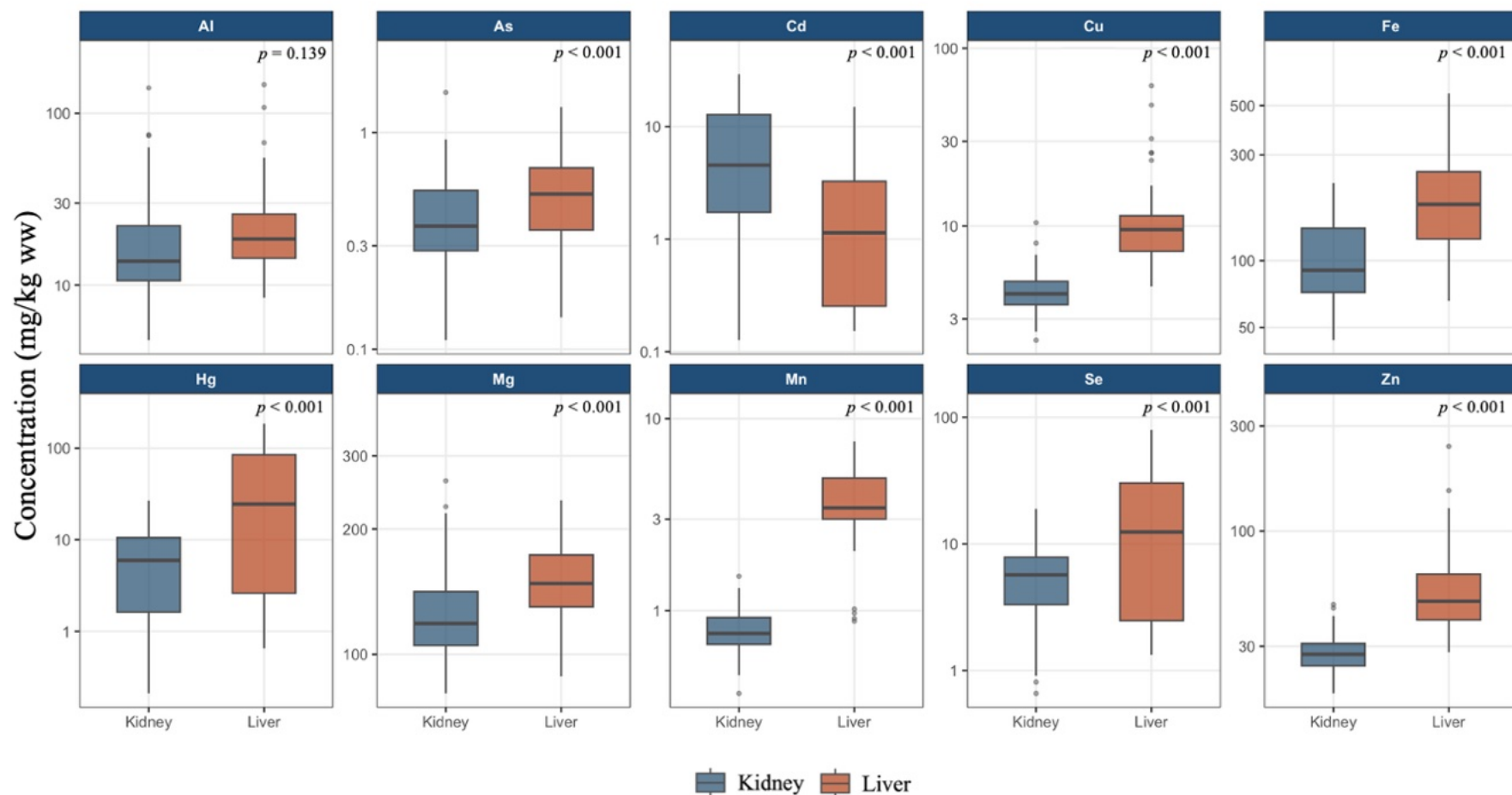


Figure 3.25 Distribution of trace element concentrations (mg/kg ww) in kidney (blue) and liver (orange) tissues. Boxes represent the IQR with median values indicated by horizontal lines; whiskers extend to $1.5 \times$ IQR, and points denote outliers. Concentrations are displayed on a log scale. Pb, Sr, and Co were excluded as mean concentrations were below analytical limits of detection. Pb, Co, and Sr excluded (*a priori*); Cr excluded ($>30\%$ LOD/ $\sqrt{2}$ substitution in liver). *p*-values are shown in the upper right corner of each panel.

Table 3.21 Kidney versus liver trace element concentrations (mg/kg ww) for the ten elements meeting the $\leq 30\%$ LOD/ $\sqrt{2}$ substitution threshold in both tissues. Elements ranked by magnitude of percent difference

Metal	n (pairs)	Kidney mean	Liver mean	Kidney median	Liver median	Percent difference	Fold change	p value	Prominenc e
Hg	71	6.96 $\pm$ 6.38	45.80 $\pm$ 48.10	5.96	24.60	+558.00	6.58	<0.001	Liver
Mn	71	0.79 $\pm$ 0.22	3.88 $\pm$ 1.52	0.76	3.43	+389.00	4.89	<0.001	Liver
Se	71	6.14 $\pm$ 3.59	18.80 $\pm$ 18.70	5.70	12.40	+206.00	3.06	<0.001	Liver
Cu	71	4.38 $\pm$ 1.30	11.50 $\pm$ 8.94	4.15	9.56	+162.00	2.62	<0.001	Liver
Zn	71	28.20 $\pm$ 5.37	59.30 $\pm$ 33.80	27.60	48.10	+110.00	2.10	<0.001	Liver
Fe	71	106.00 $\pm$ 44.10	200.00 $\pm$ 96.60	90.70	179.00	+89.30	1.89	<0.001	Liver
Cd	71	7.87 $\pm$ 8.10	2.17 $\pm$ 2.68	4.53	1.14	-72.40	0.28	<0.001	Kidney
As	71	0.43 $\pm$ 0.24	0.53 $\pm$ 0.23	0.37	0.52	+23.00	1.23	<0.001	Liver
Mg	71	129.00 $\pm$ 34.80	153.00 $\pm$ 29.90	119.00	148.00	+18.80	1.19	<0.001	Liver
Al	71	21.30 $\pm$ 20.90	24.10 $\pm$ 21.30	13.80	18.6	+13.50	1.14	0.139	—

3.6.2 *Metal covariation structure and z-score indices*

Seventy-one animals had complete concentration data for all 10 elements in both kidney and liver, yielding 106 glands for index-based modelling. Principal component analysis (PCA) of the full 10-element panels revealed that the first two principal components explained approximately half of the total variance in metal covariation in both tissues (kidney: PC1 = 30.5%, PC2 = 19.0%; liver: PC1 = 32.7%, PC2 = 15.1%; Figure 3.26-3.27). In both tissues, the bioaccumulative elements (Se, Cd, Hg) loaded strongly and consistently on PC1. The essential elements (Cu, Zn, Mg) loaded primarily on PC2 in both tissues, though with greater variability in loading patterns across tissues and with contributions from additional elements, reflecting the more complex covariation structure of physiologically regulated metals (Figure 3.28).

To confirm that the z-score indices captured the same dominant axes of variation as the PCA, each index was correlated with its corresponding principal component. The kidney bioaccumulative index correlated strongly with kidney PC1 ($r = 0.965$) and the kidney essential index with PC2 ($r = 0.943$); correspondingly, the liver bioaccumulative index correlated with liver PC1 ($r = 0.968$) and the liver essential index with PC2 ($r = 0.851$), confirming that the indices provided a valid summary of collective metal burden for use in subsequent models. Individual animals are coloured by C:M ratio in the biplots, with no clear visual separation apparent along either principal component.

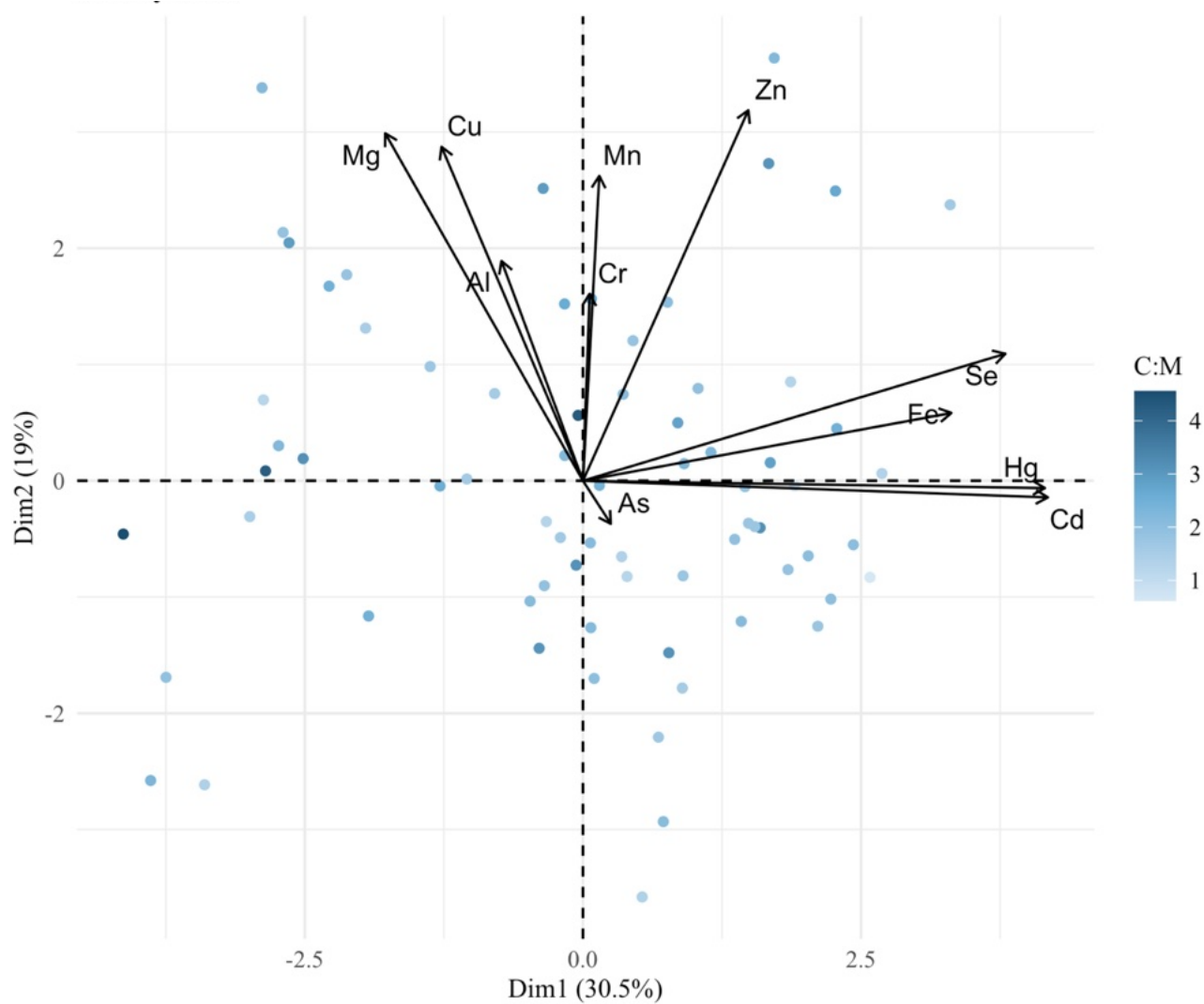


Figure 3.26 Principal component analysis biplots of 10 trace element concentrations (log-transformed, standardised) in kidney tissue. Individual animals (points) are coloured by C:M ratio (light blue = low, dark blue = high). Element loading vectors (arrows) indicate the direction and magnitude of each element's contribution to the first two principal components.

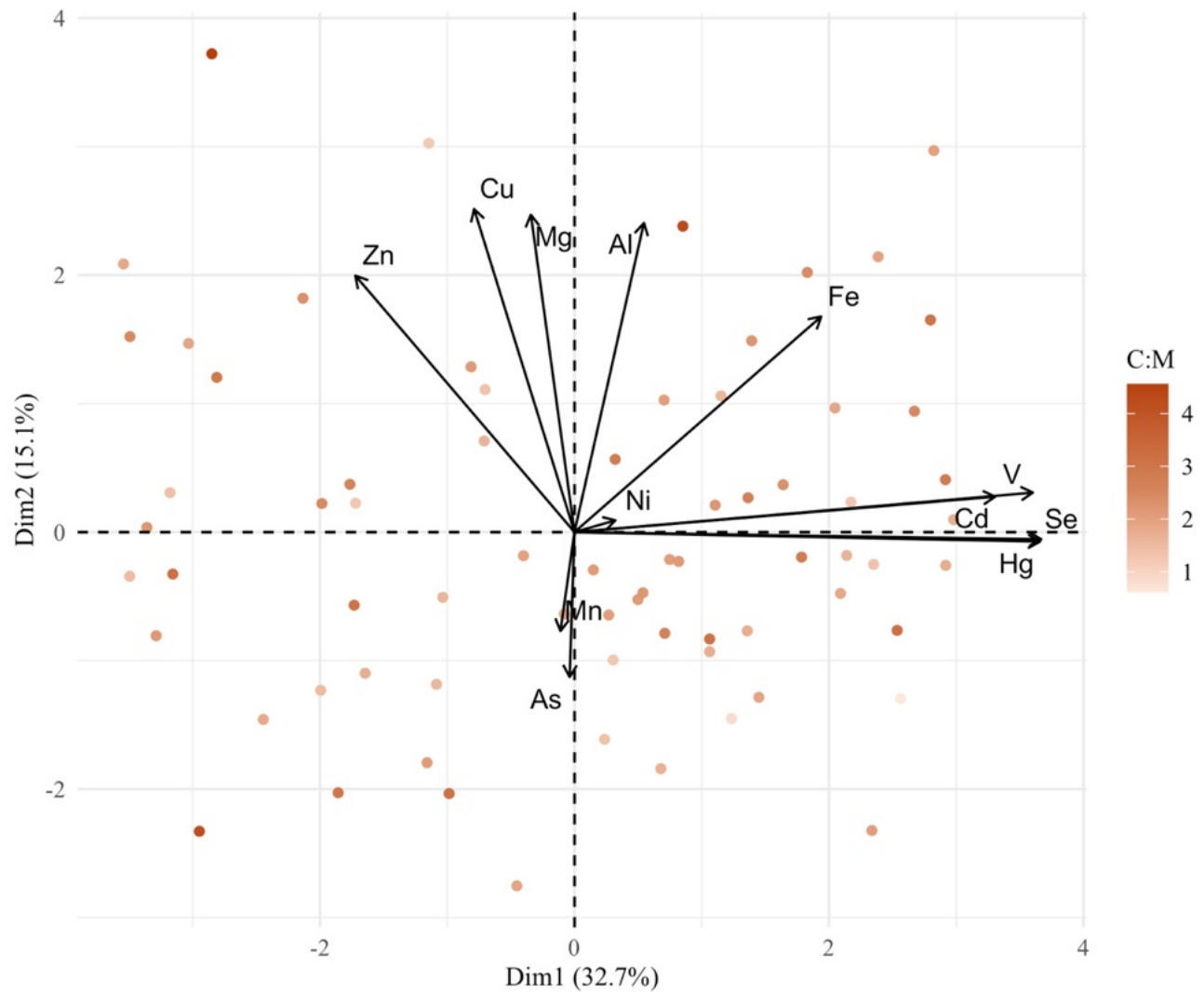


Figure 3.27 Principal component analysis biplots of 10 trace element concentrations (log-transformed, standardised) in liver tissue. Individual animals (points) are coloured by C:M ratio (light orange = low, dark orange = high). Element loading vectors (arrows) indicate the direction and magnitude of each element's contribution to the first two principal components.

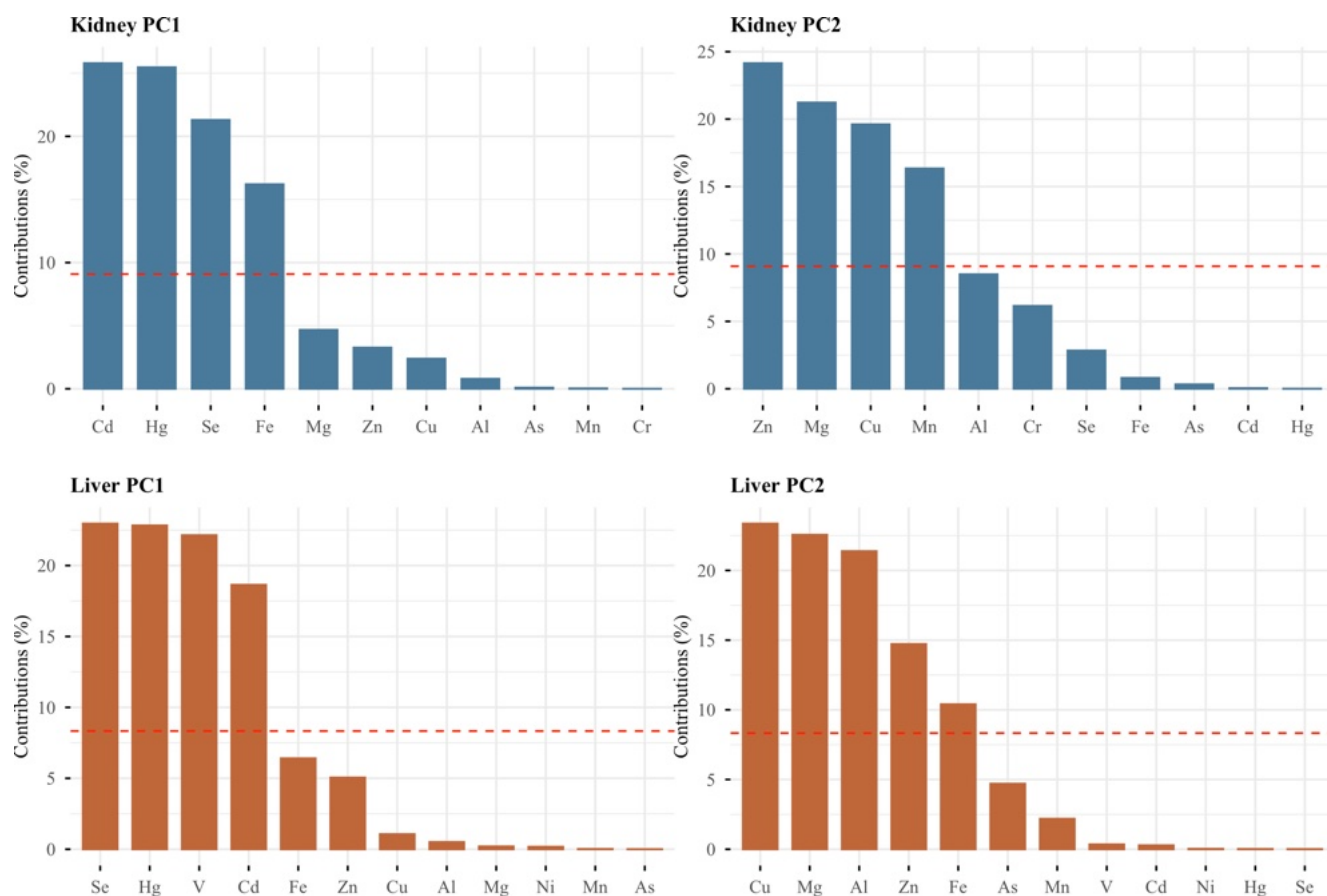


Figure 3.28 Variable contributions (%) to the first two principal components of trace element concentrations in kidney (blue) and liver (orange) tissue. Left panels show contributions to PC1; right panels show contributions to PC2. The dashed red line indicates the expected contribution under a uniform distribution ($1/10 = 10\%$). Elements exceeding this threshold contribute above average to the respective component.

3.6.3 Group comparisons

3.6.3.1 Metal indices by sex, maturity, and age

To determine whether life history variables were associated with metal burden prior to index-based modelling, kidney and liver bioaccumulative and essential z-score indices were compared by sex and sexual maturity using Wilcoxon rank-sum tests, and correlated with age using Spearman rank correlations, at the animal level ($n = 71$).

No significant sex differences were found for any index (kidney bioaccumulative: $W = 526.0$, $p = 0.234$; liver bioaccumulative: $W = 547.0$, $p = 0.343$; kidney essential: $W = 717.0$, $p = 0.320$; liver essential: $W = 643.0$, $p = 0.886$; Figure 3.29A-D), indicating that metal burden as captured by these indices did not differ between males and females in this sample.

In contrast, sexual maturity was strongly associated with bioaccumulative metal burden. Mature animals had substantially higher bioaccumulative indices than immature animals in both kidney (mature mean = 0.687 ± 0.373 , immature mean = -0.631 ± 0.824 ; $W = 80.0$, $p < 0.001$) and liver tissue (mature mean = 0.779 ± 0.446 , immature mean = -0.716 ± 0.666 ; $W = 49.0$, $p < 0.001$; Figure 3.30A, B). These differences were consistent with the known lifetime accumulation dynamics of bioaccumulative metals, such as mercury and cadmium, which increased progressively with age in marine mammals. Essential metal indices did not differ significantly by maturity status (kidney essential: $W = 757.0$, $p = 0.142$; liver essential: $W = 741.0$, $p = 0.199$; Figure 3.30C, D), consistent with the homeostatic regulation of these elements.

Bioaccumulative element indices showed very strong positive correlations with age in both tissues (kidney: $\rho = 0.869$, $p < 0.001$; liver: $\rho = 0.876$, $p < 0.001$; Figure 3.31A, B), confirming progressive accumulation of Se, Cd, and Hg with increasing age. The essential indices were not significantly correlated with age in kidney tissue ($\rho = -0.179$, $p = 0.136$; Figure 3.31C), though a weak negative correlation was observed in liver tissue ($\rho = -0.315$, $p = 0.008$; Figure 3.31D), suggesting a modest age-related decline in relative essential metal status in the liver that may reflect dilution effects associated with hepatic growth or shifting metabolic demands with age. These findings indicated that age and maturity were important covariates of bioaccumulative metal burden and should be considered when interpreting associations between metal indices and adrenal architecture in subsequent models. These age and maturity effects on metal burden

paralleled the life history associations observed for adrenal composition, where capsule proportion increased significantly with age ($p = 0.30$, $p = 0.002$; Section 3.5.5.1) and was greater in mature than immature animals (Section 3.5.5.3), while the C:M ratio showed a marginal sex effect with females having higher ratios than males (Section 3.5.5.2). The convergence of age and maturity effects on both bioaccumulative metal burden and capsule proportion raised the possibility that associations between metal indices and adrenal architecture detected in subsequent models may partly reflect shared age-related trajectories rather than direct metal effects on glandular tissue.

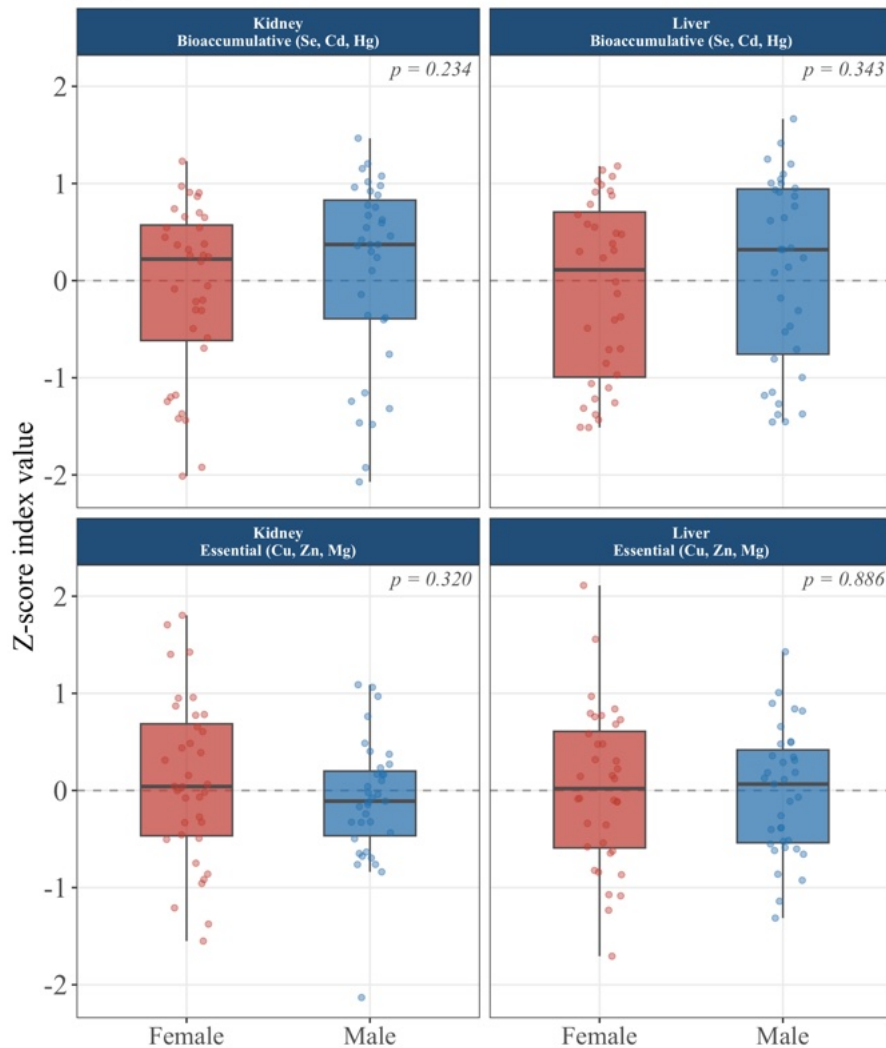


Figure 3.29 Element z-score indices by sex (females, red; males, blue). (A) Kidney bioaccumulative (Se, Cd, Hg), (B) liver bioaccumulative, (C) kidney essential (Cu, Zn, Mg), and (D) liver essential indices ($n = 71$ animals). Boxes represent the IQR with median indicated by the horizontal line; jittered individual data points are overlaid. The dashed horizontal line indicates an index value of zero. p -values are shown in the upper right corner of each panel.

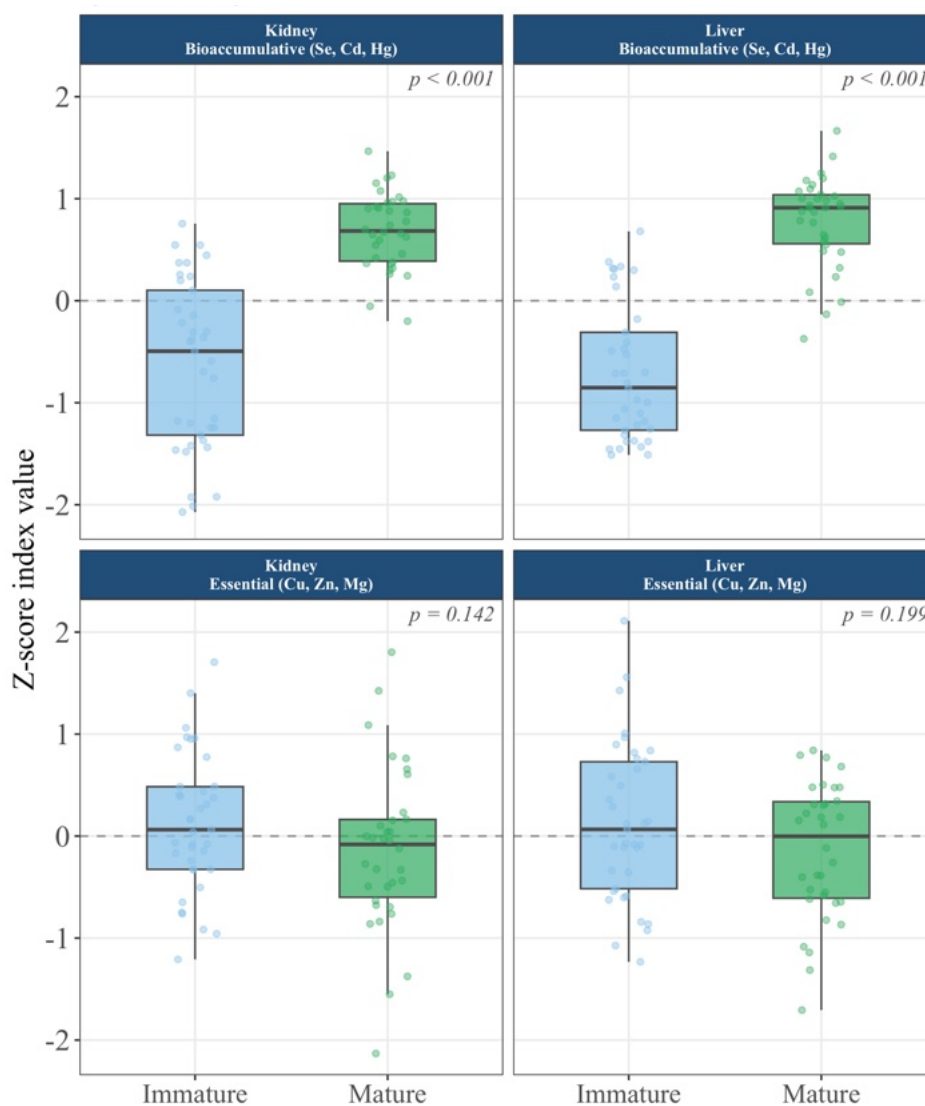


Figure 3.30 Element z-score indices by sexual maturity (immature, blue; mature, green). (A) Kidney bioaccumulative (Se, Cd, Hg), (B) liver bioaccumulative, (C) kidney essential (Cu, Zn, Mg), and (D) liver essential indices ($n = 71$ animals). Boxes represent the IQR with median indicated by the horizontal line; jittered individual data points are overlaid. The dashed horizontal line indicates an index value of zero. p -values are shown in the upper right corner of each panel.

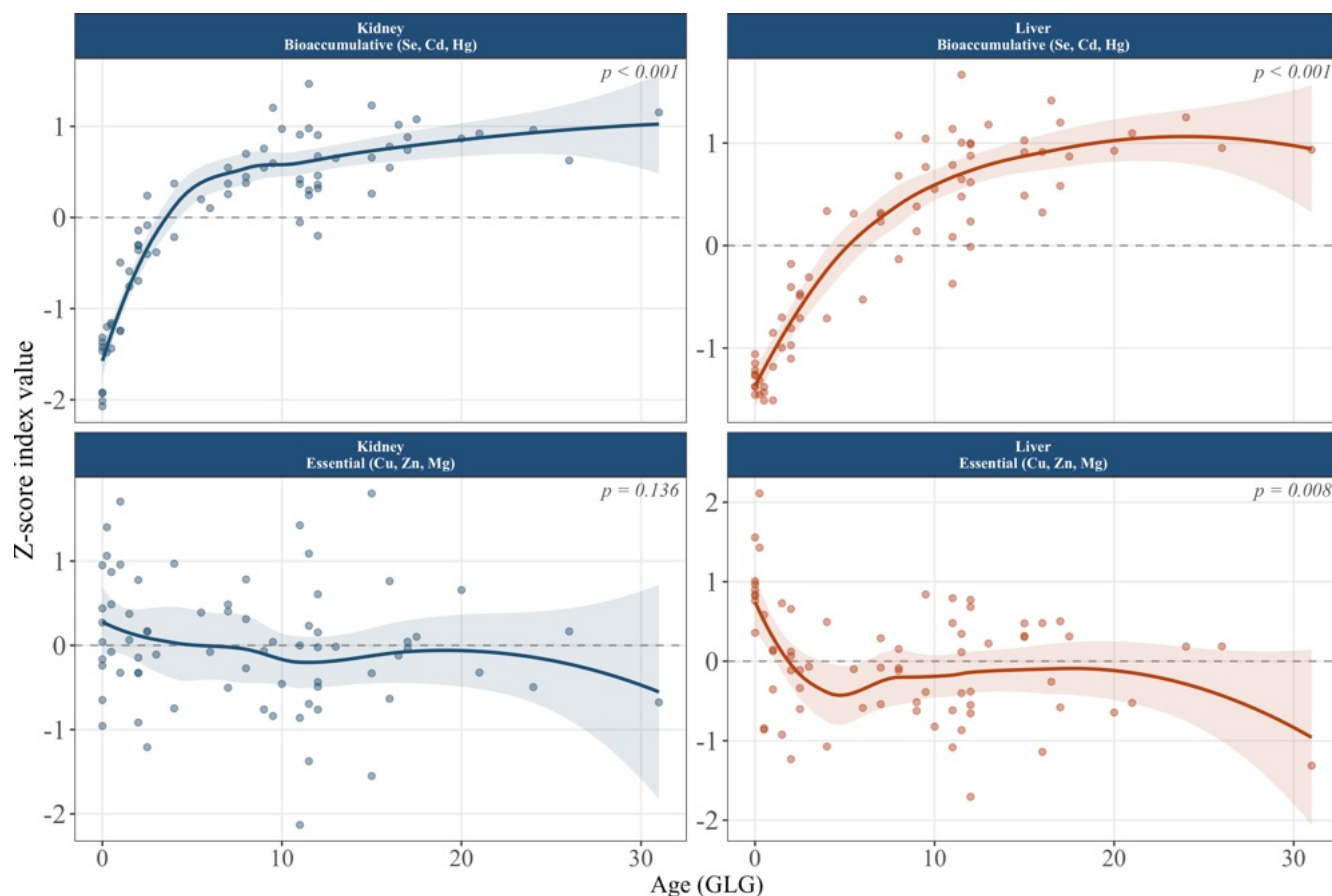


Figure 3.31 Relationship between estimated age and element z-score indices in kidney (blue) and liver (orange). Panels show kidney bioaccumulative (Se, Cd, Hg), liver bioaccumulative, kidney essential (Cu, Zn, Mg), and liver essential indices. LOESS smoothing curves with 95% confidence intervals are overlaid. The dashed horizontal line indicates an index value of zero. Spearman correlation coefficients and p -values are shown in the upper right corner of each panel.

3.6.3.2 Metal indices by pathology presence, disease score, and stress type

Prior to index-based modelling, kidney and liver bioaccumulative and essential z-score indices were compared across pathological and stress type groupings at the animal level ($n = 71$) using Wilcoxon rank-sum and Kruskal-Wallis tests, to determine whether cumulative metal burden differed systematically between groups before proceeding to linear mixed-effects models.

No significant differences in any index were found between animals with pathology absent and present (kidney bioaccumulative: $W = 403.0$, $p = 0.173$; Figure 3.32A; liver bioaccumulative: $W = 425.0$, $p = 0.280$; Figure 3.32B; kidney essential: $W = 549.0$, $p = 0.623$; Figure 3.32C; liver

essential: $W = 615.0, p = 0.182$; Figure 3.32D). When compared by disease classification, both bioaccumulative indices showed marginal trends toward higher values in D1 animals relative to D0 animals, though neither reached conventional significance (kidney bioaccumulative: $W = 133.0, p = 0.089$; liver bioaccumulative: $W = 127.0, p = 0.069$; Figure 3.33A, B). The essential indices showed no such trend (kidney essential: $W = 259.0, p = 0.457$; liver essential: $W = 262.0, p = 0.422$; Figure 3.33C, D). The marginal elevation of bioaccumulative burden in D1 animals is consistent with the pattern observed for liver Hg:Se ratios (Section 3.6.3.4), and together these findings motivated further examination of the relationship between bioaccumulative metal burden and adrenal architecture in the LMM framework.

No significant differences among the three stress type groups were found for any index (kidney bioaccumulative: $H = 1.67, df = 2, p = 0.435$; liver bioaccumulative: $H = 1.10, p = 0.570$; Figure 3.34A, B; and kidney essential: $H = 1.77, p = 0.414$; liver essential: $H = 2.20, p = 0.334$; Figure 3.34C, D). Pairwise comparisons restricted to acute versus chronic animals were similarly non-significant for all four indices (kidney bioaccumulative: $p = 0.581$; liver bioaccumulative: $p = 0.755$; kidney essential: $p = 0.260$; liver essential: $p = 0.204$). These results indicate that overall metal burden does not differ systematically across stress type classifications in this sample.

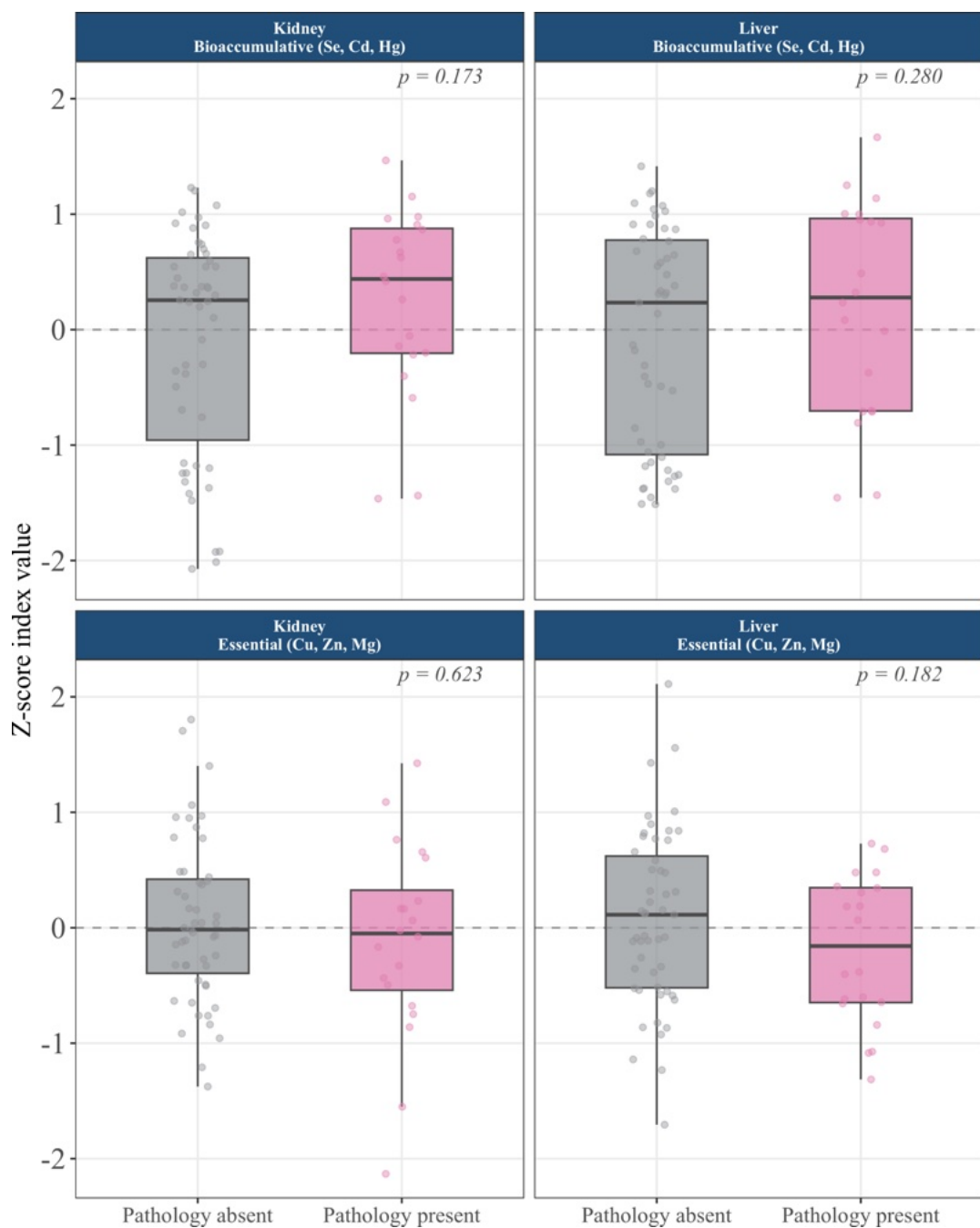


Figure 3.32 Metal z-score indices by pathology presence (pathology absent, $n = 50$, grey; pathology present, $n = 21$ animals with complete metal data; pink). Panels show (A) kidney bioaccumulative (Se, Cd, Hg), (B) liver bioaccumulative, (C) kidney essential (Cu, Zn, Mg), and (D) liver essential indices. Boxes represent the IQR with median indicated by the horizontal line; whiskers extend to $1.5 \times$ IQR. Jittered individual data points are overlaid. The dashed horizontal line indicates an index value of zero. p -values are shown in the upper right corner of each panel.

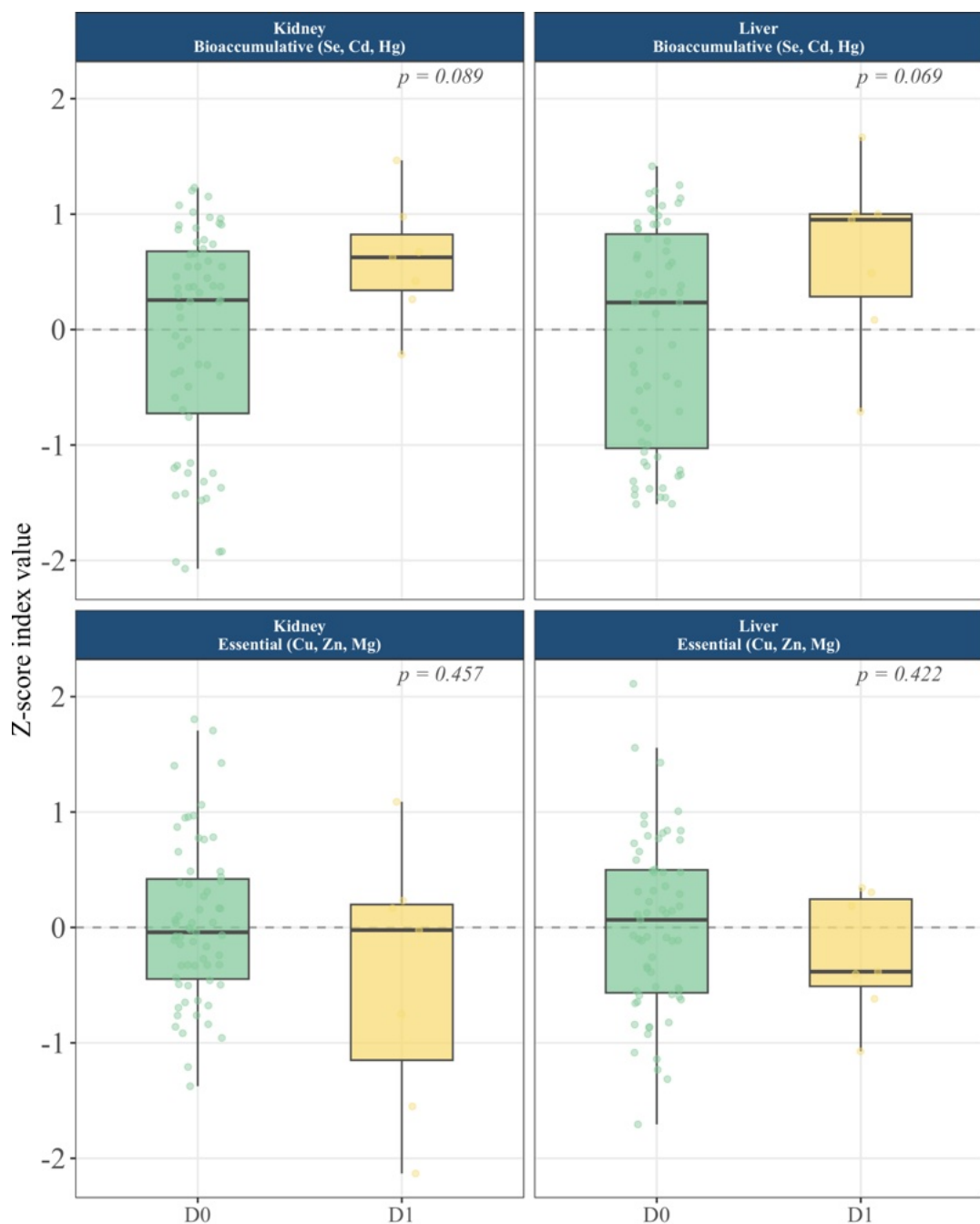


Figure 3.33 Metal z-score indices by disease classification (D0: non-diseased, $n = 64$, green; D1: disease present but unlikely significant, $n = 7$, yellow) in animals with complete metal data. Panels show kidney bioaccumulative (Se, Cd, Hg), liver bioaccumulative, kidney essential (Cu, Zn, Mg), and liver essential indices. Boxes represent the IQR with median indicated by the horizontal line; jittered individual data points are overlaid. The dashed horizontal line indicates an index value of zero. p -values are shown in the upper right corner of each panel.

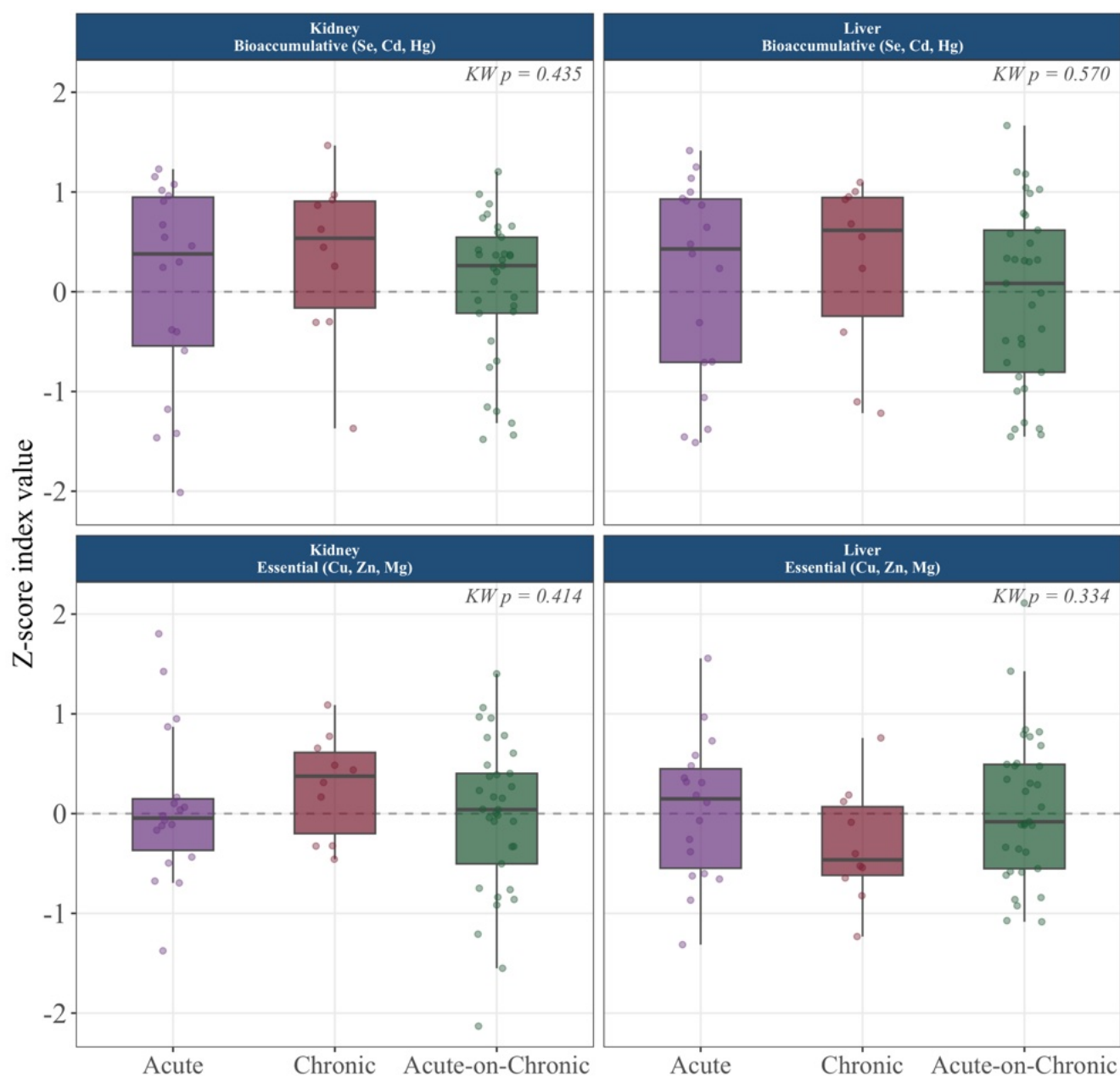


Figure 3.34 Element z-score indices by stress type classification (Acute, $n = 19$, purple; Chronic, $n = 10$, dark red; Acute-on-Chronic, $n = 35$ animals with complete metal data, dark green). (A) kidney and (B) liver bioaccumulative index (Se, Cd, Hg); (C) kidney and (D) essential index (Cu, Zn, Mg). Left panels show kidney indices, right panels show liver indices. Boxes represent the IQR with median indicated by the horizontal line; jittered individual data points are overlaid. The dashed horizontal line indicates an index value of zero. p -values are shown in the upper right corner of each panel.

3.6.3.3 Hg:Se by sex, maturity, and age

No significant sex differences were found for either kidney or liver Hg:Se ratios (kidney: female mean = 0.390 ± 0.263 , male mean = 0.396 ± 0.256 , $W = 624.0$, $p = 0.950$; liver: female mean = 0.756 ± 0.358 , male mean = 0.774 ± 0.343 , $W = 614.0$, $p = 0.859$; Figure 3.35A, B, indicating that the relative balance of mercury to selenium did not differ between sexes.

Sexual maturity was strongly associated with Hg:Se ratios in both tissues. Mature animals had substantially higher Hg:Se ratios than immature animals in kidney tissue (mature mean = 0.584 ± 0.213 , immature mean = 0.217 ± 0.147 ; $W = 93.0$, $p < 0.001$; Figure 3.36A) and liver tissue (mature mean = 1.028 ± 0.176 , immature mean = 0.524 ± 0.287 ; $W = 90.0$, $p < 0.001$; Figure 3.36B). Notably, the mean liver Hg:Se ratio in mature animals exceeded 1.0, indicating that on average mature individuals had reached or surpassed mercury-selenium molar equivalence in the liver, while immature animals remained well below this threshold. This pattern reflected the cumulative nature of mercury accumulation over the life course and was consistent with the strong maturity and age effects observed for the bioaccumulative indices.

Hg:Se ratios were strongly and positively correlated with age in both kidney ($\rho = 0.751$, $p < 0.001$; Figure 3.37A) and liver tissue ($\rho = 0.697$, $p < 0.001$; Figure 3.37B), confirming progressive increase in relative mercury burden with age. Together with the bioaccumulative index results, these findings demonstrated that mercury burden, whether measured as a composite index or as the molar ratio relative to selenium, increased substantially across the life course in this population. This age and maturity dependency should be considered when interpreting associations between mercury-related metrics and adrenal architecture in the LMM analyses that follow, as any apparent relationship between bioaccumulative burden and adrenal morphology may partly reflect underlying age-related variation in both variables.

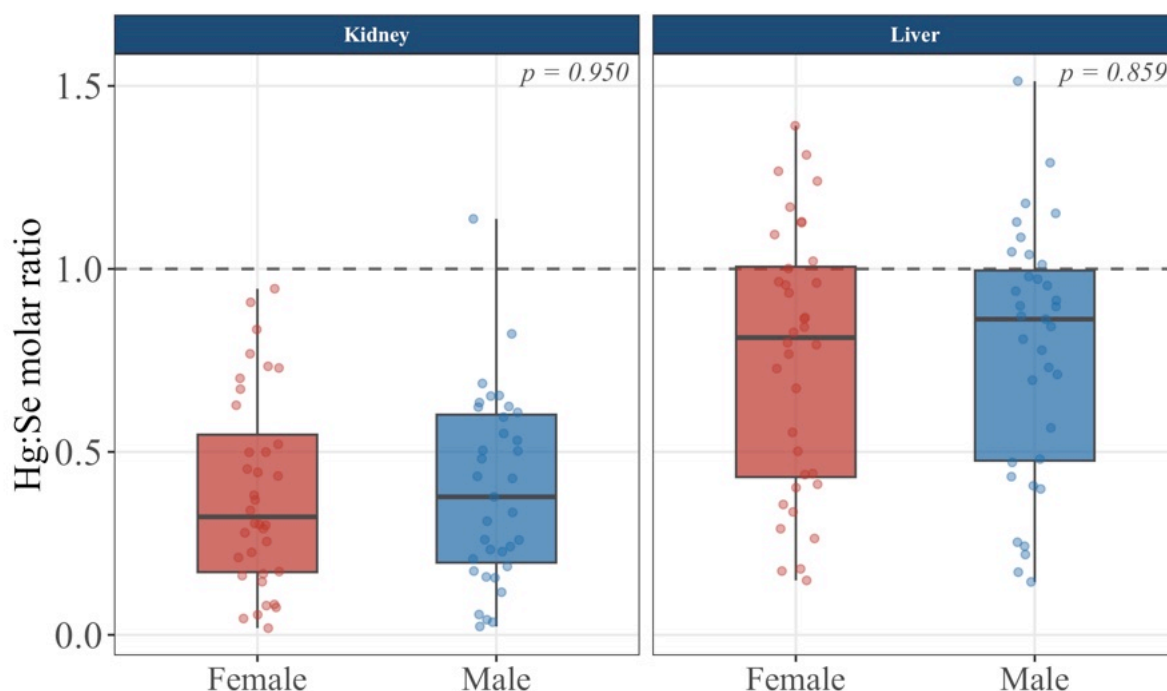


Figure 3.35 Hg:Se molar ratios by sex (females, red: males, blue) in (A) kidney and (B) liver tissue ($n = 71$ animals). The dashed horizontal line indicates Hg:Se = 1.0. No significant differences were found between sexes in either tissue (kidney: $p = 0.950$; liver: $p = 0.859$; Wilcoxon rank-sum tests). p -values are shown in the upper right corner of each panel.

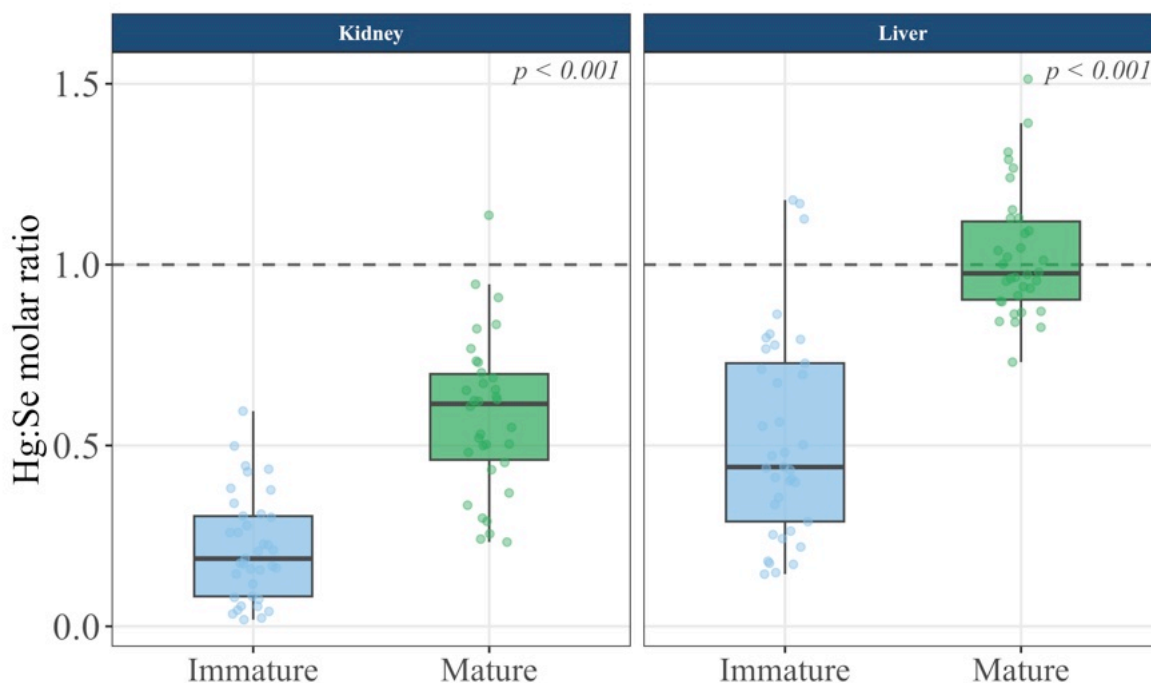


Figure 3.36 Hg:Se molar ratios by sexual maturity (immature, blue; mature, green) in (A) kidney and (B) liver tissue ($n = 71$ animals). The dashed horizontal line indicates Hg:Se = 1.0. p -values are shown in the upper right corner of each panel.

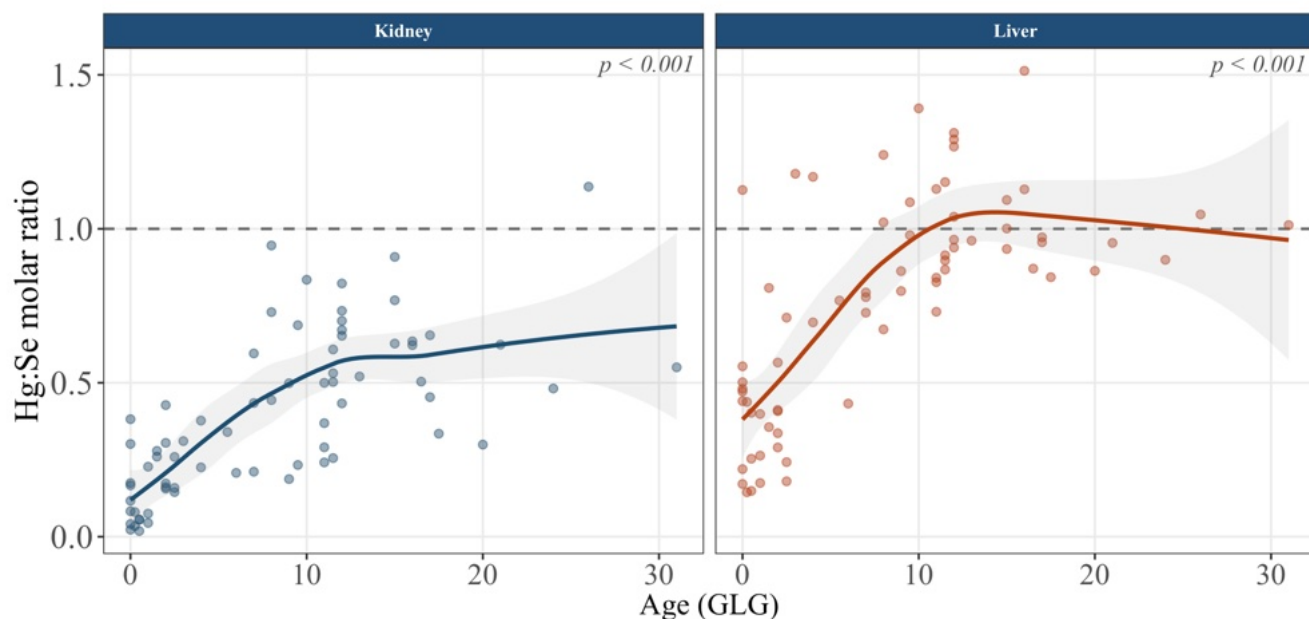


Figure 3.37 Relationship between estimated age and Hg:Se molar ratio in (A) kidney (blue) and (B) liver (orange) tissue. LOESS smoothing curves with 95% confidence intervals are overlaid. The dashed horizontal line indicates a Hg:Se ratio of 1.0 (mercury-selenium molar equivalence). Spearman correlation coefficients and p-values are shown in the upper right corner of each panel.

3.6.3.4 Hg:Se by pathology presence, adrenal disease score, and gross stress type

Hg:Se ratios did not differ significantly between animals with pathology absent and present for either kidney ($W = 436.0$, $p = 0.348$; Figure 3.38A) or liver tissue ($W = 363.0$, $p = 0.061$; Figure 3.38B), though the liver comparison showed a marginal trend toward higher Hg:Se ratios in animals with pathology present that did not reach conventional significance.

When compared by disease classification, liver Hg:Se ratios were significantly higher in D1 animals than in D0 animals ($W = 92.0$, $p = 0.012$; Figure 3.39A), indicating greater relative mercury burden in animals with recorded disease. The kidney comparison showed a similar but non-significant trend ($W = 122.0$, $p = 0.055$; Figure 3.39B). These findings suggest that liver mercury burden relative to selenium may be elevated in animals with systemic disease, though the small D1 sample size ($n = 7$ with complete metal data) warrants caution in interpretation. Taken together with the marginal D0 versus D1 differences in the bioaccumulative indices, this pattern suggested a broader association between bioaccumulative metal burden and disease status that was most clearly expressed in the liver Hg:Se ratio.

Neither kidney nor liver Hg:Se ratios differed significantly across stress type classifications (Kruskal-Wallis: kidney $H = 0.452$, $df = 2$, $p = 0.798$; liver $H = 0.443$, $p = 0.801$; Figure 3.40A), and pairwise comparisons restricted to acute versus chronic animals were similarly non-significant (kidney: $p = 0.719$; liver: $p = 0.829$; Figure 3.40B). Fisher's exact tests indicated no significant association between stress type and pathology presence ($p = 0.462$) or disease classification ($p = 0.406$), and these relationships remained non-significant when restricted to acute versus chronic comparisons (pathology: $p = 0.694$; disease: $p = 0.267$). Collectively, these results indicated that neither metal index values nor Hg:Se ratios vary systematically with stress chronicity, and that the most consistent signal relating metal burden to health status was the elevation of liver Hg:Se in D1 relative to D0 animals.

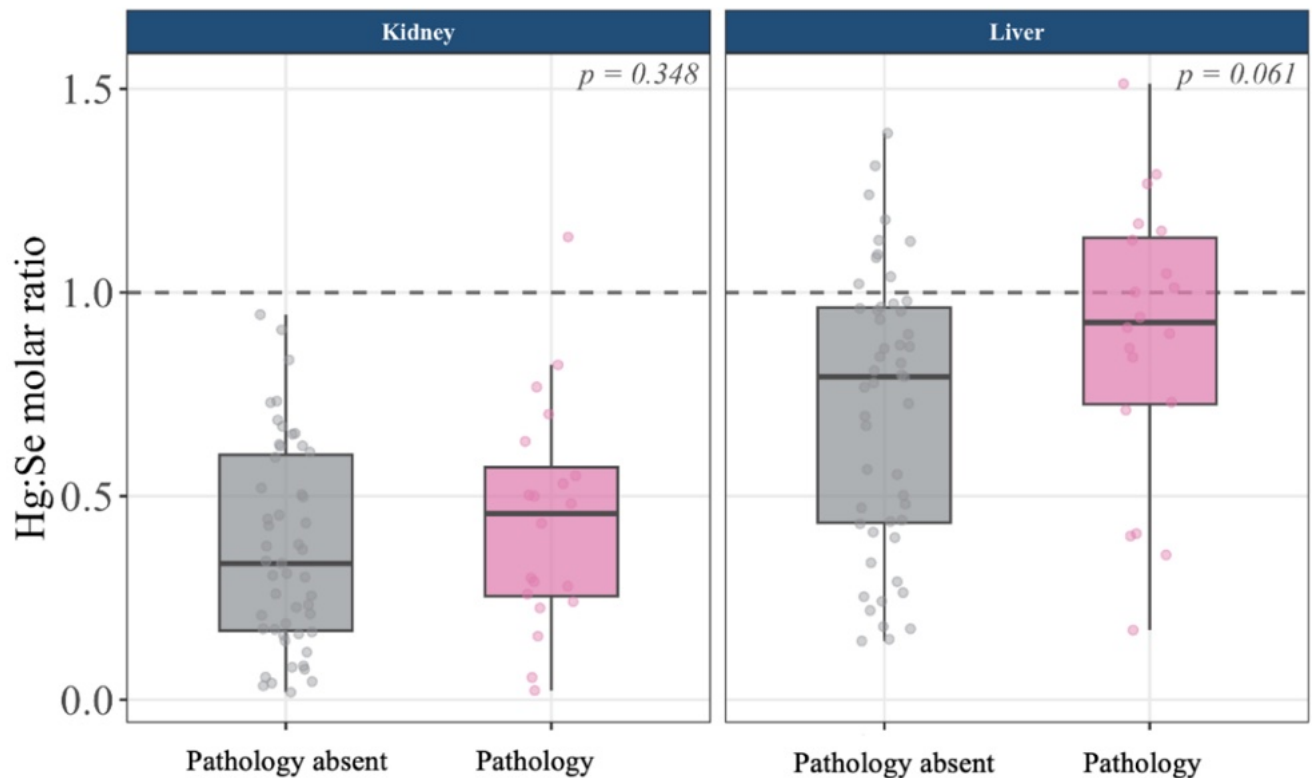


Figure 3.38 Hg:Se molar ratios by pathology presence (absent, $n = 50$, grey; present, $n = 21$, pink) in (A) kidney and (B) liver tissue. The dashed horizontal line indicates Hg:Se = 1.0. p -values are shown in the upper right corner of each panel.

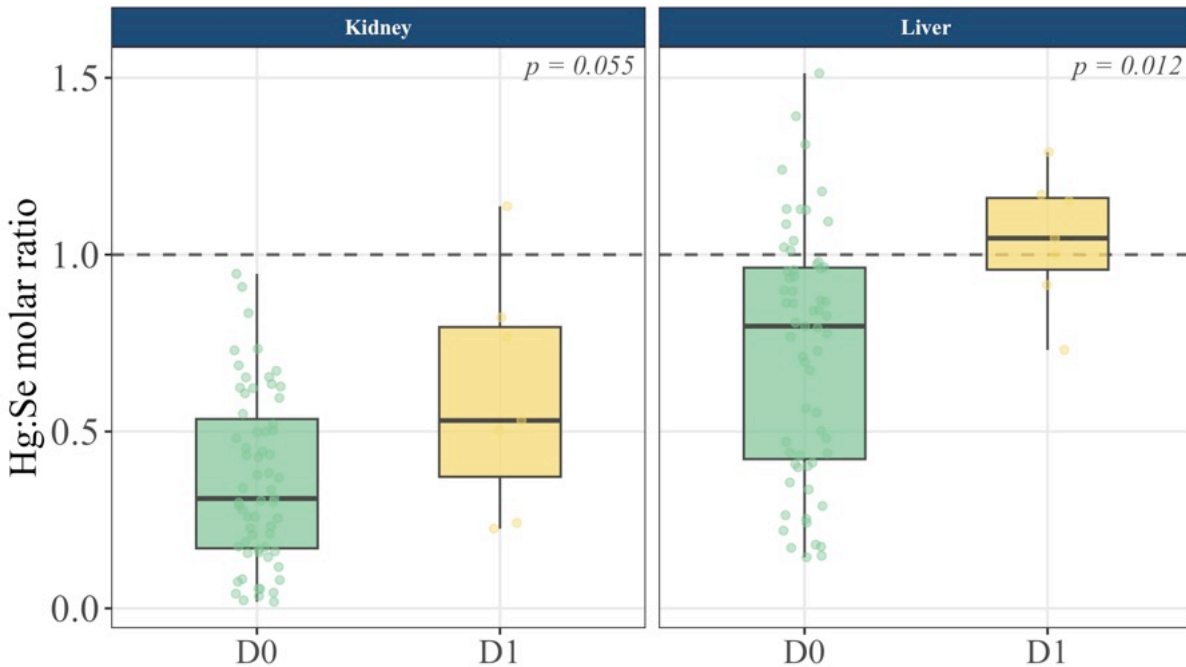


Figure 3.39 Hg:Se molar ratios by disease classification (D0: non-diseased, $n = 64$, green; D1: disease present, $n = 7$, yellow) in (A) kidney and (B) liver tissue. The dashed horizontal line indicates Hg:Se = 1.0. p -values are shown in the upper right corner of each panel.

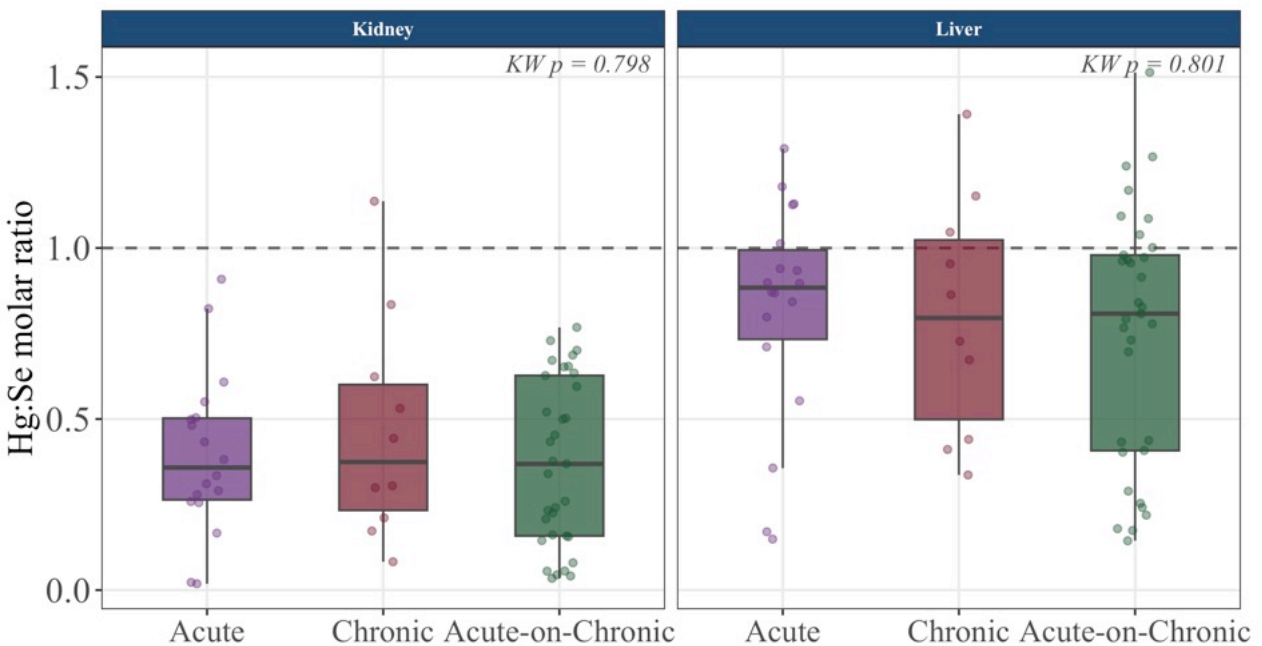


Figure 3.40 Hg:Se molar ratios by stress type classification (Acute, $n = 19$, purple; Chronic, $n = 10$, dark red; Acute-on-Chronic, $n = 35$ animals with complete metal data, dark green) in (A) kidney and (B) liver tissue. Boxes represent the IQR with median indicated by the horizontal line; jittered individual data points are overlaid. The dashed horizontal line indicates a Hg:Se ratio of 1.0. p -values are shown in the upper right corner of each panel.

3.6.4 Metal indices and Hg:Se ratios with adrenal composition

C:M ratio showed no significant correlations with any bioaccumulative index or Hg:Se ratio (all $p > 0.18$). The liver essential index showed a significant positive correlation ($\rho = 0.234$, $p = 0.050$), suggesting animals with higher essential metal status had greater relative cortical tissue, though this borderline result should be interpreted cautiously.

Capsule percent showed the strongest and most consistent pattern across predictors. Both bioaccumulative indices were significantly correlated with capsule proportion (kidney: $\rho = 0.427$, $p < 0.001$; liver: $\rho = 0.361$, $p = 0.003$; Figure 3.41A, B), as were both Hg:Se ratios (kidney: $\rho = 0.362$, $p = 0.003$; liver: $\rho = 0.326$, $p = 0.002$; Figure 3.42A, B). The liver essential index showed a significant correlation ($\rho = -0.260$, $p = 0.019$; Figure 3.41C), with the kidney essential index showing a marginal negative trend ($\rho = -0.203$, $p = 0.089$). These findings indicated that animals with higher bioaccumulative metal burden and higher Hg:Se ratios tended to have proportionally more capsular tissue, while those with higher essential metal status tended toward less.

Cortex percent showed a significant negative correlation with the kidney bioaccumulative index ($\rho = -0.279$, $p = 0.008$; Figure 3.41E) and a significant positive correlation with the liver essential index ($\rho = 0.318$, $p = 0.011$; Figure 3.41D), with marginal trends for the kidney essential index ($\rho = 0.211$, $p = 0.077$) and liver Hg:Se ratio ($\rho = -0.204$, $p = 0.088$). These patterns were directionally consistent with capsule percent, suggesting that higher bioaccumulative burden was associated with a shift away from cortical toward capsular tissue.

Medulla percent was not significantly correlated with any predictor (all $\rho < 0.16$, all $p > 0.20$), indicating that medullary tissue proportion was unrelated to metal burden or Hg:Se ratio at the animal level.

Collectively, these screening correlations indicated that capsule and cortex proportions were the most informative adrenal outcomes in relation to metal burden, and that the bioaccumulative index, essential index, and Hg:Se ratio all showed biologically coherent associations with these outcomes. These correlations served as a screening step to identify biologically meaningful associations prior to formal modelling.

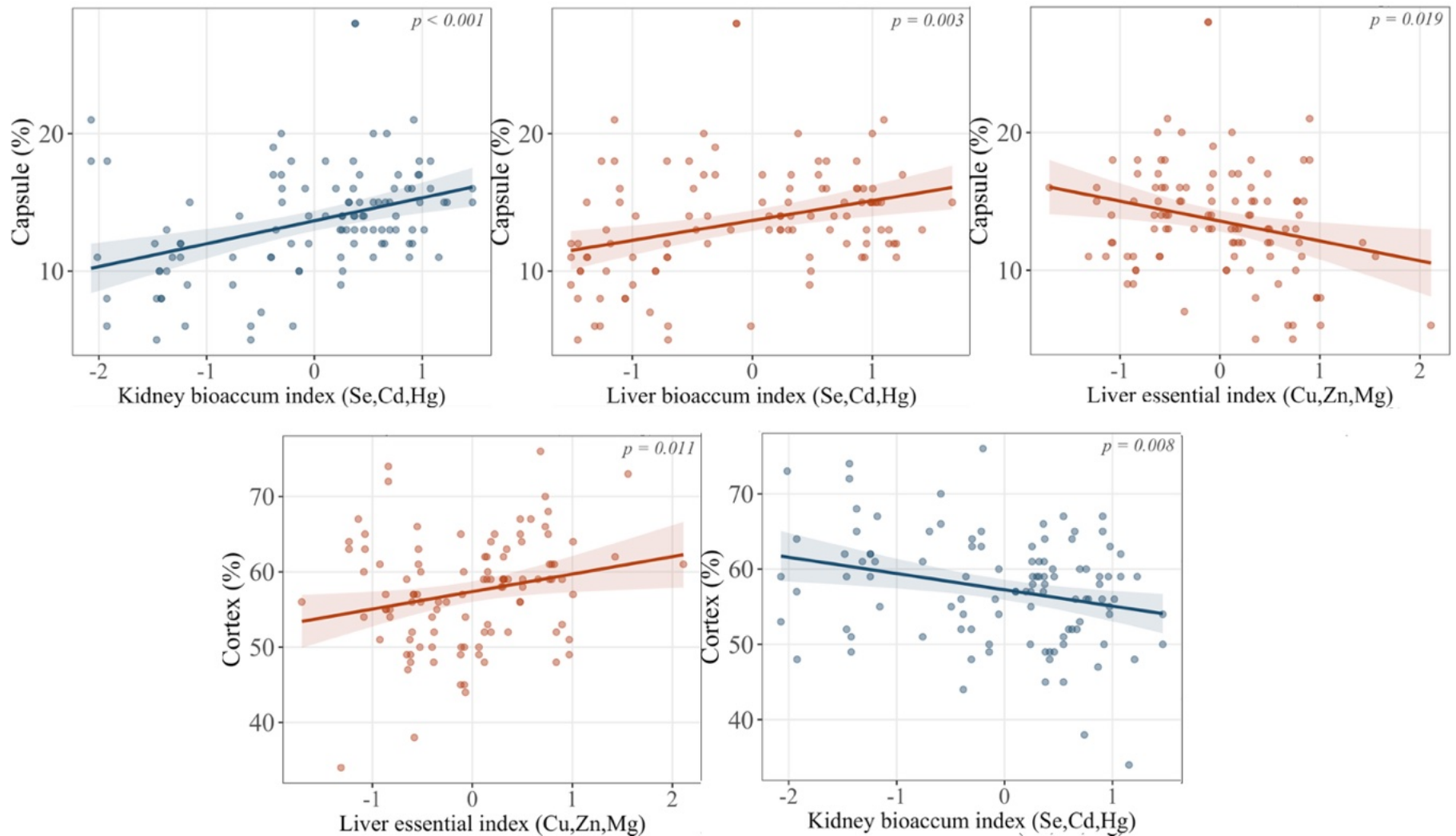


Figure 3.41 Predicted zonal proportions by metal z-score index ($n = 106$ glands, 71 animals). (A) kidney bioaccumulative index (Se, Cd, Hg) with capsule %; (B) liver bioaccumulative index with capsule %; (C) liver essential index (Cu, Zn, Mg) with capsule %; (D) liver essential index with cortex %; (E) kidney bioaccumulative index with cortex %. Lines show LMM population-level predictions; shaded ribbons represent 95% confidence intervals. Model coefficients and p -values are shown in the upper right corner of each panel.

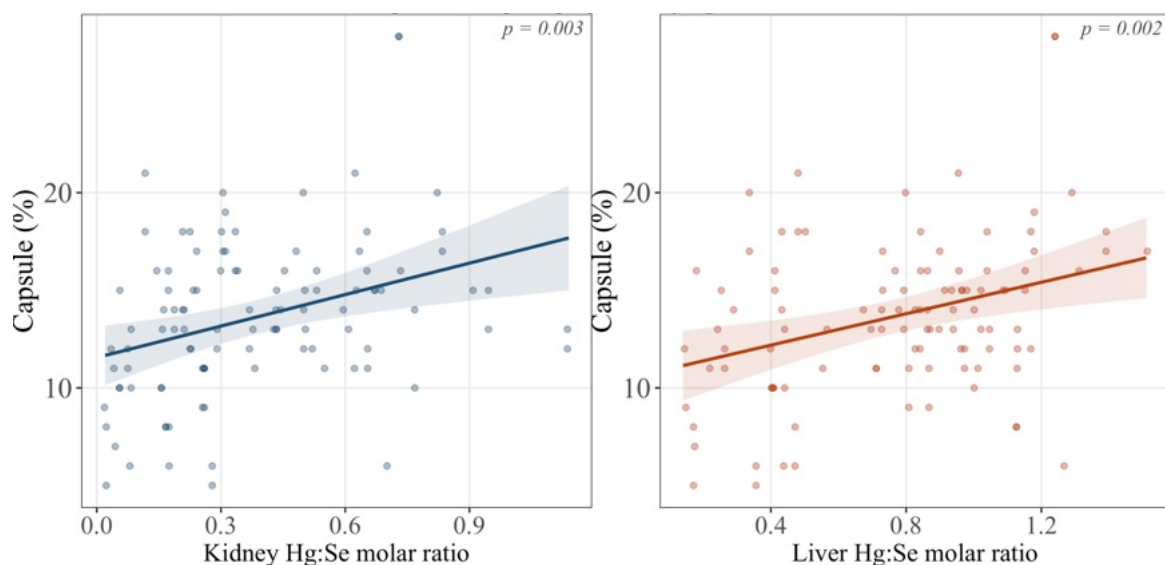


Figure 3.42 Predicted capsule proportion by Hg:Se molar ratio ($n = 106$ glands, 71 animals). (A) Kidney (blue) and (B) liver (orange). Lines show population-level predictions from linear mixed-effects models; shaded ribbons represent 95% confidence intervals. Model coefficients and p -values are shown in the upper right corner of each panel.

3.6.5 Metal index models

3.6.5.1 Bioaccumulative and essential element indices as predictors of C:M ratio

The kidney bioaccumulative index showed a marginal negative association with $\log(\text{C:M ratio})$ ($\beta = -0.076$, $SE = 0.043$, $df = 67.5$, $t = -1.74$, $p = 0.086$; Table 3.22), suggesting that animals with higher kidney concentrations of Se, Cd, and Hg tended toward lower C:M ratios, though this association did not reach conventional significance. The 95% confidence interval for the slope (-0.161 to 0.009) narrowly included zero. The ICC was 0.639, indicating that 63.9% of the variance in $\log(\text{C:M ratio})$ was attributable to between-individual differences (Table 3.23). The marginal R^2 was 0.037, indicating that the kidney bioaccumulative index explained approximately 3.7% of the fixed-effect variance.

The liver bioaccumulative index was not significantly associated with $\log(\text{C:M ratio})$ ($\beta = -0.041$, $SE = 0.043$, $df = 68.1$, $t = -0.95$, $p = 0.345$; Table 3.22). The direction of the effect was consistent with the kidney model (negative), but the magnitude was approximately half that of the kidney estimate and the confidence interval was wider (-0.126 to 0.044). The ICC was 0.645 and the marginal R^2 was 0.011, indicating negligible explanatory power (Table 3.23).

The kidney essential metal index was not significantly associated with log(C:M ratio) ($\beta = 0.075$, $SE = 0.054$, $df = 64.1$, $t = 1.40$, $p = 0.165$; Table 3.22). The effect was small and positive, with a wide confidence interval spanning zero (-0.030 to 0.180). The ICC was 0.637 and the marginal R^2 was 0.025 , indicating that kidney essential metal status explained very little variation in adrenal zonal proportions (Table 3.23).

The liver essential metal index showed a marginal positive association with log(C:M ratio) ($\beta = 0.099$, $SE = 0.055$, $df = 69.7$, $t = 1.79$, $p = 0.078$; Table 3.22), with the confidence interval narrowly including zero (-0.009 to 0.207). The ICC was 0.642 and the marginal R^2 was 0.038 (Table 3.23). While not statistically significant, this was the second strongest association among the four metal index models, suggesting a possible positive relationship between liver essential metal status and C:M ratio.

Table 3.22 Linear mixed-effects model predicting log(C:M ratio) from kidney (blue) and liver (orange) bioaccumulative element z-score index (Se, Cd, Hg) and essential element z-score index (Cu, Zn, Mg).

Predictor	β (Estimate)	SE	df	t	p	95% CI
Model: log(C:M) ~ K_bioaccum + (1 AnimalID)						
Intercept	0.697	0.040	66.8	17.54	<0.001	0.619 to 0.775
K_bioaccum	-0.076	0.043	67.5	-1.74	0.086	-0.161 to 0.009
Model: log(C:M) ~ K_essential + (1 AnimalID)						
Intercept	0.699	0.040	65.8	17.50	<0.001	0.621 to 0.777
K_essential	+0.075	0.054	64.1	1.40	0.165	-0.030 to 0.180
Model: log(C:M) ~ L_bioaccum + (1 AnimalID)						
Intercept	0.698	0.040	66.3	17.31	<0.001	0.619 to 0.777
L_bioaccum	-0.041	0.043	68.1	-0.95	0.345	-0.126 to 0.044
Model: log(C:M) ~ L_essential + (1 AnimalID)						
Intercept	0.699	0.040	67.4	17.56	<0.001	0.621 to 0.777
L_essential	+0.099	0.055	69.7	1.79	0.078	-0.009 to 0.207

Table 3.23 Variance components for individual ID random effects in the kidney (blue) and liver (orange) bioaccumulative and essential element index model of log(C:M ratio)

Random effect	Variance	SD
Model: log(C:M) ~ K_bioaccum + (1 AnimalID)		
Individual ID (random intercept)	0.0795	0.282
Residual	0.0449	0.212
Model: log(C:M) ~ K_essential + (1 AnimalID)		
Individual ID (random intercept)	0.0800	0.283
Residual	0.0455	0.213
Model: log(C:M) ~ L_bioaccum + (1 AnimalID)		
Individual ID (random intercept)	0.0823	0.287
Residual	0.0453	0.213
Model: log(C:M) ~ L_essential + (1 AnimalID)		
Individual ID (random intercept)	0.0800	0.283
Residual	0.0446	0.211

3.6.5.2 Bioaccumulative and essential element indices as predictors of adrenal composition

The bioaccumulative metal index showed the most consistent associations with adrenal zonal architecture. Higher kidney bioaccumulative burden (Se, Cd, Hg) was significantly associated with greater capsule percent ($\beta = 1.64$, $SE = 0.47$, $p < 0.001$) and lower cortex percent ($\beta = -2.45$, $SE = 0.89$, $p = 0.008$), with the liver bioaccumulative index showing the same directional pattern for both outcomes (capsule: $\beta = 1.46$, $p = 0.003$; cortex: $\beta = -1.68$, $p = 0.067$; Table 3.24). These associations suggested that animals with higher bioaccumulative metal burdens tended toward relatively greater capsular tissue at the expense of cortical tissue.

The essential metal index showed a contrasting pattern. Higher liver essential metal status was significantly associated with greater cortex percent ($\beta = 2.98$, $SE = 1.14$, $p = 0.011$) and lower capsule percent ($\beta = -1.49$, $SE = 0.62$, $p = 0.019$), suggesting a positive relationship between essential metal homeostasis and cortical tissue proportion. The kidney essential index showed trends in the same direction but did not reach significance. No significant associations were found between any metal index and medullary percentage (all $p > 0.20$; Table 3.24, 3.25), suggesting that medullary tissue proportion was not strongly related to metal burden in this sample.

Table 3.24 Linear mixed-effects model predicting adrenal composition from kidney (blue) and liver (orange) bioaccumulative (Se, Cd, Hg) and essential (Cu, Zn, Mg) element z-score indices

Predictor	β (Estimate)	SE	df	t	p	95% CI
Model: %Capsule ~ K_bioaccum + (1 AnimalID)						
Intercept	13.668	0.431	70.2	31.721	<0.001	12.823 to 14.512
K_bioaccum	1.6378	0.47	70.6	3.484	0.0009	0.717 to 2.559
Model: %Cortex ~ K_bioaccum + (1 AnimalID)						
Intercept	57.262	0.818	66.9	69.963	<0.001	55.658 to 58.866
K_bioaccum	-2.4519	0.8935	67.4	-2.744	0.0078	-4.203 to -0.701
Model: %Medulla ~ K_bioaccum + (1 AnimalID)						
Intercept	29.08	0.785	67	37.035	<0.001	27.541 to 30.619
K_bioaccum	0.7782	0.8581	67.8	0.907	0.3677	-0.904 to 2.460
Model: %Capsule ~ L_bioaccum + (1 AnimalID)						
Intercept	13.67	0.438	70.4	31.224	<0.001	12.812 to 14.528
L_bioaccum	1.4588	0.4693	71.1	3.109	0.0027	0.539 to 2.379
Model: %Cortex ~ L_bioaccum + (1 AnimalID)						
Intercept	57.261	0.841	66.6	68.118	<0.001	55.614 to 58.909
L_bioaccum	-1.6802	0.9027	67.9	-1.861	0.067	-3.449 to 0.089
Model: %Medulla ~ L_bioaccum + (1 AnimalID)						
Intercept	29.076	0.788	66.5	36.886	<0.001	27.531 to 30.621
L_bioaccum	0.1946	0.8486	68.5	0.229	0.8193	-1.469 to 1.858
Model: %Capsule ~ K_essential + (1 AnimalID)						
Intercept	13.66	0.461	70.2	29.604	<0.001	12.756 to 14.565
K_essential	-0.8236	0.6217	69.6	-1.325	0.1896	-2.042 to 0.395
Model: %Cortex ~ K_essential + (1 AnimalID)						
Intercept	57.289	0.842	66.3	68.045	<0.001	55.639 to 58.939
K_essential	1.996	1.1321	65.1	1.763	0.0826	-0.223 to 4.215
Model: %Medulla ~ K_essential + (1 AnimalID)						
Intercept	29.062	0.781	66.3	37.234	<0.001	27.532 to 30.591
K_essential	-1.1994	1.0469	64.4	-1.146	0.2561	-3.251 to 0.852
Model: %Capsule ~ L_essential + (1 AnimalID)						
Intercept	13.658	0.449	70.3	30.43	<0.001	12.778 to 14.537
L_essential	-1.4929	0.6196	71.2	-2.409	0.0186	-2.707 to -0.278
Model: %Cortex ~ L_essential + (1 AnimalID)						
Intercept	57.294	0.824	67.7	69.493	<0.001	55.678 to 58.910
L_essential	2.9802	1.1411	69.3	2.612	0.011	0.744 to 5.217
Model: %Medulla ~ L_essential + (1 AnimalID)						
Intercept	29.061	0.782	67.5	37.155	<0.001	27.528 to 30.594
L_essential	-1.3876	1.0859	69.9	-1.278	0.2055	-3.516 to 0.741

Table 3.25 Variance components for individual ID random effects in the kidney (blue) and liver (orange) bioaccumulative and essential element index model of adrenal composition

Random effect	Variance	SD
Model: %Capsule ~ K_bioaccum + (1 AnimalID)		
Individual ID (random intercept)	11.527	3.395
Residual	2.226	1.492
Model: %Cortex ~ K_bioaccum + (1 AnimalID)		
Individual ID (random intercept)	37.538	6.127
Residual	13.622	3.691
Model: %Medulla ~ K_bioaccum + (1 AnimalID)		
Individual ID (random intercept)	30.104	5.487
Residual	18.803	4.336
Model: %Capsule ~ L_bioaccum + (1 AnimalID)		
Individual ID (random intercept)	11.957	3.458
Residual	2.222	1.491
Model: % Cortex ~ L_bioaccum + (1 AnimalID)		
Individual ID (random intercept)	40.097	6.332
Residual	13.671	3.698
Model: % Medulla ~ L_bioaccum + (1 AnimalID)		
Individual ID (random intercept)	30.344	5.509
Residual	18.933	4.351
Model: %Capsule ~ K_essential + (1 AnimalID)		
Individual ID (random intercept)	13.459	3.669
Residual	2.228	1.493
Model: % Cortex ~ K_essential + (1 AnimalID)		
Individual ID (random intercept)	40.218	6.342
Residual	13.722	3.704
Model: % Medulla ~ K_essential + (1 AnimalID)		
Individual ID (random intercept)	29.457	5.427
Residual	18.984	4.357
Model: %Capsule ~ L_essential + (1 AnimalID)		
Individual ID (random intercept)	12.646	3.556
Residual	2.226	1.492
Model: % Cortex ~ L_essential + (1 AnimalID)		
Individual ID (random intercept)	38.339	6.192
Residual	13.476	3.671
Model: % Medulla ~ L_essential + (1 AnimalID)		
Individual ID (random intercept)	29.860	5.465
Residual	18.665	4.320

3.6.5.3 Hg:Se ratios

Selenium molar excess relative to mercury was consistent across this sample (Table 3.26). In kidney tissue, the mean Hg:Se ratio was 0.393 ± 0.258 , and only one of 71 animals exceeded a Hg:Se ratio of 1.0, indicating that mercury molar dominance over selenium was essentially absent in kidney tissue. Liver Hg:Se ratios were markedly higher (mean = 0.765 ± 0.348), with 19 of 71 animals exceeding the equimolar threshold. This pattern was consistent with the substantially greater liver enrichment of Hg relative to Se where Hg concentrations were 6.6-fold higher in liver than kidney, whereas Se was only 3.1-fold higher (Table 3.21).

Table 3.26 Hg:Se molar ratios in kidney (blue) and liver (orange) tissue

Ratio	<i>n</i>	Mean $\pm$ SD	Median	Min	Max	<i>n</i> (ratio > 1)
Kidney Hg:Se	71	0.393 ± 0.258	0.34	0.019	1.14	1 of 71
Liver Hg:Se	71	0.765 ± 0.348	0.841	0.144	1.51	19 of 71

3.6.5.4 Hg:Se ratios as predictors of adrenal composition

Hg:Se ratio was significantly and positively associated with capsule percent in both kidney and liver tissue. Higher kidney Hg:Se ratio was associated with greater capsule proportion ($\beta = 5.309$, $SE = 1.705$, $df = 69.6$, $t = 3.115$, $p = 0.003$; Table 3.27), and the liver Hg:Se ratio showed a comparable association ($\beta = 4.036$, $SE = 1.264$, $df = 70.9$, $t = 3.194$, $p = 0.002$; Table 3.27). The marginal R^2 for these models was 0.120 and 0.117 respectively, indicating that Hg:Se ratio explained approximately 12% of the fixed-effect variance in capsule proportion. These findings suggested that animals with greater mercury burden relative to selenium had proportionally more capsular tissue, consistent with the pattern observed for the bioaccumulative metal index.

No significant associations were found between Hg:Se ratio and cortex percent in either kidney ($p = 0.258$) or liver tissue ($p = 0.096$), though the liver estimate was directionally negative ($\beta = -4.139$, $p = 0.096$), suggesting a marginal trend toward lower cortical proportion at higher liver Hg:Se ratios that did not reach conventional significance. Medullary proportion was not associated with Hg:Se ratio in either tissue (kidney: $p = 0.649$; liver: $p = 0.991$; Table 3.27). Across all zonal proportion models, between-individual variation accounted for the majority of total variance, as reflected in the consistently high conditional R^2 values (range: 0.615-0.862; Table 3.28) relative to marginal R^2 values.

Table 3.27 Mixed-effects model predicting adrenal composition from kidney (blue) and liver (orange) Hg:Se ratios

Predictor	β (Estimate)	SE	df	t	p	95% CI
Model: %Capsule ~ K_HgSe_ratio + (1 AnimalID)						
Intercept	11.581	0.799	69.4	14.499	<0.001	10.015 to 13.147
K_HgSe_ratio	5.309	1.705	69.6	3.115	0.003	1.968 to 8.650
Model: %Cortex ~ K_HgSe_ratio + (1 AnimalID)						
Intercept	58.758	1.553	64.8	37.831	<0.001	55.714 to 61.802
K_HgSe_ratio	-3.783	3.316	65.2	-1.141	0.258	-10.282 to 2.716
Model: %Medulla ~ K_HgSe_ratio + (1 AnimalID)						
Intercept	29.619	1.431	64.3	20.704	<0.001	26.814 to 32.424
K_HgSe_ratio	-1.397	3.057	64.9	-0.457	0.649	-7.389 to 4.594
Model: %Capsule ~ L_HgSe_ratio + (1 AnimalID)						
Intercept	10.571	1.061	71.0	9.961	<0.001	8.491 to 12.651
L_HgSe_ratio	4.036	1.264	70.9	3.194	0.002	1.560 to 6.513
Model: %Cortex ~ L_HgSe_ratio + (1 AnimalID)						
Intercept	60.449	2.057	67.7	29.392	<0.001	56.417 to 64.481
L_HgSe_ratio	-4.139	2.448	67.5	-1.691	0.096	-8.937 to 0.659
Model: %Medulla ~ L_HgSe_ratio + (1 AnimalID)						
Intercept	29.053	1.931	69.1	15.045	<0.001	25.268 to 32.838
L_HgSe_ratio	0.027	2.298	68.8	0.012	0.991	-4.456 to 4.510

Table 3.28 Variance components for individual ID random effects in kidney (blue) and liver (orange) Hg:Se ratio on adrenal composition

Random effect	Variance	SD
Model: %Capsule ~ K_HgSe_ratio + (1 AnimalID)		
Individual ID (random intercept)	11.9280	3.4537
Residual	2.2285	1.4928
Model: %Cortex ~ K_HgSe_ratio + (1 AnimalID)		
Individual ID (random intercept)	41.4168	6.4356
Residual	13.7583	3.7092
Model: %Medulla ~ K_HgSe_ratio + (1 AnimalID)		
Individual ID (random intercept)	30.2236	5.4976
Residual	18.9435	4.3524
Model: %Capsule ~ L_HgSe_ratio + (1 AnimalID)		
Individual ID (random intercept)	11.8184	3.4378
Residual	2.2354	1.4951
Model: %Cortex ~ L_HgSe_ratio + (1 AnimalID)		
Individual ID (random intercept)	40.2282	6.3426
Residual	13.7866	3.7130
Model: %Medulla ~ L_HgSe_ratio + (1 AnimalID)		
Individual ID (random intercept)	30.3232	5.5067
Residual	18.9611	4.3544

3.6.5.5 Hg:Se ratios as predictors of C:M ratio

Neither kidney nor liver Hg:Se molar ratio was significantly associated with log(C:M ratio). The kidney Hg:Se model yielded a small negative and non-significant estimate ($\beta = -0.033$, $SE = 0.157$, $df = 64.5$, $t = -0.210$, $p = 0.834$; Table 3.29), with a confidence interval spanning zero (-0.342 to 0.275) and a marginal R^2 of less than 0.001, indicating no explanatory power. The liver Hg:Se model was similarly non-significant ($\beta = -0.076$, $SE = 0.118$, $df = 68.1$, $t = -0.649$, $p = 0.518$; Table 3.29), with a marginal R^2 of 0.005. These findings indicated that while Hg:Se ratio was significantly associated with capsule proportion (Section 3.6.5.4), it was not associated with the relative balance of cortical to medullary tissue, suggesting that the capsule responded independently to mercury-selenium dynamics from the deeper cortical and medullary zones (Table 3.30).

Table 3.29 Linear mixed-effects model predicting C:M ratios from kidney (blue) and liver (orange) Hg:Se ratio

Predictor	β (Estimate)	SE	df	t	p	95% CI
Model: log(C:M) ~ K_HgSe_ratio + (1 AnimalID)						
Intercept	0.711	0.074	64.0	9.653	<0.001	0.566 to 0.856
K_HgSe_ratio	-0.033	0.157	64.5	-0.210	0.834	-0.342 to 0.275
Model: log(C:M) ~ L_HgSe_ratio + (1 AnimalID)						
Intercept	0.757	0.099	68.4	7.654	<0.001	0.563 to 0.951
L_HgSe_ratio	-0.076	0.118	68.1	-0.649	0.518	-0.307 to 0.155

Table 3.30 Variance components for individual ID random effects in the kidney (blue) and liver (orange) Hg:Se ratio on C:M ratio

Random effect	Variance	SD
Model: log(C:M) ~ K_HgSe_ratio + (1 AnimalID)		
Individual ID (random intercept)	0.0833	0.2886
Residual	0.0455	0.2132
Model: log(C:M) ~ L_HgSe_ratio + (1 AnimalID)		
Individual ID (random intercept)	0.0826	0.2874
Residual	0.0455	0.2133

Chapter 4 – Discussion

4.1 Adrenal gland structure and life history variation in common dolphins

This study provided the first morphometric and histological characterisation of adrenal gland structure in common dolphins (*Delphinus delphis*) from New Zealand waters, establishing comprehensive baseline reference values for gross morphology, zonal composition, and bilateral variation. The significant findings related primarily to anatomy and architecture, particularly the dominant influence of age on gland growth, metric-specific bilateral asymmetry, and sex-based differences in zonal proportions. In contrast, stress-type and adrenal-specific pathology presence were largely non-significant predictors of adrenal composition. These patterns are discussed in the context of known cetacean and broader mammalian adrenal biology.

4.1.1 Gross anatomy and age-related growth

The gross morphology of common dolphin adrenal glands in the present study was consistent with descriptions from other delphinid species, with glands positioned cranial to the kidneys and exhibiting clear corticomedullary differentiation on cross-section (Clark et al., 2005, 2008). The shape of common dolphin adrenal glands ranged from thin and round to oblong, which was broadly consistent with descriptions of elongated to oval glands in other odontocetes, though gland shape has been noted to have high intra- and interspecific variability (Alonso-Almorox et al., 2026; Cozzi et al., 2017).

Mean adrenal weight (2.77 ± 0.15 g) and length (35.4 ± 0.6 mm) in common dolphins examined in this study were lower than values reported for common dolphins from Irish waters (acute: 5.93 ± 0.67 g; chronic: 6.70 ± 0.76 g; Santana et al., 2025), Atlantic bottlenose dolphins (R: 4.99 ± 0.51 g, L: 5.36 ± 0.56 g; (Clark et al., 2005), and common dolphins from the Canary Islands (sexually mature: 7.22-7.44 g; (Alonso-Almorox et al., 2026), though values were broadly consistent with the general range described for small odontocetes (Cozzi et al., 2017). Two explanations may account for the lower values observed here. First, the demographic composition of the present sample, which included 52.4% ($n = 54$) immature animals, likely lowered mean gland mass, given the strong age-driven growth documented in this study and previous literature (Clark et al., 2005). The exclusively mature composition of the Canary Islands

sample likely contributed to the particularly elevated values reported there. Alternatively, population-level and geographic differences in body size, diet, nutritional condition, or environmental stressor burden between New Zealand and northeast Atlantic common dolphin populations could not be excluded.

Within the present sample, age was the strongest predictor of adrenal weight (0.20 g per year; $p < 0.001$) and length (0.80 mm per year; $p < 0.001$), consistent with the well-documented pattern of continuous adrenal growth throughout life in cetaceans (Clark et al., 2005; Santana et al., 2025). Sex was not a significant predictor of adrenal weight, which was further consistent with findings in Atlantic bottlenose dolphins (Clark et al., 2005) and Irish common dolphins (Santana et al., 2025). However, a marginal positive association between female sex and adrenal length was observed. The elevated glucocorticoid demands associated with female reproductive investment, including the energetic costs of pregnancy and lactation, may have driven modest but detectable differences in gland dimensions without producing equivalent differences in mass.

Sex-based adrenal size dimorphism, favouring females, has been documented in rodents, where female rats (*Rattus norvegicus*) consistently exhibited larger adrenal cortices than males, driven by expansion of the *zona fasciculata* and *zona reticularis* (Leśniewska et al., 1990; Malendowicz, 1987), with analogous patterns in female mice (*Mus musculus*; Bielohuby et al., 2007). In cetaceans, cortisol rose significantly during late gestation in bottlenose dolphins (Steinman et al., 2016), consistent with chronically-elevated steroidogenic demand during pregnancy. Whether this translated to adrenal dimensional changes in female common dolphins warrants further investigation in reproductively characterised samples.

Lastly, glands with marked adrenal pathology were significantly heavier than non-diseased glands. This was consistent with cortical hyperplasia and glandular enlargement documented in association with chronic disease in odontocetes (Clark et al., 2006).

4.1.2 Histological organisation and life history influence on zonal composition

The histological organisation of common dolphin adrenal glands was consistent with the cetacean patterns described across odontocete species. This comprised a pseudolobulated appearance, cortex with three histologically discernible zones, a central medulla, and a prominent medullary band at the corticomedullary junction (Alonso-Almorox et al., 2026; Clark

et al., 2005, 2008; Cozzi et al., 2017; Santana et al., 2025; Vuković et al., 2010).

Pseudolobulation, arising from septal projections from the capsule into the cortical parenchyma, was evident in a subset of individuals and has been consistently reported across delphinids, varying in degree from minimal to pronounced (Clark et al., 2005, 2008; Vuković et al., 2010). The two chromaffin cell populations that were distinguishable within the medulla, with more darkly staining cells forming a peripheral medullary band and lighter cells occupying the central region, were consistent with the epinephrine- and norepinephrine-producing cell populations identified in bottlenose, spotted, and spinner dolphins (Clark et al., 2005, 2008; Vuković et al., 2010). Medullary protrusions and cortical invaginations, observed in the majority of individuals in the present study, were similarly noted in other delphinids and were interpreted as growth-related structural variants rather than pathological changes (Alonso-Almorox et al., 2026; Clark et al., 2005; Vuković et al., 2010).

Mean C:M ratios in common dolphins from New Zealand waters were higher than those reported for Atlantic bottlenose dolphins (*Tursiops truncatus*) under baseline conditions (R: 1.49 ± 0.14 , L: 1.61 ± 0.22 ; Clark et al., 2005), with elevated bilateral values in the present study (R: 2.11 ± 0.11 , L: 2.18 ± 0.12). The overall mean ratio of 2.15 for common dolphins fell between values reported for acutely- and chronically-stressed (1.96 ± 0.23 , 2.89 ± 0.19 , respectively) common dolphins in Irish waters (Santana et al., 2025). This may suggest that the New Zealand sample, which comprised a mixture of stress classifications, occupied an intermediate position consistent with a population not dominated by either acute or chronic stress pathology. A median C:M ratio of 1.04 reported in sexually mature common dolphins from the Canary Islands (Alonso-Almorox et al., 2026) was notably lower than both the present study and the Irish population. This is likely due to methodological differences, as derived C:M from linear cortical and medullary thickness measurements (Alonso-Almorox et al., 2026), rather than proportional area-based assessment done in this study. This suggested that intraspecific geographic variability in corticomedullary proportions across common dolphins warrants further investigation across standardised methods.

When compared by sex, female C:M ratios in this study (2.32 ± 0.10) were similar to female spinner dolphins (*Stenella longirostris*; 2.46 ± 0.18), while male ratios (1.95 ± 0.09) were notably lower than male spinner dolphins (3.21 ± 0.24 ; Clark et al., 2005). In spotted dolphins (*Stenella attenuata*), no significant sex difference in C:M ratio was detected (Clark et al., 2008),

contrasting with the female-dominant dimorphism observed here and the male-dominant pattern observed in spinner dolphins. This highlighted that the directionality of sexual dimorphism in adrenal composition was not consistent across delphinid species. While Santana et al. (2025) did not report C:M ratio by sex, precluding a direct intraspecific comparison between common dolphin populations, the sex-based difference documented here represents a novel finding for this species. Sex-based differences in adrenal zonal proportions may have reflected divergent steroidogenic and catecholaminergic demands between males and females, driven by reproductive investment, social behaviour, and life-history strategy (Ely & Henry, 1978; Haller et al., 1998; Handa et al., 1994; Lyraki & Schedl, 2021).

In common dolphins, the higher cortex proportion in females was consistent with elevated glucocorticoid and adrenal androgen demands associated with pregnancy and lactation (Gittleman & Thompson, 1988; Mann, 2000; Steinman et al., 2016, 2021), while the higher medulla proportion in males may reflect greater acute catecholaminergic requirements linked to male-male competition and agonistic behaviour (Ely & Henry, 1978; Haller et al., 1998; Murphy et al., 2005). The directionality of dimorphism variability across species, female-dominant in common dolphins, male-dominant in spinner dolphins and monomorphism in spotted dolphins, suggested that species-specific social systems, mating strategies, and ecological pressures likely shaped adrenal architecture in ways that are not yet fully understood.

The higher cortical proportion in female common dolphins compared to males was consistent with the elevated glucocorticoid demands associated with cetacean reproduction. This included cortisol increasing significantly during late gestation in bottlenose dolphins (Steinman et al., 2016), and during lactation, which may extend up to several years in odontocetes (Mann, 2000), representing the most energetically expensive phase of the mammalian reproductive cycle (Oftedal, 1985). This pattern was reflected also in mice, where female mice (*Mus musculus*) exhibited approximately twice the adrenal cortex volume of males (Bielohuby et al., 2007) and consistently showed greater HPA axis activation in response to stressors (Handa et al., 1994).

Conversely, the relatively higher medullary proportion in male mice may have reflected greater sympathoadrenal demand associated with agonistic and competitive behaviour, with dominant individuals showing preferential activation of the adrenomedullary axis during agonistic

encounters (Ely & Henry, 1978). This was consistent with the well-established role of catecholamines in preparing mammals for agonistic confrontation (Haller et al., 1998). Whether analogous patterns of sex-differentiated SAM axis engagement occurred in common dolphins remains untested, but the directionality of the morphological dimorphism observed here was consistent with this broader mammalian framework. Notably, no sex-age interaction was detected, indicating that this compositional dimorphism was stable across the lifespan, rather than emerging or intensifying with age. The weakening of the sex effect on C:M ratio in the linear mixed-effect model relative to the univariate comparison suggested that within-individual bilateral variability was attenuated, once accounting for individual identity.

Absolute cross-sectional area increased strongly with age (~5% per year), consistent with continuous adrenal growth throughout life in cetaceans (Clark et al., 2005; Santana et al., 2025). Proportional zonal composition, including C:M ratio, cortex percentage, and medulla percentage, was not associated with age, indicating that cortical and medullary compartments expanded at equivalent rates and that overall architecture was maintained across the lifespan. This pattern was consistent with isometric scaling of adrenal tissue, suggesting tightly regulated developmental programming of zonal architecture that was independent of gland size (Bielohuby et al., 2007; Clark et al., 2005). The exception was capsule percentage, which showed a modest but significant positive association with age (~ 0.13% per year) and was higher in sexually mature individuals. This suggested progressive capsular thickening consistent with connective tissue remodelling described in ageing mammalian adrenal glands (Dribben & Wolfe, 1947; Herbach et al., 2016). However, the elevated capsule proportion in sexually mature animals most likely reflected their older average age, rather than a maturity-specific biological transition. The high collinearity between the two variables and the likelihood ratio test confirmed that removing maturity from the model did not significantly reduce fit. The association between capsule proportion and age, therefore, remained the more robust finding. Whether this reflected a genuine fibrotic process requires histochemical confirmation.

Animals with a prominent medullary band exhibited significantly lower C:M ratios and higher medulla percentage, suggesting that band prominence reflected a genuinely larger epinephrine-producing compartment. The medullary band has been identified as the exclusive site of adrenaline-secreting chromaffin cells in odontocetes, with noradrenaline-producing cells

distributed more broadly throughout the medulla (Clark et al., 2005). That chronically-stressed bottlenose dolphins showed a significantly higher proportion of epinephrine-producing cells than acutely-stressed individuals (15.93% versus 6.7%; Clark et al., 2006) suggested that band prominence may have represented a morphological correlate of sustained sympathoadrenal demand, though immunohistochemical confirmation in common dolphins remains lacking.

4.1.3 Intraspecific variability

Across all models, individual identity was a consistently dominant source of variation, with the proportion of variance attributable to individual identity ranging from 47.5% for adrenal weight to 86.3% for cross-sectional area. Accordingly, adrenal morphology was more strongly predicted by which animal a gland originated than by any measured biological variable. This was further supported by bilateral glands from the same individual being more similar to each other than glands from different individuals. The pattern was robust across models differing substantially in their fixed-effect structure, suggesting that the sources of inter-individual variation were pervasive rather than attributable to any single unmeasured covariate. Correspondingly, the proportion of total variance explained by the measured fixed effects was low across all models, indicating that individual identity accounted for the majority of variance in adrenal morphology.

The lowest individual-level variance was adrenal weight, likely reflecting the susceptibility of this measure to transient physiological post-mortem factors, such as vascular congestion, hydration status, and tissue oedema, which may vary independently of underlying gland architecture (Brooks, 2016). Among the histological proportions, medulla percentage showed the lowest individual-level variance (61.2%). This was consistent with the more dynamic nature of catecholaminergic signalling through the SAM axis, in which medullary activity may have fluctuated more rapidly in response to acute stimuli than cortical steroidogenesis (Romero & Wingfield, 2016; Sapolsky et al., 2000). Conversely, the high individual-level variance for cross-sectional area (86.3%) suggested that overall gland size reflected a stable constitutional set-point maintained across the animal's lifetime. This possibly reflected heritable developmental trajectories and early-life programming of the HPA axis, rather than the transient physiological fluctuations that may have contributed to short-term variation in gland weight (Harris & Seckl, 2011; Weaver et al., 2004).

As such, the sources of this high inter-individual variation remained incompletely characterised. This likely reflected the cumulative and interacting effects of genetic background, nutritional history, reproductive demands, lifetime disease burden, and environmental stressor exposure, none of which were fully captured in the present cross-sectional dataset. In common dolphins specifically, organochlorines, such as polychlorinated biphenyls (PCBs) and climate-mediated changes to prey availability, have been shown to produce compounding effects on immune function and health outcomes (Santana et al., 2025; Williams et al., 2025). The influence of these stressors on adrenal morphology remains unquantified.

4.1.4 Adrenal pathology in comparative context

The 24.8% adrenal pathology prevalence documented in the present study, with lesion severity increasing with age and sexual maturity, was broadly consistent with the pattern of cumulative adrenal pathology reported in other cetacean populations. Adrenal cortical hyperplasia in 23-25% of rough-toothed dolphins (*Steno bredanensis*) during a mass stranding event (Ewing et al., 2020) was comparable to the present study, while 71% of St Lawrence Estuary beluga whales (*Delphinapterus leucas*) exhibited adrenal lesions, with cortical hyperplasia present in approximately 80% of affected animals (Lair et al., 1997). The substantially higher prevalence in St Lawrence belugas likely reflected the exceptional organochlorine burden characteristic of that population, though the available evidence linking contaminant exposure to adrenal lesion development remained associative rather than causal (Lair et al., 1997; Fair & Houde, 2023). Direct comparison across studies was limited by differences in lesion classification criteria, species, and population-level stressor burden, and no adrenal-specific prevalence data were currently available for common dolphins from other populations.

Cortical cysts, documented in 19 of 35 St Lawrence beluga whales (Lair et al., 1997) and in beluga adrenal nodules in an earlier survey (Lair et al., 1997), were not identified in any individual in the present study. This most plausibly reflected the differing contaminant profile of the New Zealand marine environment relative to the heavily contaminated St Lawrence Estuary, though differences in tissue preservation or sampling strategy, or a genuinely lower baseline prevalence of cystic lesions in this species, could not be excluded.

Neoplastic and hyperplastic adrenal changes were identified in two individuals (1.3% of assessed adrenal glands). One case (KS23-25Dd L) comprised cortical hyperplasia, consistent with

previously documented stress-associated adrenocortical responses in odontocetes (Kuiken et al., 1993; Clark et al., 2006). The second case (KS15-16Dd L) presented a medullary neoplastic lesion suggestive of a pheochromocytoma. Pheochromocytomas are tumours of the chromaffin cells of the adrenal medulla and are rare across mammalian species, with an estimated incidence of 2-8 per million in the human general population (National Cancer Institute, 2024) and 0.1-0.6% in hypertensive patients (Lenders et al., 2005). In domestic animals, the majority are incidental discoveries without overt clinical signs (Barthez et al., 1997; Luethy et al., 2016). Moreover, definitive classification of malignancy requires histological evidence of local invasion or distant metastasis rather than cytological features alone (National Cancer Institute, 2024). These tumours are typically categorised as functional or non-functional, depending on whether they secrete significantly elevated catecholamine concentrations.

In cetaceans, published reports remain sparse. De Guise et al. (1994) described a lesion, suggestive of pheochromocytoma, in a beluga whale characterised by nodular medullary expansion. Estep et al. (2005) reported a pheochromocytoma in a spotted dolphin (*Stenella frontalis*), comprising packets and cords of polygonal cells with granular argyrophilic cytoplasm. Thayer et al. (2018) documented bilateral pheochromocytomas in a white-beaked dolphin (*Lagenorhynchus albirostris*), with plasma normetanephrine and metanephrine concentrations substantially elevated relative to reference ranges for other cetacean species. They proposed that the tumour may have contributed to the animal stranding outside its typical geographic range.

The marked unilateral left adrenal enlargement in KS15-16Dd (20.95 g), substantially exceeding the sample mean, was consistent with a space-occupying neoplastic process. Whether the lesion was functional could not be determined from post-mortem data alone. Functional pheochromocytomas overproduce catecholamines and, in other species, were associated with cardiovascular sequelae, including cardiomyopathy and arrhythmia (Thayer et al., 2018). Histopathological examination of other tissues in this individual revealed mild myocardial lipofuscinosis and testicular atrophy, both of which could indicate chronic catecholamine excess. However, these findings were also consistent with advanced age, and lipofuscin accumulation in cardiomyocytes is a well-recognised age-related change in mammals. Without plasma metanephrine or normetanephrine measurements, definitive assignment of functional status was not possible. This case highlighted the value of comprehensive post-mortem adrenal screening

and tissue archiving, and underscored the importance of considering adrenal neoplasia as a differential in individuals exhibiting marked unilateral adrenal asymmetry.

Four of the pathological findings identified in the present study are currently undergoing further sectioning and immunohistochemical confirmation. Preliminary data are suggestive of toxoplasmosis, cortical hyperplasia and medullary neoplastic proliferation, and putative lipid infiltration. These findings are, therefore, provisional and further characterisation will be informed by the results of ongoing analyses.

4.2 Bilateral morphological variation and functional lateralisation

The left and right adrenal glands are anatomically distinct. The right adrenal vein drains directly into the caudal vena cava via a short vessel, while the left drains into the left renal vein via a longer course shared with the inferior phrenic vein (Cesmebasi et al., 2014). This asymmetric venous drainage imposes different haemodynamic conditions on each gland, providing a plausible anatomical basis for the bilateral morphological differences observed in the present study.

4.2.1 Gross-level asymmetry

The linear mixed-effects model indicated that left glands were significantly heavier than their right counterparts by approximately 0.42 g (15.1%). Conversely, right glands were longer than left by approximately 1.10 mm (3.2%). Notably, both differences were confirmed by paired analysis and robust to the exclusion of a single pathological outlier. No bilateral differences were detected in width or depth. The bilateral asymmetry was therefore metric-specific rather than reflecting a uniform scaling difference between sides. The left gland was heavier while the right was longer, indicating that each side developed a distinct morphological profile, rather than both dimensions scaling proportionally in the same direction. A left-heavier trend has been reported across cetacean studies, with Clark et al. (2005) noting that the left gland was typically heavier in bottlenose dolphins, and Alonso-Almorox et al. (2026) reporting numerically greater left adrenal mass in both sexes of common dolphins from the Canary Islands, though neither study detected a statistically significant bilateral mass difference. The present study, therefore, provided the first

statistically supported evidence of left-dominant adrenal mass asymmetry in a delphinid. A comparable pattern has been described in mice, where control animals consistently exhibited larger left than right adrenal glands (Droste et al., 2003). This asymmetry may be due to the higher venous back-pressure in the left gland, resulting from drainage into the renal vein rather than directly into the caudal vena cava (Cesmebasi et al., 2014), potentially promoting fluid retention and tissue engorgement and contributing to greater mass without proportional elongation. This mechanism has not been directly tested in cetaceans.

The directionality of the length asymmetry, favouring the right gland despite its lower mass, was not readily explained by venous haemodynamic differences alone. Alonso-Almorox et al. (2026) noted that the left gland tended to appear more slender in gross contour in common dolphins from the Canary Islands. This qualitative observation contrasted with the present finding of greater left mass alongside greater right length, suggesting that subjective shape impression and quantitative metric asymmetry may not align. Thus, the relationship between gross contour and linear dimensions warrants systematic investigation. The right-dominant length asymmetry may instead have reflected the distinct anatomical relationships of the right gland, which sat in closer proximity to the liver and caudal vena cava than the left (Cozzi et al., 2017). In humans, bilateral shape asymmetry has been described, with the right adrenal typically pyramidal and the left more semilunar, a difference attributed to the differing anatomical constraints imposed by adjacent structures on each side of the body (Cesmebasi et al., 2014). Whether analogous shape asymmetry exists in common dolphins has not been assessed in any published cetacean adrenal study, including those on bottlenose, spinner, spotted, or striped dolphins, and gross weight and length measurements alone could not resolve this question. However, differential spatial constraint on the right side may contribute to the observed metric-specific lateralisation. Beyond haemodynamic and anatomical factors, asymmetric splanchnic innervation of the adrenal glands has been demonstrated in mice, with greater supraspinal representation of the left adrenal than the right (Tóth et al., 2008), contributing to differential stimulation between sides. Developmental left-right patterning established during embryogenesis represented a further candidate, as visceral asymmetries in vertebrates were known to arise from conserved molecular cascades active prior to any postnatal functional influence (Levin, 2005).

4.2.2 Histological-level symmetry

Despite the gross-level asymmetries in weight and length, the bilateral asymmetry was one of scale rather than architecture. Both glands shared the same relative zonal composition, regardless of which was larger, with C:M ratio, capsule percentage, cortex **percentage**, and medulla **percentage** all showing no significant bilateral divergence. Left glands had significantly larger absolute cross-sectional and cortical areas, consistent with their greater overall mass, but effect sizes were small relative to inter-individual variability. The practical implication was that either adrenal gland may be used as a representative sample in zonal proportion analyses, without systematic bias being introduced by side of collection. This bilateral histological equivalence aligned with findings of no significant bilateral difference in C:M ratio in bottlenose dolphins (Clark et al., 2005, 2006), common dolphins from both Irish waters (Santana et al., 2025), and the Canary Islands (Alonso-Almorox et al., 2026). Across these studies, proportional composition was preserved despite bilateral differences in absolute size. This suggested that cortical and medullary compartments scaled proportionally under the same growth signals, irrespective of which side was larger, and that bilateral histological proportional equivalence may be a robust feature of adrenal organisation across delphinids.

4.3 Structure-function relationship in the adrenal cortex and medulla

The proportional composition of common dolphin adrenal glands, with the cortex constituting 57.5% and the medulla 28.9% of total cross-sectional area, provided a structural basis for inferring the relative contribution of steroidogenic and catecholaminergic pathways to adrenal function in this species. While direct measurement of hormone output, as well as zonal measurements within the cortex, was beyond the scope of the present study, the relative sizes of functional compartments were widely used as proxies for the physiological capacity of each axis (Clark et al., 2005; Cozzi et al., 2017). The following sections interpret these proportions in that context.

4.3.1 Cortical zonation and steroidogenic function

The cortical dominance observed in common dolphins from New Zealand waters, accounting for 57.5% of total cross-sectional area and a C:M ratio of 2.15, indicated that steroidogenic capacity

represented the primary functional output of the common dolphin adrenal gland. This ratio substantially exceeded the baseline values reported for Atlantic bottlenose dolphins under non-stressed conditions (R: 1.49 ± 0.14 , L: 1.61 ± 0.22 ; Clark et al., 2005), and as discussed in Section 4.1.2, fell intermediate between values reported for acutely- and chronically-stressed common dolphins from Irish waters (1.96 ± 0.23 and 2.89 ± 0.19 , respectively; Santana et al., 2025). This was consistent with a sample spanning a range of stress states rather than one dominated by either acute or chronic stress pathology. A higher C:M ratio implied proportionally greater steroidogenic tissue mass (Clark et al., 2005; Cozzi et al., 2017), suggesting that common dolphins allocated a larger fraction of adrenal mass to cortical hormone production than bottlenose dolphins, under equivalent baseline conditions.

This may be explained by species-level differences in HPA axis demand, cortical zonation, or the relative contribution of adrenal androgens to the hormonal milieu. Alternatively, the elevated C:M ratio may be due to differences in sample composition or population-level allostatic load rather than a fixed species-level architectural set-point. Alonso-Almorox et al. (2026) reported substantially lower C:M ratios in Canary Islands common dolphins and demonstrated that sexually mature individuals had significantly higher ratios than immature animals. If the present sample differed in age structure or sex ratio, this could have contributed to the elevated values independently of any stress-related effect. A population-level explanation also remained plausible, as New Zealand common dolphins were subject to documented bycatch pressure and environmental stressors that may have sustained elevated HPA axis activation and driven cortical hypertrophy relative to conspecifics in other regions (Clark et al., 2006; Santana et al., 2025). Distinguishing between these explanations would require comparative sampling across regions under standardised methods.

The cortex was functionally tripartite, and the functional significance of the cortical compartment depended on the relative contribution of each zona. The *zona glomerulosa* was of particular relevance in a marine context, as aldosterone-mediated renal sodium retention was integral to managing the chronic osmotic challenge of a hypertonic environment (Hart et al., 2015; Crocker, 2018; McClain et al., 2020). How mineralocorticoid demand varied across cetacean species inhabiting environments of differing salinity, remains poorly characterised. The *zona fasciculata*, broadly the largest cortical zone across mammals (Cozzi et al., 2017),

underpinned glucocorticoid output and HPA axis responsiveness. Its contribution to total cortical area was likely the dominant driver of the elevated C:M ratios observed in this population relative to bottlenose dolphins under baseline conditions (Clark et al., 2005). The *zona reticularis* contributed adrenal androgens, including DHEA and its precursors, which in odontocetes acted in concert with gonadal production to regulate reproductive function (Clark et al., 2008; Vuković et al., 2010).

The sex-based difference in cortical proportion (see section 4.1.2) was further interpretable through the function of the *zona reticularis*. As the primary source of adrenal androgen precursors, including DHEA and androstenedione, the *zona reticularis* may have contributed disproportionately to the greater cortical proportion observed in females, given the role of adrenal-derived androgens in supplementing circulating sex steroid production in female mammals (Clark et al., 2008; Vuković et al., 2010).

Discrete histological identification of zona boundaries was not systematically attempted in the present study, since reliable delineation of the *zona fasciculata* and *zona reticularis* required immunohistochemical markers, such as cytochrome P450 17A1 (CYP17A1) or 3-beta-hydroxysteroid dehydrogenase (3 β -HSD) in cetacean tissue (Vuković et al., 2010; Clark et al., 2008). Without zona-specific quantification, it was not possible to determine which cortical zone accounted for the greater female cortical proportion. Future studies incorporating zonal immunohistochemistry would allow this to be addressed directly and would permit the relative contribution of androgen-producing capacity to be compared across sex and reproductive state.

4.3.2 Medullary structure and catecholamine production

The adrenal medulla, comprising 28.9% of total cross-sectional area, represented the catecholaminergic compartment of the gland, with chromaffin cells producing adrenaline and noradrenaline in response to acute neural activation via the SAM axis. In odontocetes, epinephrine-producing chromaffin cells have been localised to the peripheral medullary band, with noradrenaline-producing cells distributed more broadly across the inner medulla (Clark et al., 2005; Vuković et al., 2010), though this cellular organisation was not directly assessed in the present study. The relative size of the medullary compartment and the prominence of the

medullary band were therefore used here as structural proxies for sympathoadrenal capacity, with the important caveat that morphometric proportion does not directly measure catecholamine output or the functional ratio of epinephrine to noradrenaline production.

The subjective scoring system for medullary band prominence employed here and in previous cetacean adrenal research received important methodological validation from the present findings. Animals classified as having a prominent band showed a medulla accounting for approximately 3.2% more of total cross-sectional area and a significantly lower C:M ratio, confirming that qualitative band assessment corresponded to genuine and measurable differences in medullary compartment size. The finding confirmed that qualitative assessment of band prominence corresponded to genuine and measurable differences in medullary compartment size. This had direct practical value for future comparative work, as band prominence can be used as a reliable proxy for elevated medullary proportion in studies where quantitative image analysis was not feasible. This pattern was further supported by findings in bottlenose dolphins, where chronically stressed animals showed thicker medullary bands and a significantly higher proportion of epinephrine-producing chromaffin cells (15.93% versus 6.7%; $p = 0.021$; Clark et al., 2006), suggesting that band prominence may represent a cumulative morphological response to sustained sympathoadrenal demand. Immunohistochemical confirmation of chromaffin cell phenotype distribution in common dolphins has not yet been performed and would be necessary to determine whether band prominence in this species similarly reflected epinephrine-producing cell expansion.

The higher medullary proportion in male compared to female (30.5% versus 27.6%) common dolphins, discussed in the context of sex-differentiated catecholaminergic demand in section 4.1.2, was further notable here. Superficially, this sex differences coincided with the validation of band prominence as a structural proxy for medullary size, suggesting that future studies scoring band prominence in males versus females may capture meaningful sex-based differences in sympathoadrenal capacity, without requiring quantitative image analysis. Furthermore, given the significant association between band prominence and C:M ratio, C:M ratio itself may have served as an indirect indicator of medullary band status in cases where histological scoring was not feasible, such as in degraded tissue samples or gross-only examinations.

4.4 Metal burden and adrenal health

Contaminant burden has demonstrable population-level health consequences in common dolphins. In a three-decade study of United Kingdom-stranded conspecifics, PCB blubber concentration was significantly associated with increased infectious disease mortality risk. This was independent of body condition, nutritional status, and disease state (Williams et al., 2025), establishing that contaminant burden operated on health outcomes through pathways that were not simply reducible to observable nutritional or pathological condition. The present study extended this framework by examining whether element burden, a class of contaminants characterised by lifelong bioaccumulation, was associated with structural remodelling of the adrenal gland, an organ central to stress physiology, immune regulation, and endocrine homeostasis in cetaceans.

4.4.1 *Tissue-specific metal partitioning and population burden*

The distribution of trace elements between kidney and liver in this New Zealand common dolphin population followed patterns broadly consistent with established toxicokinetics in marine mammals. Of the ten elements meeting detection thresholds in both tissues, nine showed statistically significant tissue-specific enrichment. Cadmium was the only element showing significantly higher concentrations in kidney than liver (7.87 ± 8.10 vs. 2.17 ± 2.68 mg/kg ww), reflecting its high-affinity binding to renal metallothionein and progressive accumulation across the lifespan. Mercury showed the greatest liver enrichment (6.6-fold higher in liver than kidney), consistent with hepatic demethylation of methylmercury and co-accumulation with selenium as insoluble HgSe granules (Kershaw & Hall, 2019). The essential elements, iron, zinc, copper, and magnesium, were all significantly enriched in liver tissue, consistent with the hepatic roles of these metals in haematopoiesis, enzymatic catalysis, and metabolism (Hansen et al., 2016). Aluminium was the sole element showing no significant tissue difference, consistent with its predominantly dietary and environmental source rather than active biological accumulation (Garcia-Garin et al., 2023). These patterns were consistent with the explanation that tissue-specific accumulation in this population reflected fundamental toxicokinetic properties of each element, rather than population-specific exposure conditions. The elevated mercury burden discussed below specifically warrants consideration in relation to the New Zealand marine environment.

4.4.2 Hepatic mercury in a comparative context

The mean liver Hg concentration in this population (45.8 ± 48.1 mg/kg ww; median 24.6 mg/kg ww; range 0.65-185 mg/kg ww, $n = 71$) was comparable in range to values reported from other common dolphin populations, where liver tissue has been directly measured. The most directly comparable dataset was that of Stockin et al. (2023), who reported liver Hg of 34.49 ± 50.28 mg/kg ww (range 2.10-210 mg/kg ww) in New Zealand common dolphins that were sampled between 2011 and 2013. However, 11 of their 20 individuals were represented in the present dataset, and these values, therefore, reflected shared rather than independent samples. Atlantic populations of common dolphins consistently reported lower hepatic Hg concentrations than the present study, ranging from 0.63-15 mg/kg ww on the Portuguese coast in 1998–2003 (Cecilio, 2006) to 0.5-66.0 mg/kg ww in a more recent Portuguese sample (Monteiro et al., 2016), and 1.53-48.48 mg/kg ww in Patagonian individuals (Machovsky-Capuska et al., 2020). Mediterranean populations were more comparable, with Sicilian individuals averaging 26.85 ± 5.32 mg/kg ww (Naccari et al., 2024) and Murcia coast animals ranging from 1.6-95.4 mg/kg ww (Martínez-López et al., 2019). The elevated Mediterranean values have been attributed to the generally higher mercury bioavailability in the semi-enclosed Mediterranean basin.

The most plausible explanation for the elevated hepatic burden observed here is the naturally elevated mercury loading characteristic of the New Zealand marine environment, where active volcanism and hydrothermal activity contribute geogenic mercury inputs to coastal and offshore waters (Stoffers et al., 1999). Trophic differences between common dolphin populations are comparatively modest and less likely to account for the magnitude of the geographic difference observed. An alternative explanation is that the high individual variation in both this study and Stockin et al. (2023), reflected in the large standard deviations relative to means, indicated that age structure and individual foraging history were stronger determinants of hepatic Hg than geographic location, a pattern consistent with the strong age effects documented below.

4.4.3 Bioaccumulative versus essential metal dynamics

The metal dataset revealed a clear distinction in accumulation dynamics between bioaccumulative and essential elements, consistent with their fundamentally different toxicokinetic properties. This was consistent with the known lifetime accumulation dynamics of

mercury and cadmium in marine mammals, and paralleled age-related bioaccumulative patterns reported in common dolphins from the Celtic Seas, Portuguese coast, and Patagonia (Gosnell et al., 2024; Monteiro et al., 2016; Machovsky-Capuska et al., 2020). In contrast, essential element indices showed no significant age or maturity effects, reflecting the tight homeostatic regulation of these metals, in which absorption, tissue distribution, and excretion were actively controlled to maintain physiological concentrations, irrespective of dietary intake or age (Hansen et al., 2016; Das et al., 2003). This distinction between dose-dependent bioaccumulative burden and regulated essential metal status underpinned the index-based modelling in subsequent sections. The explanation that bioaccumulative and essential elements represented fundamentally different biological processes, dose-dependent accumulation versus physiological regulation, was supported by the divergent age trajectories observed, where essential elements were equally dietary in origin yet show no age-related accumulation. This pattern could not be explained by differences in dietary source and was more consistent with homeostatic control (Hansen et al., 2016).

Furthermore, the strong covariation of bioaccumulative elements documented in the PCA structure indicated that mercury, cadmium, and selenium accumulate as a mixture in this population. The biological effects of simultaneous exposure to co-accumulated contaminants that may interact in additive or synergistic ways are more difficult to assess than single-element effects, but are likely to be more toxicologically significant than any single element considered in isolation (Kershaw & Hall, 2019; Williams et al., 2025). Notably, both bioaccumulative burden and adrenal capsule proportion increased significantly with age in this population, raising the possibility that associations between metal indices and adrenal architecture may partly reflect shared age-related trajectories rather than direct contaminant effects on glandular tissue. However, the opposing directional associations of essential metal indices, which share the same age-related dietary trajectory but showed no accumulation, provided a structural counter-argument to this interpretation. This suggested that the bioaccumulative associations point to something beyond a shared covariate with age.

4.4.4 Metal burden indices and Hg:Se ratios as predictors of adrenal architecture

Both metal burden and adrenal capsule proportion increased with age. While this raised the possibility of a shared age-related trajectory, direct contaminant effects could not be excluded. Several features of the data supported a genuine biological effect beyond age-related covariation.

Capsule proportion was the primary and most robust adrenal correlate of metal burden. Bioaccumulative burden in both kidney and liver predicted greater capsular tissue, with effect sizes exceeding those of age in the life history model. This represented the strongest associations that were observed across any adrenal composition model in this study. In contrast, higher essential metal status was associated with less capsular tissue, both in liver and kidney. This opposing directionality, bioaccumulative burden driving capsular expansion while essential metal status was associated with less capsule, was biologically meaningful. Essential and bioaccumulative elements share the same dietary route of exposure but followed fundamentally different accumulation trajectories, and their opposing associations with capsule proportion could not be explained by a shared confounding variable. The pattern was instead consistent with a dose-dependent toxic response driving connective tissue proliferation, rather than a non-specific correlate of overall metal exposure. A complementary and reciprocal pattern was evident in cortical tissue: higher bioaccumulative burden was associated with proportionally less cortex, while higher essential metal status predicted more cortex. This directional pairing, more capsule and less cortex with increasing bioaccumulative burden, less capsule and more cortex with higher essential status, was consistent with a structural shift in which connective tissue proliferated at the expense of functional steroidogenic parenchyma under increasing contaminant load. The bioaccumulative index of the kidney also showed a marginal negative association with C:M ratio, suggesting a trend toward lower relative cortical tissue with increasing cumulative burden.

The Hg:Se molar ratio provided a mechanistically specific refinement of the metal burden signal. Mercury burden, expressed as its molar ratio to selenium, was significantly associated with capsular expansion in both kidney and liver tissue, without producing detectable shifts in cortical, medullary, or C:M proportions. This was consistent with the known dual role of selenium in mercury toxicology: selenium sequestered mercury as insoluble HgSe, reducing its

acute bioavailability, but in doing so was progressively rendered unavailable for antioxidant selenoprotein synthesis (Kershaw & Hall, 2019). As the Hg:Se ratio approached equimolarity, oxidative defences within the fibrous capsule may be progressively undermined, accelerating connective tissue remodelling. In the present population, 19 of 71 individuals exceeded the equimolar Hg:Se threshold in liver tissue (Hansen et al., 2016), indicating that a substantial proportion of animals carried mercury burdens sufficient to compromise selenium-mediated protection. Capsular expansion is consistent with contaminant-driven connective tissue remodelling (Page-Karjian et al., 2020). Fibrotic lesions across the liver, kidney, lung, and thyroid have been documented concurrently with elevated metal burdens in stranded cetaceans, though causality remained difficult to establish in field settings. Documented associations between contaminant burden and adrenal pathology in marine mammals were more broadly consistent with this interpretation (Fair and Houde, 2023). The prominent fibrous capsule and septal architecture described in common dolphins (Alonso-Almorox et al., 2026) provided the structural context within which such remodelling may have occurred.

The medullary compartment showed no association with any metal index or Hg:Se ratio in either tissue. This suggested that catecholaminergic architecture was resistant to the contaminant burden levels documented here.

Overall, the directional pattern of higher bioaccumulative burden associated with greater capsular and lower cortical tissue and higher essential metal status with less capsule and more cortex pointed to two structurally distinct processes. Cortical reduction was consistent with experimental evidence of cadmium-induced adrenocortical cell apoptosis and necroptosis in rodent and porcine models (Zhao et al., 2008; Qi et al., 2021) and mercury-mediated impairment of adrenal steroidogenesis in experimental mice (Rana, 2014). Contaminant-associated cortical atrophy has also been documented in vivo in bottlenose dolphins following petroleum hydrocarbon exposure (Venn-Watson et al., 2015). This was consistent with progressive parenchymal loss under increasing bioaccumulative burden. Taken together, these patterns suggested that bioaccumulative metal burden may simultaneously drive connective tissue proliferation at the capsule and parenchymal loss within the cortex. This produced structural remodelling that was directionally opposed to the ACTH-driven cortical hyperplasia characteristic of chronic physiological stress.

4.4.5 Metal burden, stress, adrenal pathology and disease

Pathology presence, disease classification, and stress type were not significant predictors of adrenal composition, in contrast to the significant capsular and cortical associations with bioaccumulative metal indices. This dissociation indicated that the adrenal structural changes associated with metal burden were not simply a reflection of chronic physiological stress. In other cetacean species, chronic stress produced measurable increases in cortical mass via ACTH-driven adrenocortical hyperplasia (Kuiken et al., 1993; Clark et al., 2006; Santana et al., 2025). Here, stress classification was unrelated to adrenal composition while metal burden was associated with capsular expansion and cortical reduction, a directionally opposite pattern. This suggested contaminants were acting through a mechanism distinct from the classical HPA stress response. In a recent histomorphometric study of common dolphins from the Canary Islands, corticomedullary ratio did not differ significantly between animals classified as acute and non-acute cause of death (Alonso-Almorox et al., 2026). The authors concluded that corticomedullary ratio was more strongly influenced by developmental processes than by terminal pathological context, and that its interpretation as a direct structural proxy for chronic stress required caution.

To our knowledge, no prior study has directly linked tissue metal burden indices to quantitative histomorphometric adrenal outcomes in cetaceans. The primary aim advanced here was that chronic bioaccumulative burden drives structural remodelling of the adrenal capsule as a connective tissue response to metal-induced oxidative or inflammatory stress, with a reciprocal reduction in functional cortical parenchyma. This was consistent with known metal toxicokinetic and the broader mammalian literature on contaminant-driven adrenal pathology (Rana, 2014). An alternative explanation was that associations reflected shared age-related trajectories, rather than direct contaminant effects. This was substantially addressed by the opposing directional associations of both essential and bioaccumulative metal indices. Further support was provided by ontogenetic differences in corticomedullary ratio in common dolphin around the Canary Islands, where sexual maturity, rather than pathological context, was the primary driver of adrenal proportional architecture (Alonso-Almorox et al., 2026). One caveat is that a contribution from shared age-related trajectories could not be fully excluded from cross-sectional data alone.

4.4.6 Interpreting stress chronicity in stranded common dolphins

Interpreting adrenal morphology in the context of physiological stress required careful consideration of how stress chronicity was defined in post-mortem cetacean studies (Clark et al., 2006; Santana et al., 2025). The conventional framework of classifying stranding events as reflecting acute or chronic stress, based on cause of death, or stranding circumstance, carried inherent constraints (Kuiken et al., 1993; Clark et al., 2006; Santana et al., 2025). Animals rarely presented with pathological profiles that fall cleanly into a single category, and the interpretation of circumstantial evidence is inherently subjective. An animal classified with acute mortality (e.g., boat strike) may have harboured chronic underlying disease or infection, while an animal classified as chronically-stressed may have experienced acute deterioration that was superimposed on pre-existing conditions.

Furthermore, the distinction between acute and chronic stress may be more reliably assessed in living animals through behavioural and endocrinological monitoring, than via post-mortem pathological classification, where the temporal relationship between lesion development and death was often unclear (Romero & Wingfield, 2016; Atkinson et al., 2015). The present study attempted to address this in two ways: by assessing inflammatory cues from tissues across the entire animal, and by incorporating an acute-on-chronic category that captured concurrent evidence of both acute and chronic disease processes. However, these approaches operate within the same fundamental constraint. Stress state in stranded cetaceans was inferred rather than directly measured, and individuals may not have conformed neatly to any single category.

This methodological challenge was not unique to the present study. In a dedicated histomorphometric investigation of common dolphin adrenal architecture, Alonso-Almorox et al. (2026) found no significant difference in corticomedullary ratio between animals that died from acute events and those with progressive pathological conditions. They explicitly noted that cause-of-death classification may not fully capture the complexity of an individual's endocrine history. Similarly, Williams et al. (2025) found that body condition was not a significant predictor of infectious disease mortality risk in UK-stranded common dolphins. They concluded that contaminant burden and health outcomes in this species were not straightforwardly mediated by observable nutritional or pathological state.

Thus, the non-significant association between stress classification and adrenal composition should not be interpreted as evidence that stress has no adrenal effect in this species. Instead, it should be viewed as a reflection of the inherent difficulty of reliably characterising stress chronicity from post-mortem data. If anything, it makes the identification of significant metal burden associations against the same background noise more, rather than less, compelling.

4.5 Integrating structure, pathology and environmental exposure

The findings of this study converge on a coherent picture in which adrenal architecture in New Zealand common dolphins was shaped by a hierarchy of biological influences. Life history variables, principally age, sex, and maturity, were the dominant predictors of gross morphology and zonal proportions, consistent with well-characterised developmental and reproductive drivers of adrenal structure across cetaceans (Clark et al., 2005, 2006; Santana et al., 2025; Alonso-Almorox et al., 2026). Pathology, disease classification, and stress type were largely non-significant predictors of adrenal composition, a pattern now documented independently in common dolphins from the Canary Islands (Alonso-Almorox et al., 2026). This was consistent with the broader argument that cause-of-death classification was a poor proxy for endocrine history in stranded cetaceans. Against this background, the identification of significant bioaccumulative metal burden associations with capsular and cortical architecture represented a distinct and biologically coherent signal, one that was directionally opposed to the stress-driven pattern and robust to the same life history and pathology covariates that failed to explain adrenal composition.

It is worth noting that structural remodelling of the adrenal gland and functional compromise were not necessarily concurrent. Harvey and Sutcliffe (2010) demonstrated that normal circulating glucocorticoid levels were maintained in the face of impaired adrenocortical steroidogenesis through compensatory elevation of ACTH. This means that structural remodelling and functional compromise were not necessarily concurrent and that morphometric findings alone could not confirm functional adequacy.

Adrenal cortical atrophy in bottlenose dolphins appeared clinically silent, until animals were challenged by additional stressors, including cold temperatures, infection, or pregnancy (Venn-

Watson et al., 2015). At which point, adrenal crisis became life-threatening. The subclinical structural remodelling documented here, capsular expansion and modest cortical reduction, may therefore have represented an early or intermediate stage of contaminant-driven adrenal disruption, which has not yet translated into measurable functional compromise (Venn-Watson et al., 2015). The population-level significance of this was contextualised by evidence from other common dolphin populations. PCB burden was significantly associated with infectious disease mortality risk in United Kingdom-stranded common dolphins, independently of body condition (Williams et al., 2025). This established that chemical contaminant burden in this species had measurable health consequences, that were not straightforwardly captured by observable condition or disease state.

Whether the metal-associated adrenal structural changes documented here carry analogous long-term consequences for stress responsiveness, immune regulation, or reproductive capacity in free-ranging New Zealand common dolphins remains unknown. Longitudinal integration of structural assessment with functional endocrine biomarkers would be required to determine whether remodelling of this kind progressed toward functional insufficiency under sustained contaminant pressure, and represents an important direction for future research.

4.6 Study limitations

This study relies on adrenal gland samples collected from stranded and bycaught common dolphins, which introduces several inherent limitations associated with opportunistic post-mortem sampling. The availability of samples was constrained temporally and spatially, reflecting patterns of stranding effort, reporting, and carcass recovery, rather than systematic sampling across the population. As a result, the dataset may be biased toward specific geographic regions, seasons, or mortality events, potentially limiting the generalisability of findings to the broader population.

The use of stranded animals also introduced a potential health bias, as individuals that strand were more likely to have experienced underlying disease, injury, or physiological compromise prior to death (Díaz-Delgado et al., 2018). The inclusion of commercially bycaught animals and mass stranding events broadened the range of mortality contexts represented in the dataset

beyond individually stranded animals, which may have reduced but could not eliminate the potential for health-related sampling bias (Clark et al., 2006; Santana et al., 2025). The classification of stress chronicity from post-mortem data introduced further uncertainty, as stress state is inferred rather than directly measured, and individuals may not have conformed neatly to any single category (Alonso-Almorox et al., 2026).

Another limitation relates to carcass condition and tissue preservation. Variable decomposition states could have influenced histological integrity, tissue architecture, and the reliability of both morphometric and pathological assessments. Although samples with advanced autolysis were excluded, subtle cellular alterations associated with early decomposition could not be entirely ruled out and may have confounded interpretation of adrenal cortical and medullary features (Brooks, 2016; IJsseldijk et al., 2019). Similarly, post-mortem changes may have influenced the precision of regional quantification and corticomedullary ratio measurements (Clark et al., 2005, 2006; Santana et al., 2025; Alonso-Almorox et al., 2026). All planimetric annotations were performed by a single observer (SSM). While this eliminated inter-observer variability, it precluded quantification of intra-observer measurement error. A formal intra-observer repeatability assessment, for example re-annotation of a randomly selected subset of slides, was not undertaken, and future applications of this digital planimetric approach would benefit from repeated annotations to quantify measurement reliability.

Sample size constraints also limited the power of certain comparative analyses. While this study represented one of the more comprehensive assessments of adrenal gland structure in common dolphins to date, subdivision of the dataset by sex, sexual maturity, age class, mortality event, disease status, and bilateral comparisons reduced statistical power in some groups. As a result, mixed-effects modelling was employed to account for hierarchical structure and repeated measures where possible. However, some biologically relevant trends may have been undetected, due to limited representation within specific categories. Many statistical comparisons were conducted across multiple response variables and predictors, and no correction for multiple testing, such as Bonferroni or false discovery rate adjustment, was applied. Several associations rested on borderline p -values ($p < 0.10$) and should be interpreted with caution, as a proportion may not persist under correction. These results are therefore, considered suggestive rather than confirmatory and warrant confirmation in larger or independent datasets.

Finally, assessment of heavy metal and trace element exposure relied on hepatic and renal concentrations as proxies for whole-body and endocrine tissue exposure. While liver and kidney tissues were well-established indicators of contaminant burden (Frodello et al., 2000; Lischka et al., 2021; Stockin et al., 2021), direct measurement of metals within adrenal tissue was not feasible in this study. Furthermore, hepatic and renal metal concentrations represented the burden present at the time of death, rather than a direct measure of lifetime cumulative exposure. For mercury in particular, hepatic concentrations may have been influenced by nutritional condition and mobilisation of stored burden during periods of nutritional stress. This meant that concentrations in animals that experienced emaciation prior to death may not be directly comparable to those in animals in good body condition. While the strong age correlations documented for bioaccumulative indices were consistent with genuine lifetime accumulation, the possibility that condition-related redistribution contributed to metal burden estimates in some individuals could not be fully excluded.

In particular, Hg:Se molar ratios were calculated from liver and kidney tissue rather than adrenal tissue, and adrenal-specific accumulation patterns and selenium availability within the gland itself remain unmeasured. This limited the precision with which selenium-mediated oxidative defence within the capsular compartment could be inferred. The composite index approach, while biologically justified by the PCA covariation structure and validated against dominant PCA axes, meant that the contributions of individual elements to adrenal structural change could not be separated. Mercury, cadmium, and selenium co-vary strongly in this population, and it was not possible to determine whether any single element or specific combination drives the associations observed.

As samples represented a single time point per individual, the temporal sequence of contaminant accumulation and structural change could not be established, and associations between metal burden and adrenal architecture remained inferential. Therefore, inferred relationships between contaminant exposure and adrenal structural or pathological outcomes may have underestimated tissue-specific effects or failed to capture fine-scale adrenal accumulation patterns.

The absence of functional endocrine data represented a further limitation. No measurements of cortisol, aldosterone, or ACTH were available for the animals in this study, meaning that the

structural remodelling documented here could not be directly linked to functional adrenal compromise or insufficiency. It was, therefore, not possible to determine from morphometric data alone whether animals were experiencing subclinical adrenal insufficiency or maintaining functional adequacy through compensatory mechanisms.

Despite these limitations, this study provides valuable insight into adrenal gland structure, pathology, and potential environmental influences in a sentinel delphinid species. Importantly, the integration of histological, pathological, life history, and contaminant data offered a robust framework for interpreting adrenal health in stranded marine mammals and aimed to highlight key priorities for future research, including direct endocrine tissue contaminant analyses; longitudinal integration of structural assessment with functional endocrine biomarkers; and investigation of whether the subclinical adrenal remodelling documented here carried consequences for stress responsiveness, immune function, or reproductive capacity in free-ranging common dolphins.

4.7 Future research

This study provided the first comprehensive morphometric and pathological assessment of adrenal glands in New Zealand common dolphins, establishing baseline reference values for zonal composition, bilateral morphological variation, and pathological prevalence against which future changes could be measured. However, several limitations of the present dataset constrained interpretation and suggested clear avenues for further investigation.

First, the marginal association between kidney bioaccumulative metal burden and C:M ratio, together with the significant associations between Hg:Se molar ratio in kidney and liver and capsule proportion, suggested that contaminant exposure may have influenced adrenal architecture in ways not fully captured by the sample size available here. Larger datasets incorporating a wider range of contaminant burdens would help clarify whether these associations reflect genuine biological effects or artefacts of limited sample size. Direct measurement of metal concentrations within adrenal tissue itself, rather than relying on hepatic and renal proxies, would allow adrenal-specific accumulation patterns to be characterised and would strengthen inference about tissue-level contaminant effects.

Second, histological characterisation of capsule composition using Masson's trichrome or Sirius red staining to quantify collagen density and fibroblast activity would help determine whether the capsular expansion associated with bioaccumulative burden reflected a genuine fibrotic process. This could be paired with immunohistochemical markers of adrenal steroidogenic activity, such as CYP11A1 or 3 β -HSD, to link structural and functional changes. Integration of functional endocrine data, including cortisol, aldosterone, and ACTH measurements from blubber or adrenal tissue, alongside morphometric assessment would allow the relationship between structural remodelling and functional adrenal output to be directly evaluated. This would also help determine whether the subclinical changes documented here were associated with measurable endocrine compromise.

Finally, this study was limited to common dolphins from New Zealand waters. Comparative studies across common dolphin populations from different geographic regions and contaminant exposure contexts would help determine whether the structural patterns and metal-adrenal associations observed here were consistent across populations or reflected conditions specific to the New Zealand marine environment. The 24.8% pathology prevalence documented here, and the environmental and anthropogenic pressures that may have underpinned it, warrant investigation across other New Zealand cetacean species to determine whether these pressures produce comparable adrenal and pathological signatures.

Broader comparative work across odontocete species sharing New Zealand waters, and extending globally across delphinid species from ocean basins with contrasting contaminant regimes, would further help establish whether the metal-adrenal associations described here represent a conserved biological response to bioaccumulative burden or were specific to populations experiencing the elevated mercury loading characteristic of this region. Such comparisons are currently constrained by the scarcity of adrenal histomorphometric baseline data across cetacean species. Comprehensive quantitative characterisation of adrenal zonal composition existed for only a handful of delphinid species. Going forward, establishing species-specific reference frameworks is a prerequisite for meaningful comparative and pathological interpretation across the broader cetacean community.

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Appendices

Table A1 Summary of published cetacean adrenal gland morphometric, histological, and pathological data. Panel A includes studies reporting cortex:medulla (C:M) ratios, tissue proportions, or volumetric estimates. Panel B includes studies reporting gross morphology, descriptive histology, or pathological findings only, without quantitative compositional data. Composition is expressed as percentage of total cross-sectional area: C, cortex; M, medulla; Cap, capsule; O, other tissue including capsule and vasculature. Values are presented as mean $\pm$ *SE* unless otherwise stated. R, right adrenal gland; L, left adrenal gland; SBL, standard body length; —, not reported. Sei whale primary source inaccessible

Species	<i>n</i>	C:M Ratio	Composition	Adrenal Mass	Source
Panel A: Morphometric studies (C:M ratios, tissue proportions, or volumetric estimates)					
Common dolphin (acute; Ireland)	14	1.96 $\pm$ 0.23	66%C / 31%M / 3%O	5.93 $\pm$ 0.67 g	Santana et al., 2025
Common dolphin (chronic; Ireland)	19	2.89 $\pm$ 0.19	71%C / 25%M / 4%O	6.70 $\pm$ 0.76 g	Santana et al., 2025
Common dolphin (female; New Zealand)	R:32 L: 30	R: 2.23 $\pm$ 0.13 L: 2.41 $\pm$ 0.16	59%C / 28%M / 14%Cap	R: 2.70 $\pm$ 0.19 g L: 2.77 $\pm$ 0.25 g	This study
Common dolphin (male; New Zealand)	R:28 L: 23	R: 1.92 $\pm$ 0.08 L: 1.99 $\pm$ 0.16	56%C / 30%M / 14%Cap	R: 2.47 $\pm$ 0.25 g L: 3.17 $\pm$ 0.61 g	This study
Bottlenose dolphin (baseline; Atlantic)	R: 28 L:31	R: 1.49 $\pm$ 0.141 L: 1.61 $\pm$ 0.219	48%C / 41%M / 11%O	R: 4.99 $\pm$ 0.513 g L: 5.36 $\pm$ 0.558 g	Clark et al., 2005
Bottlenose dolphin (acute; Atlantic)	31	1.22 $\pm$ 0.059	48%C / 41%M / 11%O	5.2 $\pm$ 0.51 g	Clark et al., 2006

Bottlenose dolphin (chronic; Atlantic)	37	1.61 ± 0.125	53%C / 36%M / 11%O	11.01 ± 0.57 g	Clark et al., 2006
Spotted dolphin	18	2.34 ± 0.79	64.4%C / 29.4%M / 6.2%O	—	Clark et al., 2008
Spinner dolphin (female)	10	2.46 ± 0.18	65%C / 22.7%M / 7.7%O	—	Clark et al., 2008
Spinner dolphin (male)	4	3.21 ± 0.24	71%C / 27.3%M / 5.6%O	—	Clark et al., 2008
Harbour porpoise	28	—	volumetric estimates only	0.90–14.70 g	Kuiken et al., 1993
Chinese river dolphin	6	6.59	cortex-dominated gland	—	Zhongjie, 1988
Sei whale	—	2.65 (1.09–10.1)	—	—	Miyazawa & Usui, 1983 (as cited in Clark et al., 2005)
Pygmy sperm whale	2	$\geq 4:1$	>80% cortex	—	Carballeira et al., 1987
Gervais' beaked whale	2	$\geq 4:1$	>80% cortex	—	Carballeira et al., 1987
Panel B: Gross morphology, descriptive histology, and pathology studies					
Bottlenose dolphin (<175 cm SBL)	R: 7 L: 7	— —	— —	R: 2.5 ± 1.2 g L: 2.6 ± 1.2 g	Turner et al., 2006
Bottlenose dolphin (175–225 cm SBL)	R: 19 L: 17	— —	— —	R: 6.0 ± 1.5 g L: 6.3 ± 1.9 g	Turner et al., 2006

Bottlenose dolphin (>225 cm SBL)	R: 36 L: 36	— —	— —	R: 11.5 ± 3.8 g L: 11.3 ± 3.3 g	Turner et al., 2006
Bottlenose dolphin (Adriatic)	11	—————	Descriptive histology only	—————	Vuković et al., 2010
Striped dolphin	5	—————	Descriptive histology only	—————	Vuković et al., 2010
Hourglass dolphin	R: 1 L: 1	— —	— —	R: 8 g L: 7 g	Graïc et al., 2025
Spectacled porpoise	R: 3 L: 3	— —	— —	R: 7–19.2 g L: 8–18.4 g	Graïc et al., 2025
Blue whale	—	—	—	600–1200 g	Burn et al., 1951
Fin whale	—	—	—	600–800 g	Burn et al., 1951
Rough-toothed dolphin	46	—————	Pathology only	—————	Ewing et al., 2020
Beluga whale	35	—————	Pathology only	—————	Lair et al., 1997

Table A2 Individual sample data for common dolphins (*Delphinus delphis*) that were examined post-mortem in this study (n = 103). Age is reported in growth layer groups (GLG); individuals with unerupted teeth were assigned an age of 0. Disease score is reported separately for right (R) and left (L) adrenal glands where available. D0, non-diseased; D1, disease present but unlikely significant to the animal; D2, disease likely to have had a clinical effect on the animal; UK, unknown due to marked autolysis; PM, post-mortem; DCC, decomposition condition category; —, data unavailable

ID	Age	Sex	Sexual maturity	DCC code	Stress type	Disease score (R)	Disease score (L)
W08-28Dd	2	Female	Immature	3	Indeterminate	D0 - Non-Diseased	D0 - Non-Diseased
KS10-15Dd	0.75	Male	Immature	2	Chronic	UK (Autolysis)	UK (Autolysis)
KS11-10Dd	14	Male	Mature	4	Chronic	UK (Autolysis)	UK (Autolysis)
KS11-13Dd	17	Male	Mature	2	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS11-14Dd	12	Female	Mature	3	Acute-on-Chronic	D2 - Disease	D2 - Disease
KS11-27Dd	2	Male	Immature	1	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS11-39Dd	2	Female	Immature	2	Acute	D0 - Non-Diseased	D0 - Non-Diseased
KS11-50Dd	14	Female	Mature	2	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS11-50aDd	0	Female	Immature	2	Acute	D0 - Non-Diseased	D0 - Non-Diseased
KS11-52Dd	8	Male	—	3-4	Indeterminate	UK (Autolysis)	D0 - Non-Diseased
KS12-13Dd	8	Female	Mature	3	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS12-14Dd	11	Male	Immature	2	Indeterminate	D0 - Non-Diseased	D0 - Non-Diseased
KS12-15Dd	20	Female	Mature	2	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS12-17Dd	1.5	Male	Immature	2	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS12-18Dd	1	Female	Immature	2-3	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS12-23Dd	2.5	Male	Immature	2	Acute	D0 - Non-Diseased	D0 - Non-Diseased
KS13-03Dd	12	Female	—	4-5	Indeterminate	UK (Autolysis)	UK (Autolysis)

KS13-07Dd	0.5	Male	Immature	3	Acute-on-Chronic	Not sampled PM	D0 - Non-Diseased
KS13-10Dd	18	Female	Mature	3-4	Acute-on-Chronic	UK (Autolysis)	UK (Autolysis)
KS13-12Dd	3	Male	Immature	3-4	Acute	D0 - Non-Diseased	D0 - Non-Diseased
KS14-34Dd	11	Female	Mature	3	Acute	D0 - Non-Diseased	D0 - Non-Diseased
KS14-38Dd	16	Male	Mature	2	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS14-39Dd	2	Female	Immature	3	Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS14-42Dd	1	Female	Immature	2	Indeterminate	D0 - Non-Diseased	D0 - Non-Diseased
KS14-62Dd	0	Female	Immature	2	Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS14-63Dd	0	Male	Immature	2-3	Indeterminate	D0 - Non-Diseased	D0 - Non-Diseased
KS15-16Dd	26	Male	Mature	3	Chronic	D1 - Diseased	D1 - Diseased
KS15-19Dd	11	Female	Mature	2	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS15-20Dd	0.5	Female	Immature	2	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS15-21Dd	4	Female	Immature	2	Acute-on-Chronic	D1 - Diseased	D0 - Non-Diseased
KS16-06Dd	0.5	Female	Immature	2-3	Indeterminate	UK (Autolysis)	UK (Autolysis)
KS17-01Dd	12	Male	Mature	2	Acute	D1 - Diseased	D1 - Diseased
KS17-02Dd	11.5	Male	Mature	2	Chronic	D1 - Diseased	D1 - Diseased
KS17-03Dd	16	Male	Mature	2	Indeterminate	D0 - Non-Diseased	D0 - Non-Diseased
KS19-10Dd	7	Female	Immature	2-3	Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS19-12Dd	9	Male	Immature	2-3	Indeterminate	D0 - Non-Diseased	D0 - Non-Diseased
KS19-13Dd	5.5	Female	Immature	2-3	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS19-14Dd	10	Female	Mature	3	Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS19-16Dd	9	Female	Immature	2	Acute	D0 - Non-Diseased	D0 - Non-Diseased

KS19-17Dd	12	Female	Mature	2-3	Indeterminate	D0 - Non-Diseased	D0 - Non-Diseased
KS19-18Dd	0.25	Male	Immature	2	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS19-19Dd	11	Female	Mature	2	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS19-20Dd	12	Female	Mature	2	Acute-on-Chronic	UK (Autolysis)	UK (Autolysis)
KS19-21Dd	15	Female	Mature	2-3	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS19-22Dd	18	Female	Mature	2	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS19-23Dd	—	Female	Mature	2-3	Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS19-26Dd	15	Female	Mature	2-3	Acute	D0 - Non-Diseased	D0 - Non-Diseased
KS19-38Dd	0	Female	Immature	2-3	Acute	Not sampled PM	D0 - Non-Diseased
KS19-39Dd	8	Female	Mature	2	Indeterminate	D0 - Non-Diseased	D0 - Non-Diseased
KS20-05Dd	9.5	Male	Mature	3	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS20-30Dd	2	Male	Immature	2-3	Indeterminate	D0 - Non-Diseased	D0 - Non-Diseased
KS21-01Dd	12	Female	Mature	3	Acute-on-Chronic	UK (Autolysis)	UK (Autolysis)
KS21-31Dd	15	Female	Mature	2	Acute-on-Chronic	D1 - Diseased	D1 - Diseased
KS21-41Dd	12	Female	Mature	1-2	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS21-42Dd	2	Female	Immature	2-3	Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS22-03Dd	9	Female	Mature	2	Acute	D0 - Non-Diseased	UK (Autolysis)
KS22-07Dd	2	Female	Immature	3-4	Acute	UK (Autolysis)	UK (Autolysis)
KS22-08Dd	2.5	Female	Immature	2	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS22-48Dd	25	Female	Mature	2	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS22-49Dd	2	Female	Immature	2	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS22-52Dd	11.5	Male	Mature	3	Acute	D0 - Non-Diseased	Not sampled PM

KS22-53Dd	1	Male	Immature	2-3	Acute-on-Chronic	D0 - Non-Diseased	Not sampled PM
KS22-59Dd	1	Male	Immature	2	Indeterminate	D0 - Non-Diseased	Not sampled PM
KS22-73Dd	0	Male	Immature	3	Acute	UK - Unknown	Not sampled PM
KS22-77Dd	0	Male	Immature	2-3	Acute	Not sampled PM	UK (Autolysis)
KS23-03Dd	1.5	Female	Immature	3	Acute	UK (Autolysis)	Not sampled PM
KS23-04Dd	0.5	Female	Immature	2	Acute	D0 - Non-Diseased	Not sampled PM
KS23-05Dd	20	Female	Mature	1	Chronic	D0 - Non-Diseased	Not sampled PM
KS23-06Dd	0.25	Female	Immature	1-2	Acute-on-Chronic	D0 - Non-Diseased	Not sampled PM
KS23-07Dd	13	Female	Mature	1	Acute-on-Chronic	D0 - Non-Diseased	Not sampled PM
KS23-08Dd	2.5	Male	Immature	2	Acute-on-Chronic	D0 - Non-Diseased	Not sampled PM
KS23-09Dd	8	Female	Immature	2	Chronic	D0 - Non-Diseased	Not sampled PM
KS23-10Dd	14	Female	Mature	2	Acute-on-Chronic	D0 - Non-Diseased	Not sampled PM
KS23-11Dd	17	Female	Mature	2	Acute-on-Chronic	D0 - Non-Diseased	Not sampled PM
KS23-12Dd	0	Male	Immature	2	Acute-on-Chronic	D0 - Non-Diseased	Not sampled PM
KS23-13Dd	3	Female	Immature	3	Acute-on-Chronic	UK	Not sampled PM
KS23-42Dd	21	Male	Mature	1	Chronic	D0 - Non-Diseased	Not sampled PM
KS23-43Dd	16.5	Male	Mature	1	Acute	D0 - Non-Diseased	Not sampled PM
KS23-44Dd	9.5	Male	Mature	2	Acute-on-Chronic	Not sampled PM	D0 - Non-Diseased
KS23-45Dd	31	Male	Mature	2	Acute	Not sampled PM	D0 - Non-Diseased
KS23-46Dd	24	Male	Mature	1	Acute	Not sampled PM	D0 - Non-Diseased
KS23-47Dd	11.5	Male	Mature	2	Acute-on-Chronic	D1 - Diseased	Not sampled PM
KS23-50Dd	12	Male	Mature	2	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased

KS23-54Dd	0.5	Female	Immature	3	Acute-on-Chronic	UK (Autolysis)	Not sampled PM
KS23-57Dd	4	Male	Immature	2-3	Acute-on-Chronic	D0 - Non-Diseased	Not sampled PM
KS23-58Dd	7	Male	Immature	2-3	Acute-on-Chronic	D0 - Non-Diseased	Not sampled PM
KS24-48Dd	0	Male	Immature	2-3	Acute	UK (Autolysis)	UK (Autolysis)
KS24-54Dd	0	Male	Immature	3	Acute	UK (Autolysis)	Not sampled PM
KS24-59Dd	10	Female	Immature	3	Acute	UK (Autolysis)	Not sampled PM
KS24-68Dd	0	Female	Immature	3	Indeterminate	Not sampled PM	D0 - Non-Diseased
KS24-81Dd	17.5	Male	Mature	2	Acute	D0 - Non-Diseased	D0 - Non-Diseased
KS24-86Dd	2	Male	Immature	2	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS24-88Dd	1.5	Female	Immature	2	Acute	D0 - Non-Diseased	D0 - Non-Diseased
KS24-96Dd	0	Male	Immature	2	Indeterminate	D0 - Non-Diseased	D0 - Non-Diseased
KS24-97Dd	6	Male	Immature	2-3	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS25-07Dd	12	Male	Mature	1	Acute	D0 - Non-Diseased	D0 - Non-Diseased
KS25-08Dd	11	Male	Mature	1	Acute-on-Chronic	D1 - Diseased	D0 - Non-Diseased
KS25-09Dd	11.5	Female	Mature	1-2	Acute	D0 - Non-Diseased	D0 - Non-Diseased
KS25-10Dd	7	Female	Immature	1-2	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS25-11Dd	0	Male	Immature	1	Acute	D0 - Non-Diseased	D0 - Non-Diseased
KS25-61Dd	2.5	Female	Immature	2-3	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased
KS25-67Dd	19	Female	Mature	2	Acute	D0 - Non-Diseased	D0 - Non-Diseased
KS25-68Dd	18	Female	Mature	3	Acute-on-Chronic	D0 - Non-Diseased	D0 - Non-Diseased

Table A3 Adrenal gland gross morphometric and histological composition variables in common dolphins (*Delphinus delphis*) from New Zealand waters, by side and sex. Gross morphometric variables (weight, length, width, depth) were reported for all 176 adrenal glands (n = 96 right, n = 80 left); histological composition variables (C:M ratio, cortex, medulla, and capsule proportions) for the subset meeting quality criteria (n = 113 glands from 75 individuals). Pooled values combine right and left measurements per sex. C:M, cortex-to-medulla ratio; SE, standard error; SD, standard deviation; R, right; L, left

Variable	Group	<i>n</i>	Mean	<i>SE</i>	<i>SD</i>	Median	Min	Max
Weight (g)								
	All samples	176	2.76	0.15	2.05	2.68	0.28	20.95
	Right	96	2.61	0.15	1.48	2.63	0.28	7.95
	Left	80	2.93	0.29	2.58	2.73	0.32	20.95
	Female R	56	2.7	0.19	1.41	2.68	0.28	7.68
	Female L	47	2.77	0.25	1.69	2.67	0.32	8.48
	Female (pooled)	103	2.73	0.15	1.54	2.67	0.28	8.48
	Male R	40	2.47	0.25	1.58	2.5	0.43	7.95
	Male L	33	3.17	0.61	3.49	3	0.46	20.95
	Male (pooled)	73	2.79	0.31	2.62	2.73	0.43	20.95
Length (mm)								
	All samples	176	35.42	0.62	8.26	36.17	13.36	59.14
	Right	96	36.21	0.83	8.12	36.55	18.64	53.9
	Left	80	34.46	0.94	8.37	35.55	13.36	59.14
	Female R	56	37.49	0.97	7.29	37.91	19.23	53.9
	Female L	47	34.71	1.18	8.06	36.14	16.36	52.47
	Female (pooled)	103	36.22	0.76	7.74	37.77	16.36	53.9

Male R	40	34.43	1.42	8.96	35.38	18.64	53.12
Male L	33	34.12	1.55	8.91	35.05	13.36	59.14
Male (pooled)	73	34.29	1.04	8.88	35.22	13.36	59.14
Width (mm)							
All samples	176	15.64	0.31	4.05	16.05	1.53	26.88
Right	96	15.5	0.41	4.01	15.78	1.53	24.56
Left	80	15.8	0.46	4.12	16.13	7.53	26.88
Female R	56	15.73	0.55	4.12	15.91	1.53	24.56
Female L	47	16.33	0.62	4.27	16.78	7.53	26.88
Female (pooled)	103	16	0.41	4.18	16.39	1.53	26.88
Male R	40	15.18	0.62	3.89	15.68	7.48	21.2
Male L	33	15.05	0.67	3.82	15.21	8.07	24.1
Male (pooled)	73	15.12	0.45	3.83	15.56	7.48	24.1
Depth (mm)							
All samples	176	8.69	0.18	2.43	8.61	3.39	17.13
Right	96	8.64	0.24	2.31	8.54	3.72	16.36
Left	80	8.74	0.29	2.57	8.67	3.39	17.13
Female R	56	8.73	0.3	2.23	8.64	3.72	16.36
Female L	47	8.75	0.32	2.2	8.64	3.82	14.24
Female (pooled)	103	8.74	0.22	2.21	8.64	3.72	16.36
Male R	40	8.52	0.39	2.44	8.17	4.27	13.52
Male L	33	8.73	0.53	3.05	8.82	3.39	17.13

Male (pooled)	73	8.61	0.32	2.72	8.24	3.39	17.13
C:M ratio							
All samples	113	2.15	0.07	0.74	2.05	0.62	4.56
Right	60	2.09	0.08	0.64	2.03	0.85	4.49
Left	53	2.23	0.12	0.84	2.17	0.62	4.56
Female R	32	2.23	0.13	0.76	2.15	0.85	4.49
Female L	30	2.41	0.16	0.86	2.37	1.17	4.56
Female (pooled)	62	2.32	0.1	0.81	2.23	0.85	4.56
Male R	28	1.92	0.08	0.44	1.88	1.26	2.83
Male L	23	1.99	0.16	0.78	1.98	0.62	4.05
Male (pooled)	51	1.95	0.09	0.61	1.92	0.62	4.05
Cortex (%)							
All samples	113	57.51	0.67	7.09	59	34	76
Right	60	57.23	0.8	6.23	57.5	38	74
Left	53	57.83	1.1	8.01	59	34	76
Female R	32	58.28	1.31	7.4	59	38	74
Female L	30	59.67	1.41	7.74	60.5	45	76
Female (pooled)	62	58.95	0.96	7.54	59.5	38	76
Male R	28	56.04	0.83	4.38	56.5	48	64
Male L	23	55.43	1.64	7.87	57	34	67
Male (pooled)	51	55.76	0.86	6.14	57	34	67
Medulla (%)							

All samples	113	28.88	0.64	6.84	28	16	55
Right	60	29.17	0.8	6.16	28	16	45
Left	53	28.55	1.04	7.57	28	16	55
Female R	32	28.22	1.23	6.93	27.5	16	45
Female L	30	26.8	1.13	6.19	25.5	16	42
Female (pooled)	62	27.53	0.83	6.57	26.5	16	45
Male R	28	30.25	0.95	5.05	29	22	40
Male L	23	30.83	1.81	8.69	29	16	55
Male (pooled)	51	30.51	0.96	6.86	29	16	55
Capsule (%)							
All samples	113	13.62	0.38	4.03	14	5	28
Right	60	13.62	0.52	4.03	14	5	28
Left	53	13.62	0.56	4.05	14	5	28
Female R	32	13.44	0.76	4.31	14	6	28
Female L	30	13.63	0.83	4.56	14	5	28
Female (pooled)	62	13.53	0.56	4.4	14	5	28
Male R	28	13.82	0.71	3.76	13.5	5	21
Male L	23	13.61	0.7	3.38	13	8	21
Male (pooled)	51	13.73	0.5	3.56	13	5	21

Table A4 Instrument limits of detection (LOD) in dry weight (dw, mg/kg) and derived wet weight (ww, mg/kg) equivalents for the 16 trace elements targeted by ICP-MS analysis. Wet weight LODs were calculated by multiplying instrument dw LODs by mean tissue-specific dw/ww conversion factors (kidney = 0.2554, n = 98; liver = 0.3088, n = 98), with one kidney outlier excluded. LOD, limit of detection; dw, dry weight; ww, wet weight; ICP-MS, inductively coupled plasma mass spectrometry

Element	Instrument LOD (mg/kg dw)	Wet weight LOD (mg/kg ww)	
		(Kidney $\times$ 0.2554)	(Liver $\times$ 0.3088)
Aluminium (Al)	7.01	1.79	2.16
Arsenic (As)	0.07	0.02	0.02
Cadmium (Cd)	0.70	0.18	0.22
Cobalt (Co)	0.07	0.02	0.02
Chromium (Cr)	0.04	0.01	0.01
Copper (Cu)	0.70	0.18	0.22
Iron (Fe)	7.01	1.79	2.16
Mercury (Hg)	0.07	0.02	0.02
Magnesium (Mg)	7.01	1.79	2.16
Manganese (Mn)	0.07	0.02	0.02
Nickel (Ni)	0.07	0.02	0.02
Lead (Pb)	0.70	0.18	0.22
Selenium (Se)	1.30	0.33	0.40
Strontium (Sr)	7.01	1.79	2.16
Vanadium (V)	0.04	0.01	0.01
Zinc (Zn)	7.01	1.79	2.16

Table A5 Trace element recoveries (%) for DORM-4 certified fish protein reference material (Fisheries and Oceans Canada) used for quality control in inductively coupled plasma mass spectrometry analyses. Mean recoveries ranged from 74% to 124% across the 16 targeted elements. Co, information value only; Pb, reference value not certified; LOD, limit of detection

Concentration (mg/kg dw)														
Sample	Mg	Al	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	Pb 208	Hg (O ₂)
LOD	7.0	7.0	0.07	7.0	0.07	0.07	0.70	7.0	0.07	1.3	7.0	0.70	0.70	0.07
CRM 1	720.9	1041.3	3.41	306.5	0.25	1.59	16.09	49.2	6.90	3.4	9.5	Below LOD	Below LOD	0.49
CRM 2	638.3	1009.4	4.74	301.8	0.25	1.25	15.40	48.9	6.92	3.5	9.9	Below LOD	Below LOD	0.49
CRM 3	539.6	882.3	3.28	272.3	0.22	1.22	14.10	45.3	6.61	3.4	10.0	Below LOD	Below LOD	0.47
CRM 4	520.7	834.7	2.97	289.4	0.23	1.19	14.81	47.4	5.80	3.1	8.8	Below LOD	Below LOD	0.46
CRM 5	511.5	798.0	2.95	287.1	0.23	1.14	14.51	47.2	5.56	3.0	9.1	Below LOD	Below LOD	0.45
CRM 6	753.4	1135.6	3.65	326.2	0.26	1.46	16.20	55.8	6.92	3.6	9.3	Below LOD	Below LOD	0.50
CRM 7	683.9	1067.0	3.65	341.6	0.25	1.33	15.90	54.5	6.93	3.7	9.5	Below LOD	Below LOD	0.45
CRM 8	612.7	1051.6	3.26	293.6	0.25	1.35	15.70	52.8	6.86	3.7	8.9	Below LOD	Below LOD	0.39

CRM 9	691.3	1052.1	3.34	313.9	0.26	1.33	16.20	55.3	6.60	3.6	9.2	Below LOD	Below LOD	0.37
CRM 10	641.7	1023.2	3.28	320.9	0.25	1.29	15.40	52.8	6.68	3.5	8.9	Below LOD	Below LOD	0.40
Recovery (%)														
Sample	Mg	Al	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	Pb 208	Hg (O ₂)
CRM 1	79.2	81.4	107.6	89.4	100.0	118.7	102.5	95.3	100.4	98.6	94.1	Below LOD	Below LOD	118.9
CRM 2	70.1	78.9	149.5	88.0	100.0	93.3	98.1	94.8	100.7	101.4	98.0	Below LOD	Below LOD	118.9
CRM 3	59.3	68.9	103.5	79.4	88.0	91.0	89.8	87.8	96.2	98.6	99.0	Below LOD	Below LOD	114.1
CRM 4	57.2	65.2	93.7	84.4	92.0	88.8	94.3	91.9	84.4	89.9	87.1	Below LOD	Below LOD	111.7
CRM 5	56.2	62.3	93.1	83.7	92.0	85.1	92.4	91.5	80.9	87.0	90.1	Below LOD	Below LOD	109.2
CRM 6	82.8	88.7	115.1	95.1	104.0	109.0	103.2	108.1	100.7	104.3	92.1	Below LOD	Below LOD	121.4
CRM 7	75.2	83.4	115.1	99.6	100.0	99.3	101.3	105.6	100.9	107.2	94.1	Below LOD	Below LOD	109.2
CRM 8	67.3	82.2	102.8	85.6	100.0	100.7	100.0	102.3	99.9	107.2	88.1	Below LOD	Below LOD	94.7

CRM 9	76.0	82.2	105.4	91.5	104.0	99.3	103.2	107.2	96.1	104.3	91.1	Below LOD	Below LOD	89.8
CRM 10	70.5	79.9	103.5	93.6	100.0	96.3	98.1	102.3	97.2	101.4	88.1	Below LOD	Below LOD	97.1
Mean	69.4	77.3	108.9	89.0	98.0	98.2	98.3	98.7	95.7	100.0	92.2	Below LOD	Below LOD	108.5

Certified reference values (DORM-4)

	Mg	Al	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Cd	Pb 208	Hg (O ₂)
Certified	910.0± 80.0	1280.0± 340.0	3.2± 0.3	343.0± 20.0	0.3	1.3± 0.1	15.7± 0.5	51.6± 2.8	6.87± 0.4	3.45± 0.4	10.1± 0.8	0.299± 0.0	N/A	0.4± 0.0