

# Trophic ecology of Indo-Pacific dolphins (*Tursiops aduncus*) in Owen Anchorage and Cockburn Sound inferred by stable isotopes

**Theme:** Apex Predators and Iconic Species  
WAMSI Westport Marine Science Program



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## ABOUT THE MARINE SCIENCE PROGRAM

The WAMSI Westport Marine Science Program (WWMSP) is a \$13.5 million body of marine research funded by the WA Government. The aims of the WWMSP are to increase knowledge of Cockburn Sound in areas that will inform the environmental impact assessment of the proposed Westport development and help to manage this important and heavily used marine area into the future. Westport is the State Government's program to move container trade from Fremantle to Kwinana, and includes a new container port and associated freight, road and rail, and logistics. The WWMSP comprises more than 30 research projects in the biological, physical and social sciences that are focused on the Cockburn Sound area. They are being delivered by more than 100 scientists from the WAMSI partnership and other organisations.

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## DATA

Finalised datasets will be released as open data, and data and/or metadata will be discoverable through Data WA and the Shared Land Information Platform (SLIP).

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## FRONT COVER IMAGE

**Theme:** Apex Predators and Iconic Species

**Front cover image:** Dolphin pod in Cockburn Sound (Photo by Delphine Chabanne, Murdoch University).

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*The WAMSI Westport Marine Science Program is a \$13.5 million body of research that is designed to fill knowledge gaps relating to the Cockburn Sound region. It was developed with the objectives of improving the capacity to avoid, mitigate and offset environmental impacts of the proposed Westport container port development and increase the WA Government's ability to manage other pressures acting on Cockburn Sound into the future. Funding for the program has been provided by Westport (through the Department of Transport) and the science projects are being delivered by the Western Australian Marine Science Institution.*

# Trophic ecology of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) in Owen Anchorage and Cockburn Sound inferred by stable isotopes

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## Project

8.3, Spatio-temporal distribution of key habitat uses and key prey species for Indo-Pacific bottlenose dolphins in Owen Anchorage and Cockburn Sound, including a fine-scale understanding of the use of the habitats in the Kwinana Shelf

## Executive Summary

This study aims to address gaps in our understanding of the ecology of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) in Owen Anchorage and Cockburn Sound to improve the scientific basis for environmental impact assessment (EIA) of the proposed Westport port development. This report presents the results of the trophic ecology of Indo-Pacific bottlenose dolphins using skin samples of a subset of individual dolphins and muscle samples of selected prey collected in 2022 and 2023 across Owen Anchorage and Cockburn Sound.

A total of 49 skin samples were obtained during the late summer/autumn and late winter/spring seasons across Owen Anchorage and Cockburn Sound, including the Kwinana Shelf. Stable analyses with mixing models were used to estimate the contribution of different feeding guilds based on prey species available during the study period.

### Key findings

Significant seasonal variation in dolphin diets were identified, with summer diets primarily comprising benthic detritivores (e.g. sea mullet *Mugil cephalus*) and winter diets dominated by benthic opportunistic species (e.g. western butterfish *Pentapodus vitta*) and invertebrate carnivores (e.g. New Holland cuttlefish *Sepia novaehollandiae*, southern squid *Sepioteuthis australis*, western rock octopus *Octopus djinda*). Pelagic prey did not significantly contribute to dolphins' diets. Additionally, the study also revealed potential complex niche partitioning among dolphins, such as differences in prey choice - particularly with resident females exhibiting higher levels of site fidelity and specialized foraging behaviours.

### Implications

The findings of this study may have implications for the proposed Westport port development. They reveal seasonal variations in dolphin diets, underscoring the importance of considering temporal changes in prey availability and habitat conditions (e.g. seasonal variation of seagrass) when assessing potential impacts. The results highlight the need to avoid or minimise seagrass disturbance, as the identified prey depend on seagrass, and habitat modifications could lead to secondary impacts through changes in prey distribution.

Furthermore, the identification of potential complex niche partitioning among dolphins suggests that the effects of port development, which could alter prey availability, may vary among different subgroups of the dolphins. Resident dolphins, in particular, may be more sensitive to local habitat loss or modification, because their carbon signatures indicate a reliance on prey from coastal and estuarine ecosystems rather than offshore source. This situation is further complicated by the intra-selection of prey among the resident females. This highlights the necessity of tailored mitigation measures that address the specific needs and vulnerabilities of different dolphin groups, ensuring effective conservation measures are implemented. These efforts should include measures to protect seagrass habitats, promote sustainable fisheries practices and integrate robust scientific monitoring to safeguard the marine ecosystem and its inhabitants.

### *Conclusions/Recommendations*

This study provides valuable insights into the trophic ecology of Indo-Pacific bottlenose dolphins in Owen Anchorage and Cockburn Sound, shedding light on their dietary habits and habitat preferences. The observed seasonal variations as well as potential interannual in dolphin diets and complex niche partitioning highlight the dynamic nature of marine ecosystems and underscore the importance of considering these factors in environmental management and conservation efforts.



# 1 Introduction

## 1.1 Background

Activities associated with the establishment and operation of a port in the Kwinana Shelf, Cockburn Sound, have the potential to adversely affect local marine biodiversity, including the Indo-Pacific bottlenose dolphins (*Tursiops aduncus*), which are recognised as apex predators and iconic species in the region. As a cetacean, the Indo-Pacific bottlenose dolphin is protected in Australia under the *Environment Protection and Biodiversity Conservation Act 1999*. In Western Australia, it is also safeguarded from immediate and measurable threats under the *Biodiversity Conservation Act 2016*. Since 2019, this species has also been listed as ‘near threatened’ globally in the IUCN Red List of Threatened Species due to several factors: 1) the formation of small resident populations in restricted near-shore areas; 2) inhabiting habitats subject to increasing anthropogenic threats leading to habitat loss and degradation; 3) being highly vulnerable to entanglement in fishing gear; and, 4) experiencing mortality rates that put populations at risk of decline (Braulik et al. 2019).

The ecological dynamics of dolphins in Perth metropolitan waters are characterised by fine-scale population structures, marked by long-term residency, strong site fidelity, and limited-ranging patterns within Cockburn Sound, Owen Anchorage, and the Swan Canning Riverpark (Finn 2005, Chabanne et al. 2012, 2017a, 2017b). The identified key habitats, characterised by the highest occurrence of resident dolphins in Owen Anchorage and Cockburn Sound were consistent with previously identified core areas (Chabanne et al. 2017a). Specifically, previous studies exhibited a moderate to very high probability of dolphin occurrence throughout the entire Owen Anchorage region, with seasonal shifts influenced by the seasonal patterns of sea surface temperature (SST) and visibility (Chabanne 2023). In Cockburn Sound, important areas were identified along the northern tip of Garden Island, Mangles Bay (i.e. the southern part of Garden Island and the southeastern coastline), and across the Kwinana Shelf, all being shallow areas (<10 m) of mixed substrate including reef, cobble, and seagrass (Chabanne 2023).

Despite several boat-based observation studies conducted in Cockburn Sound (1993-1997, 2001-2002, 2011-2015), little is known about the diet of the dolphins in the embayment, which would be beneficial for identifying important habitats. One reason for this is that it is generally rare to observe dolphins consuming prey. If observed, large prey such as salmon or octopus may be involved, as they need to be fragmented before consumption (observations from Chabanne, 2011-2015). Studies from Bunbury and the Peel-Harvey Estuary, Western Australia, also reveal diverse dietary preferences among Indo-Pacific bottlenose dolphins influenced by local habitat and social dynamics (McCluskey et al. 2021, Nicholson et al. 2021a). In Bunbury, coastal dolphins tend to favour pelagic prey, while inshore dolphins prefer nitrogen-enriched benthic prey (McCluskey et al. 2021). Similarly, dolphins in Peel-Harvey Estuary exhibit niche partitioning, with estuarine entrance inhabitants consuming benthic omnivores and carnivores, contrasting with those in estuary basins reflecting broader fish feeding guilds (Nicholson et al. 2021a). These findings underscore the adaptive dietary strategies of dolphins, shaped by their ecological niches and social dynamics. Feeding aggregations of fish observed in Cockburn Sound suggest probable prey similar to those targeted by seabirds and described as forage fish (Finn & Calver 2008). However, it is still unclear what the relative importance of those prey is to the diet of the local dolphin population, particularly in light of potential habitat modification and its effects on prey availability.

## 1.2 Stable isotopes to infer trophic ecology

Stable isotopes serve as a valuable tool for examining the food web structures, including dietary habits of dolphins and other species (e.g. Newsome et al. 2006, Giménez et al. 2018). Isotopes are different forms of the same element with slightly different weights, and these forms are absorbed into animals through their food. By comparing these isotopic signatures in different animals within an

ecosystem, we can determine who is eating whom. If two animals have similar isotopic signatures, it suggests one may be consuming the other.

The metabolic processes within an organism drive isotopic composition, which can be measured using an isotope-ratio mass spectrometer. Carbon and nitrogen isotopes are particularly useful for studying diets. Carbon ratios play a crucial role in pinpointing sources of primary production and generally exhibit consistency between producers and consumers, offering a snapshot of an individual's recent foraging habitat. This is because plants growing in different habitats have distinct carbon signatures, and these signatures remain consistent as they move through the food chain. In contrast, nitrogen ratios increase with each trophic level in the food chain, providing valuable insights into the trophic position an individual occupies (Teixeira et al. 2022).

This dynamic use of stable isotopes enables researchers to unravel intricate details about the feeding ecology of dolphins and other species, shedding light on their foraging habits and ecological interactions within the marine environment.

### 1.3 Aims and Objectives

By establishing links between habitat that provide resources and the presence of dolphins, it becomes possible to identify important areas for population viability and assess the potential adverse effects of natural impacts and human disturbance on the dolphin population. This study aimed to identify the main groups of prey (feeding guilds) consumed by dolphins in Owen Anchorage and Cockburn Sound. Feeding guilds are groups of prey that share similar feeding habits, which can be identified through variations in carbon and nitrogen signatures in stable isotope analyses. Additionally, the study investigated how the dolphins' diet may change seasonally and vary with individual traits like sex.

The specific objectives of the study were as follows:

- To identify main groups of prey (feeding guilds) consumed by dolphins, which can provide insights into their habitat requirements;
- To assess whether there was any temporal change in diet;
- To identify individual characteristics such as sex, residency status and area of use, and assess whether there were any variations in targeted feeding guilds.

The identification of significant feeding guilds can provide valuable information for inferring the important habitats for dolphins as a proxy for the importance of prey species' habitat types. The findings presented in this report, together with WAMSI Westport Marine Science Program project report *Fine-scale understanding of the use of the Kwinana Shelf by the resident dolphins in Cockburn Sound and evaluate the importance of specific habitats present in the area* (Chabanne 2024), will provide valuable information for informing the environmental impact assessment for the proposed port development.

## 2 Materials and Methods

### 2.1 Scientific permits and animal ethics

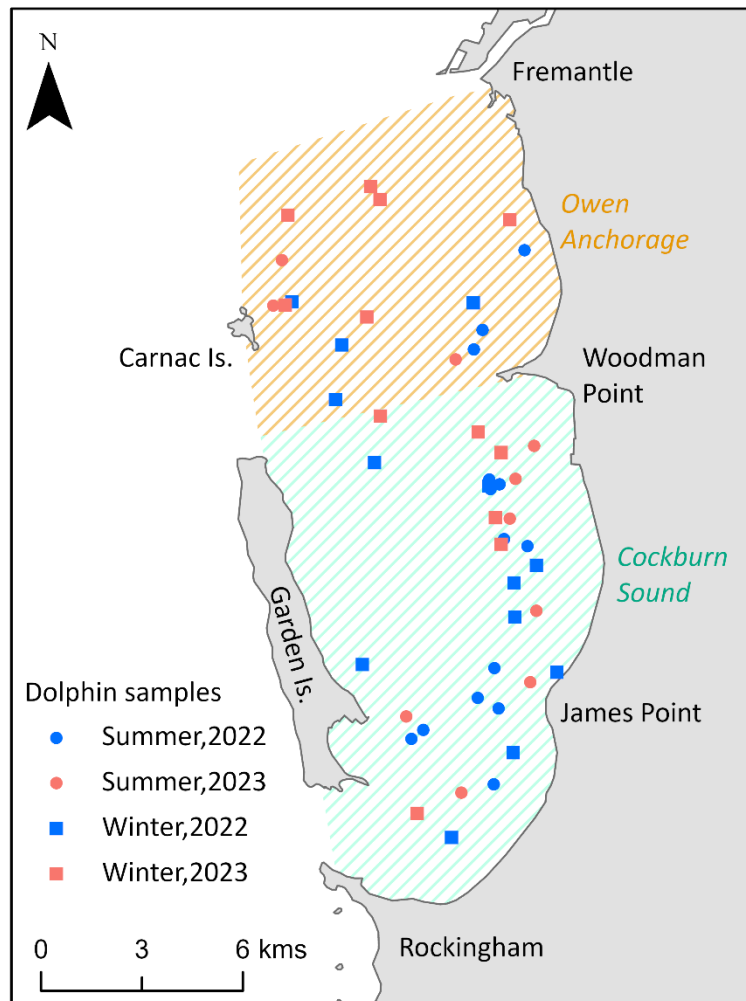
Biopsy sampling on Indo-Pacific bottlenose dolphins was conducted under Scientific Permit FO25000086-65 and FO25000086-6 issued by the Department of Biodiversity, Conservation and Attractions, and Animal Ethics approval R3365/21 from the Animal Ethics Committee of Murdoch University.

### 2.2 Data collection

The study area encompassed approximately 180 km<sup>2</sup> of coastal waters, spanning from Rockingham to Fremantle and along the eastern side of Garden and Carnac islands (**Figure 1**). Within this area, two distinct geographic regions were identified based on the topography and bathymetry of the waters:

- Cockburn Sound: This is a semi-enclosed embayment characterised by varying depths ranging from less than 2 m to over 20 m.
- Owen Anchorage: This embayment features depths of less than 10 m, except in the channel where the maximum depth reaches 14.7 m. The northern boundary of Owen Anchorage is situated at the channel that passes through the Fremantle Inner Harbour.

During systematic and opportunistic photo-identification surveys conducted across Cockburn Sound and Owen Anchorage, biopsy skin samples from adult and juvenile dolphins were collected in late summer/early autumn and late winter/early spring for both years 2022 and 2023 (**Figure 1**), using a PAXARMS remote biopsy darting system (Krützen et al. 2002). Briefly, one or two observers was obtaining photographs of dolphins while another was aiming at collecting a sample of the same individual at an approximate distance of five to 20 m. Samples were placed in empty vials stored in ice for transport and then transferred to a -80°C freezer for preservation until processing for stable isotope analyses of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (see below). Prey species were collected as part of a separate study in November 2021 and 2022 for the summer season, as well as in May 2022 and April 2023 for the winter season using a broad range of sampling techniques, including trawling and seine netting (refer to details of fish sampling in the WAMSI Westport Marine Science Program project report *Spatial distribution and temporal variability in life stages of key fish species in Cockburn Sound*, Yeoh et al. (2024)) and stored in a -20°C freezer until processing for stable isotope analyses of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .



**Figure 1.** Map of the study area showing Owen Anchorage (OA, orange hatched area) and Cockburn Sound (CS, green hatched area). Dolphin sampling locations are indicated by season (dots for summer and square for winter) and year (blue for 2022 and red for 2023). Each sample represents a different individual dolphin.

## 2.3 Stable isotope analyses

### 2.3.1 Dolphins

Tissue samples from dolphins were separated into skin and blubber to keep the skin only. The variability in skin lipid content can alter bulk tissue  $\delta^{15}\text{C}$  values (Logan et al. 2008, Newsome et al. 2018) as lipids have more negative  $\delta^{13}\text{C}$  values relative to other biochemical compounds. As recommended by de Lecea and de Charmoy (2015) and Giménez et al. (2016), for accurate measurements of both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  were analysed in lipid-free samples and  $\delta^{15}\text{N}$  in bulk (i.e. untreated) samples. The bulk samples were oven-dried at  $60^\circ\text{C}$  for 24 hours and powdered with a mortar and pestle. The other aliquot underwent lipid extraction with three consecutive 24-hour soaks in 2:1 chloroform:methanol solvent solution (as per Newsome et al. 2006). The samples were then rinsed with deionised water, oven-dried and powdered as per the bulk samples. All samples were weighed to the nearest  $10^{-2}\mu\text{g}$ , packed into tin capsules for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , arranged on a microtiter tray and delivered to the West Australian Biogeochemistry Centre (WABC) at the University of Western Australia for analyses (i.e. elemental composition and isotope ratios were determined by continuous-flow isotope ratio mass spectrometry for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ). Carbon and

nitrogen stable isotope ratios are expressed in  $\delta$  notation ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) as parts per thousand (‰) as determined from:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}})-1] * 1000$$

where X is  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  and R is the corresponding ratio  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ . The ratio is expressed as  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  in parts per thousand (‰).

In cases where there was insufficient material (skin) for lipid extraction treatment, a correction factor was applied to estimate the non-lipid-extracted  $\delta^{13}\text{C}$ . The equation used was:

$$\text{lipid-extracted } \delta^{13}\text{C} = a + b \times \text{non-lipid-extracted } \delta^{13}\text{C}$$

where values of 'a' and 'b' were obtained through linear correlation for each sampling period.

### 2.3.2 Prey

Flesh samples from fish identified as potential dolphin prey based on their availability in the area and insights from other studies (e.g. McCluskey et al. 2021, Nicholson et al. 2021a) (**Table 1**), were obtained and dried at 60°C for 24-48 h. The dried samples were finely ground into a powder. Samples were then analysed for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  with and without lipids. Lipids were removed from the fish samples following the methods described in Logan et al. (2008). The dried powdered sample was immersed in a 2:1 ratio of chloroform:methanol, centrifuged at 2500 rpm for 10 mins, and the supernatant was pipetted off and the process repeated until the supernatant became completely clear and colourless. The final sample was rinsed with chloroform-methanol and dried at 50°C before being weighed and packed into tin capsules. These comprehensive procedures mirrored those implemented for the corresponding dolphin samples, ensuring a consistent and rigorous analytical framework throughout the study.

**Table 1.** Prey species included as sources in a mixing model to estimate feeding guild contributions to Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) diet in the Cockburn Sound/Owen Anchorage waters with details of the number of samples collected per year and season. Notation: -: no samples were available or retained.

Feeding Guild		2022		2023	
Species	Common name	Summer	Winter	Summer	Winter
<b>Benthic Detritivore</b>					
<i>Mugil cephalus</i>	Sea mullet	5	-	4	-
<b>Benthic Invertivore; annalid/arthropod specialist</b>					
<i>Parequula melbournensis</i>	Silverbelly	5	5	6	14
<i>Sillago vittata</i>	Western school whiting				
<i>Sillago burrus</i>	Western trumpeter whiting	6	6	8	9
<b>Benthic Invertivore; arthropod specialist</b>					
<i>Upeneus australiae</i>	Australian goatfish	6	5	8	14
<i>Chrysophrys auratus</i>	Pink snapper	6	-	4	-
<i>Pseudocaranx wright</i>	Skipjack trevally	5	6	7	15
<b>Benthic Invertivore (Elasmobranch)</b>					
<i>Mustelus antarcticus</i>	Gummy shark	2	-	-	-
<b>Benthic Invertivore (molls/arthropod specialist)</b>					
<i>Pseudocaranx georgianus</i>	Silver trevally	4	-	6	-
<b>Benthic Omnivore</b>					
<i>Pelsartia humeralis</i>	Sea trumpeter	2	-	-	-
<i>Hyporhamphus melanochir</i>	Southern garfish	-	5	4	3
<i>Pelates octolineatus</i>	Western striped grunter (8-line)	6	9	4	5
<i>Alarichetta forsteri</i>	Yelloweye mullet	4	6	11	-
<b>Benthic Opportunist</b>					
<i>Pentapodus vitta</i>	Western butterflyfish	6	6	-	16
<b>Invertebrate - Benthic Carnivore</b>					
<i>Sepia novaehollandiae/cultrata</i>	New Holland cuttlefish	3	6	-	-
<i>Octopus djinda</i>	Western rock octopus	-	4	-	-
<i>Sepioteuthis australis</i>	Southern Squid	3	-	-	-
<b>Pelagic Carnivore</b>					
<i>Sardinella lemuru</i>	Scaly mackerel	4	-	-	5
<b>Pelagic Invertivore</b>					
<i>Atherinomorus vaigiensis</i>	Common hardyhead	4	9	5	
<i>Trachurus novaezelandiae</i>	Yellowtail scad	6	8	8	15
<b>Pelagic Piscivore</b>					
<i>Sphyrna obtusata</i>	Striped barracuda	2	4	4	-
<b>Pelagic Planktivore</b>					
<i>Engraulis australis</i>	Australian anchovy	8	2	-	2
<i>Sardinops sagax</i>	Blue sardine	-	-	4	-
<i>Spratelloides robustus</i>	Blue sprat	9	11	9	-
<i>Hyperlophus vittatus</i>	Sandy sprat	8	9	5	-
<i>Leptatherina presbyteroides</i>	Silver fish	-	-	4	-

Note: Although not reviewed here, scaly mackerels were considered closer to Pelagic Planktivore after analyses from WAMSI Westport Marine Science Program project report *Trophic pathways and food web structure of Cockburn Sound and Owen Anchorage* (pers. comment by Glenn Hyndes, 17 April 2024).

## 2.4 Drivers of Niche Partitioning

The relationship between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values and a set of individual variables such as sex, area used, and residency status was analysed.

### 2.4.1 Sex

Sex of the sampled individual dolphin was determined through one of four methods: genetic analyses conducted on biopsy samples collected during the 2011-2015 study project (Chabanne et al. 2021); visual confirmation of genital areas; observations of dependent calves for adult females; or inference based on social and behavioural observations detailing the formation of male alliances (Chabanne et al. 2022).

### 2.4.2 Primary area used

Determination of the primary area used by each biopsied dolphin was based on the frequency of sightings across the entire study area, with a caveat that only a limited portion of Cockburn Sound underwent systematic surveys (i.e. regular repeat surveys exclusively conducted across Kwinana Shelf, see the report of WAMSI Westport Marine Science Program project *Fine-scale understanding of the use of the Kwinana Shelf by the resident dolphins in Cockburn Sound and evaluate the importance of specific habitats present in the area*, Chabanne (2024)). Surveys conducted elsewhere were characterised as opportunistic. The determination process involved the delineation of three groups: Owen Anchorage (OA), Cockburn Sound (CS), and across both Cockburn Sound/Owen Anchorage (CSOA). Individuals identified as OA exhibited the majority of sightings in Owen Anchorage. For individuals classified as CS, the majority of sightings spanned the entire sound, including Kwinana Shelf and the southern section, with less than a third of the total sightings observed in OA, if any. If the latter constituted more than a third, the individual was designated as CSOA, suggesting an area spanning across both Cockburn Sound and Owen Anchorage.

### 2.4.3 Residency status

Residency status was determined by assessing both the overall daily sighting rate and the seasonal sighting rate. Additionally, residency status was supported by prior knowledge obtained from individuals re-identified from the 2011-2015 study (Chabanne et al. 2017a). The Seasonal Sighting Rate (SSR) was calculated by dividing the number of seasons in which a dolphin was sighted at least once by the total number of seasons ( $n = 8$ ) within the study period. Seasons followed the south-west Australian calendar: summer (December to February), autumn (March to May), winter (June to August), and spring (September to November). Based on the criteria applied in Chabanne et al. (2012), three distinct categories were established: resident, visitor, and transient. The criteria for each category are summarised in Table 2 below.

Table 2: Classification of residency status of dolphins based on sighting frequency (% relative to total number of survey days and encompassing both systematic and opportunistic days conducted on Kwinana Shelf) and seasonal sighting rate (SSR).

Residency status	Sighting frequency	Seasonal Sighting Rate (SSR)
Resident	Sighted >10% of survey days	High SSR (>0.75)
Visitor	Sighted <10% of survey days	Moderate SSR (0.125 - 0.75)
Transient	Sighted <10% of survey days	Low SSR (<0.125)

#### 2.4.4 Tests

All statistical analyses were performed using R 4.3.1 (R Core Team 2023b) in R Studio (Posit team 2023a).  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and their relationships with individual variables such as sex, primary area used, and residency status, as well as temporal variables season and year, were analysed using a generalised additive model (GAM) (Hastie 1990) with the *mgcv* package (Wood 2011). Twenty-eight models were built with Gaussian distribution and an identity link function for each of the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, using all possible combinations of the predictive individual variables. The optimal models were selected based on the corrected Akaike's information criterion adjusted for small sample size (AICc) (Burnham & Anderson 2002) and the percentage of deviance explained and checked for normality by visual inspection of the residuals and by using a Shapiro-Wilk test.

#### 2.5 Dietary sources

To investigate the contribution of prey sources to the diet of dolphins, a Bayesian mixing model approach was employed. Mixing models were executed separately for each season and year due to variations in prey species (see **Table 1**) and their stable isotopes across different seasons and years (results not presented in this report), although a combined year and season mixing model, omitting fish species and seasonal/yearly variations, was run for comparison. Using published knowledge of morphology and diet, prey fish species were grouped into 'feeding guilds (Hyndes et al. 2024). The identified feeding-guild groups included Benthic Detritivore (BD), Benthic Invertivore annelid/arthropod specialist (BIAAS), Benthic Invertivore arthropod specialist (BIAS), Benthic Invertivore Elasmobranch (BIE), Benthic Invertivore Mollusk/arthropod specialist (BIMAS), Benthic Omnivore (BOM), Benthic Opportunist (BOP), Invertebrate – Benthic Carnivore (IBC), Pelagic Carnivore (PC), Pelagic Invertivore (PI), Pelagic Piscivore (PPI), and Pelagic Planktivore (refer to **Table 1** for chosen species per season and Hyndes et al. (2024)). A trophic discrimination factor (i.e. the difference in the isotopic ratio between the consumer and their diet) was set to 1.01 (SD = 0.37) for  $\delta^{13}\text{C}$  and 1.57 (SD = 0.52) for  $\delta^{15}\text{N}$ , as reported by Giménez et al. (2016) in the models.

A total of 2,000 iterations of Monte Carlo simulation of mixing polygons (i.e. graphical tools used to visualise the range of potential dietary sources, such as prey species, contributing to the dolphins' isotopic signatures) were performed to verify the validity of the mixing models for correctly estimating source contribution to the consumer's isotopic value (Smith et al. 2013). This process was conducted using the packages *sp* (Bivand et al. 2022) and *splancs* (Rowlingson & Diggle 2020) in R. Model validation was considered successful when all consumers were within the 95% mixing region (Smith et al. 2013).

A set of eight Bayesian mixing models with different covariate structures was then applied using the *MixSIAR* package (Stock et al. 2018). The null model considered all individuals in the population to



share the same diet, while other fitted models allowed variation in diet according to sex, area used, residency status, or a combination of two using a nested function. The last fitted model allowed variation among groups of dolphins identified using a Ward linkage hierarchical cluster analysis based on the mean isotopic values and run separately for each season/year.

All mixing models were fitted with generalist (i.e. uninformative) priors using a Dirichlet distribution (i.e. all combinations of the proportions are equally likely) and a multiplicative error term (i.e. process\*residual). Markov Chain Monte Carlo (MCMC) simulations were employed to estimate the probability distribution, referred to as the “posterior”, for dietary contributions from each source (i.e. the proportional contribution of each feeding guild group to the dolphin’s diet). Models were executed using the ‘very long’ combination for MCMC and consisted of three chains run with a length of 1,000,000, a burnin of 500,000, and a thinning interval of 500.

Gelman–Rubin and Geweke diagnostics, along with inspection of the traces of each MCMC chain, were used to assess the convergence of the models. Gelman confidence intervals close to 1 and < 1.05 indicate model convergence, while Geweke diagnostics are standard Z-scores based on the equality of two parts of the Markov chains. At convergence, the means of the chains should be the same, with  $\leq 5\%$  of variables in each chain outside of  $\pm 1.96$  (Stock & Semmens 2016).

Since each set of models within each temporal set fit the same data, model comparison using an information criterion approach was performed, namely the leave-one-out cross-validation (LOO). LOO estimates pointwise out-of-sample prediction accuracy from a fitted Bayesian model using the log-likelihood evaluated at the posterior simulations of the parameter values, along with standard errors for the difference in LOO between two models (dLOOic) (Vehtari et al. 2016). The relative support for each model using Akaike weights was also calculated (Burnham & Anderson 2002, Vehtari et al. 2016).

### 3 Results

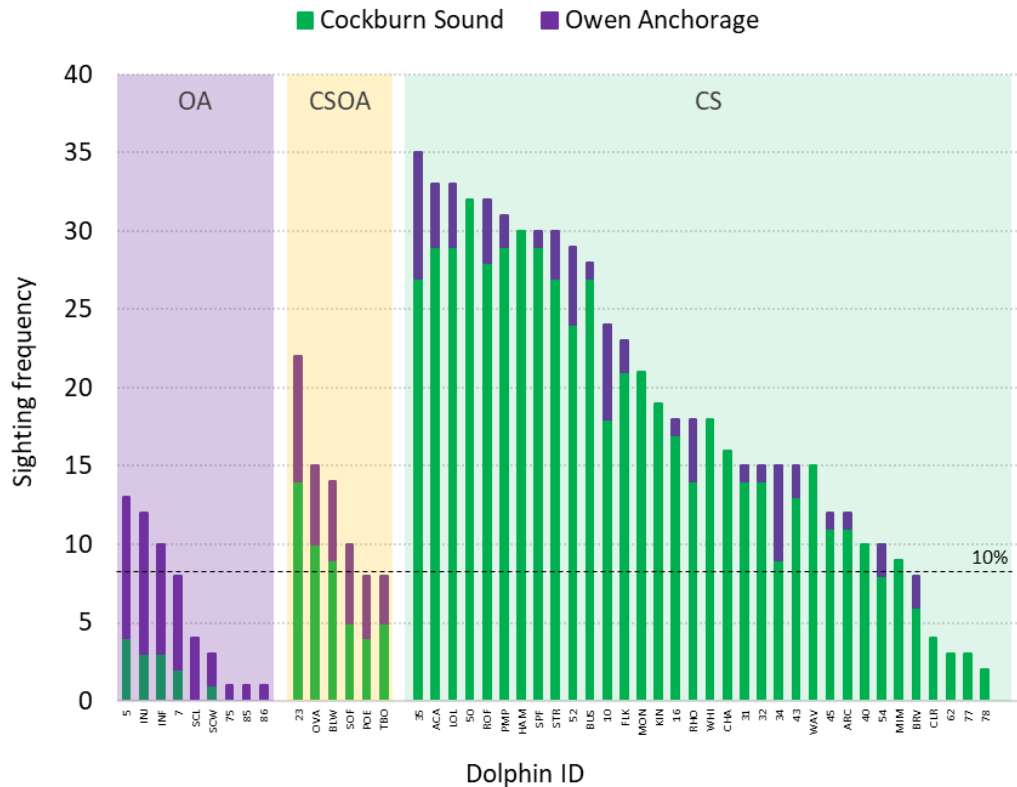
A total of 49 tissue samples from distinct individual dolphins were successfully collected across Cockburn Sound and Owen Anchorage for the examination of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  stable isotopes (see **Appendix 1**). Additionally, three samples were collected from individuals using Owen Anchorage and Cockburn Sound despite being recognised as residents in the Swan Canning estuary. A preliminary test highlighted a significant difference in the  $\delta^{15}\text{N}$  values of these three samples compared to all others. Complemented with findings from Holyoake et al. (2010), which previously indicated variation in stable isotopes between resident dolphins from the Swan Canning estuary and coastal adjacent waters, these three samples were excluded from subsequent analyses.

#### 3.1 Individual characteristics

Among the samples, 21 dolphins were identified as females and nine as males based on results from a previous genetic study (Chabanne et al 2017; 2021), the presence of a calf, or direct observations of the genital part made during the 2022-2023 surveys. Through observations of behaviour and social structure, eight females and 11 males were further identified based on their social structure (see details in **Appendix 1**).

Among the sampled individuals, 34 were described as primarily using Cockburn Sound based on 84 boat-based days conducted between 2022 and 2023 (combining systematic and opportunistic survey days). Although the number of survey days in Owen Anchorage was limited, nine individuals were identified as primarily using Owen Anchorage, while six were observed in both Cockburn Sound and Owen Anchorage (**Figure 2**).

Of the 49 biopsied individuals, three were classified as ‘transient’ because they were sighted only once. Thirteen were classified as ‘visitors’ due to a Seasonal Sighting Rate (SSR) falling between 0.75 and 0.125, and fewer sightings than 10% of the survey days conducted. Thirty-three were classified as ‘resident’ because they were sighted more than nine times (mean = 21 sightings, median = 18, range 9-35), and were observed across multiple seasons (SSR > 0.75) (**Figure 2**).



**Figure 2.** Sighting frequency per region (Owen Anchorage and Cockburn Sound) for biopsied Indo-Pacific bottlenose dolphins. The shaded background indicates the primary areas used by the sampled individual dolphins: purple for individuals primarily observed in Owen Anchorage (OA); yellow for individuals equally using Cockburn sound and Owen Anchorage (CSOA); green for individuals primarily using Cockburn Sound (CS).

### 3.2 Intraspecific variation in stable isotope values

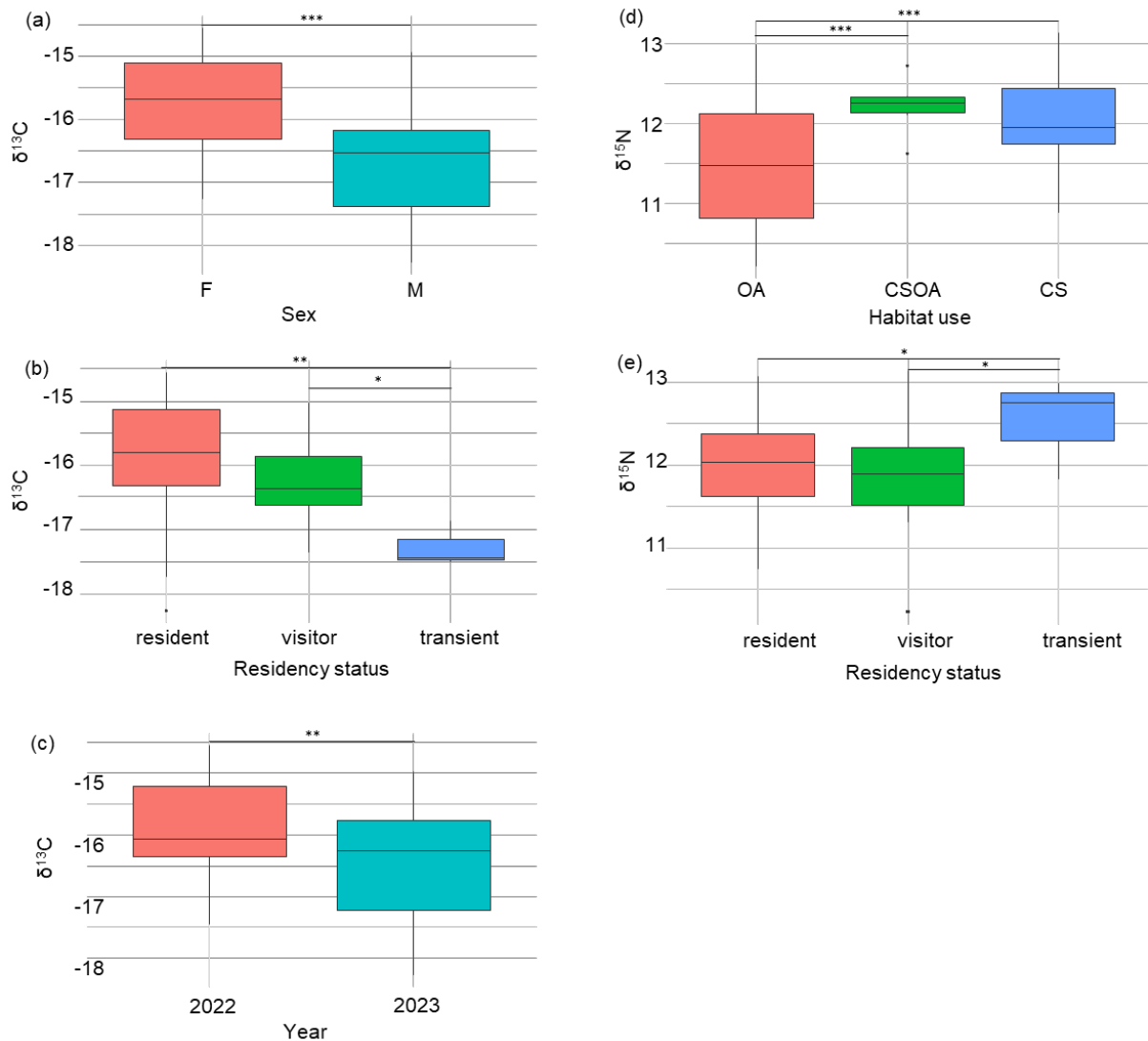
The  $\delta^{13}\text{C}$  (non-lipid) values for dolphin skin tissue ranged from -18.26 to -14.55‰ and the  $\delta^{15}\text{N}$  from 10.23 to 13.13‰. The model that best fitted the  $\delta^{13}\text{C}$  data retained sex, area used, residency status and year as covariates without interaction, explaining 55.5% of the deviance (**Table 2**). Females had significantly larger  $\delta^{13}\text{C}$  values than males (mean  $\delta^{13}\text{C}_{\text{female}}$  = -15.71‰, SE 0.14; mean  $\delta^{13}\text{C}_{\text{male}}$  = -16.66‰, SE 0.18; adjusted p-value < 0.001); resident and visitors had larger  $\delta^{13}\text{C}$  values than transients (mean  $\delta^{13}\text{C}_{\text{resident}}$  = -15.91‰, SE 0.16; mean  $\delta^{13}\text{C}_{\text{visitor}}$  = -16.30‰, SE 0.18; mean  $\delta^{13}\text{C}_{\text{transient}}$  = -17.23‰, SE 0.20; adjusted p-value < 0.040); and samples obtained in 2022 were more enriched in  $\delta^{13}\text{C}$  than those collected in 2023 (mean  $\delta^{13}\text{C}_{2022}$  = -15.85‰, SE 0.15; mean  $\delta^{13}\text{C}_{2023}$  = -16.40‰, SE 0.20; adjusted p-value = 0.003) (**Figure 3**).

The model that best fitted the  $\delta^{15}\text{N}$  data retained area used and residency status as covariates, explaining 28.7% of the deviance (**Table 2**). Samples from individuals primarily using Owen Anchorage had significantly smaller  $\delta^{15}\text{N}$  values than those primarily using Cockburn Sound or both (mean  $\delta^{15}\text{N}_{\text{OA}}$  = 11.58‰, SE 0.31; mean  $\delta^{15}\text{N}_{\text{CS}}$  = 12.03‰, SE 0.10; mean  $\delta^{15}\text{N}_{\text{CSOA}}$  = 12.21‰, SE 0.15;

adjusted p-value <0.001). Transient individuals were significantly enriched in  $\delta^{15}\text{N}$  compared to residents and visitors (mean  $\delta^{15}\text{N}_{\text{resident}} = 11.95\text{‰}$ , SE 0.11; mean  $\delta^{15}\text{N}_{\text{visitor}} = 11.88\text{‰}$ , SE 0.20; mean  $\delta^{15}\text{N}_{\text{transient}} = 12.52\text{‰}$ , SE 0.35; adjusted p-value <0.018) (**Figure 3**).

**Table 3.** Best generalised additive models for Indo-Pacific bottlenose dolphins'  $\delta^{13}\text{C}$  (a) and  $\delta^{15}\text{N}$  (b) values. Significant variables in each model are given in bold.  $R^2$  represents the adjusted coefficient of determination; % DE denotes the percentage of deviance explained;  $\Delta\text{AICc}$  refers to the difference in Akaike's information criterion (AICc) of the current and top-ranked model. Only models with  $\Delta\text{AIC} < 2$  are displayed.

#	Model	$R^2$	% DE	$\Delta\text{AICc}$
a) $\delta^{13}\text{C}$				
1	~ <b>Sex</b> + Area + <b>Status</b> + <b>Year</b>	0.49	55.5	-
b) $\delta^{15}\text{N}$				
1	~ <b>Area</b> + <b>Status</b>	0.22	28.7	-
2	~ Sex + <b>Area</b> + <b>Status</b>	0.22	29.6	1.40
3	~ <b>Area</b> + <b>Status</b> + Year	0.21	28.9	1.87
4	~ <b>Area</b> + <b>Status</b> + Season	0.21	28.8	1.92



**Figure 3.** Variation in  $\delta^{13}\text{C}$  values among sex (a), residency status (b) and year (c), and in  $\delta^{15}\text{N}$  values among primary areas used (d) and residency status (e) for Indo-Pacific bottlenose dolphin samples were collected across Owen Anchorage and Cockburn Sound.

### 3.3 Dietary sources

Opportunistic observations of dolphins with prey were limited (**Figure 4**). Nonetheless, on a few occasions, observations of dolphins tossing cobbles were made. During the winter season, aggregations of seabirds with dolphins foraging on Western striped grunters were also observed. Snack events with dolphins chasing fish while belly up just underneath the surface confirmed opportunistic catches of small Australian anchovies. In spring, although it falls outside the temporal season for stable isotope analysis in the current study, two females known as residents in Cockburn Sound were observed holding a pink snapper. Finally, other observations were made in previous studies, such as in autumn 2015, where several males were observed with large Australian salmon, although salmon were not observed in the course of this study.



**Figure 4.** Opportunistic observations of Indo-Pacific bottlenose dolphins catching prey: (a) western striped grunter; (b) Australian anchovy; (c) cobbler; and (d) pink snapper.

Australian sea lions (*Neophoca cinerea*) were also observed in Cockburn Sound, Kwinana Shelf with prey including estuary cobbler (*Cnidoglanis macrocephalus*), eagle ray (*Aetobatus* sp.) and cuttlefish (*Sepia* sp.) (see **Appendix 2** for photos and details of their locations).

All individual samples of dolphins (i.e. consumers, except for three collected in winter 2023, fell within the 95% contour of the mixing polygon of the available sources within each temporal set and indicated that the sources were probable contributors to their diets (**Appendix 3**). The three samples from winter 2023 that had isotopic signatures falling outside the 95% contour of the mixing model (i.e. outliers) were excluded from the mixing model to help maintain its accuracy and reliability.

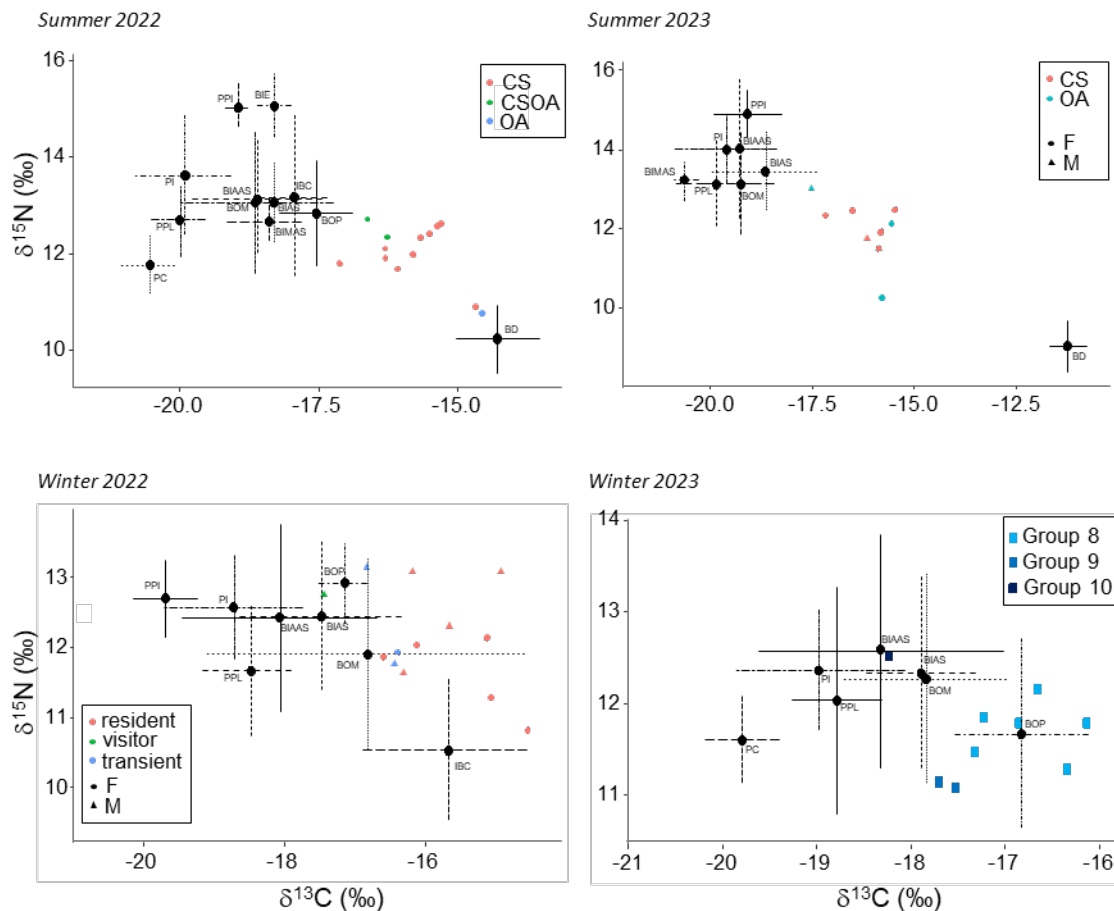
Following the Gelman–Rubin diagnostic, all models converged with no parameters having a Gelman–Rubin diagnostic of  $>1.05$ . However, based on the Geweke test, the majority of the models (20 out of 32 models) did not converge adequately in at least one chain (i.e.  $p > 0.05$ ), suggesting that models did not reach a stable and consistent pattern that would allow reliable and trustworthy estimation of model parameters. When models do not converge, it means the simulations did not settle into a

consistent and reliable pattern. This is crucial because, without convergence, the results may not accurately reflect the true relationships in the data, leading to potential errors in our understanding and predictions. Here, only results from models that converged are described but refer to **Appendix 4** for all other models.

The best model covariates varied for each temporal set. In summer 2022, the model with the lowest LOOic had a fixed effect on the area used and received 68% of the Akaike weight. Additionally, a second model with a fixed effect of residency status received 32% of the Akaike weight and therefore could not be ruled out (>20% of the weight). For summer 2023, the best model included a fixed effect of the area used, with sex nested within it, receiving 85% of the Akaike weight. In winter 2022, the best model had a fixed effect of residency status, with sex nested within it, and received 71% of the Akaike weight. Lastly, in winter 2023, the model with the lowest LOOic included a fixed effect of the grouping provided via the hierarchical cluster analysis, receiving 60% of the Akaike weight (**Table 4, Figure 5**).

**Table 4.** Comparison of the mixing models fitted with MixSIAR on Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) data per temporal set: summer 2022 (n = 14 samples); summer 2023 (n = 10 samples); winter 2022 (n = 13 samples); winter 2023 (n = 9 samples, excluding three that fell outside the 95% contour of the mixing polygon). Single covariates were included as fixed effects. A nested effect was added for the second covariate when paired. The best model for each season/year is indicated in bold text.

#	Model covariate	LOOic	SE (LOOic)	dLOOic	SE (LOOic)	Akaike weight
<i>Summer 2022</i>						
<b>5</b>	<b>Area used</b>	<b>43.1</b>	<b>6.3</b>	<b>0</b>	<b>NA</b>	<b>0.68</b>
3	Residency status	44.6	5.7	1.5	2.4	0.32
<i>Summer 2023</i>						
<b>6</b>	<b>Area used + Sex</b>	<b>23.6</b>	<b>4.2</b>	<b>0</b>	<b>NA</b>	<b>0.85</b>
1	Null	27.7	6.7	4.1	4.2	0.11
2	Sex	29.9	5.7	6.3	3.1	0.04
<i>Winter 2022</i>						
<b>4</b>	<b>Residency status + Sex</b>	<b>54.1</b>	<b>5.7</b>	<b>0</b>	<b>NA</b>	<b>0.71</b>
1	Null	57	6.1	2.9	1.3	0.17
7	Area used + Residency status	57.5	6.1	3.4	1.5	0.13
<i>Winter 2023</i>						
<b>8</b>	<b>Group</b>	<b>36.3</b>	<b>2.8</b>	<b>0</b>	<b>NA</b>	<b>0.60</b>
3	Residency status	38.8	2.1	2.5	1.3	0.17
4	Residency status + Sex	39.5	2.1	3.2	1.1	0.12
7	Area used + Residency status	39.6	2.3	3.3	1.3	0.11

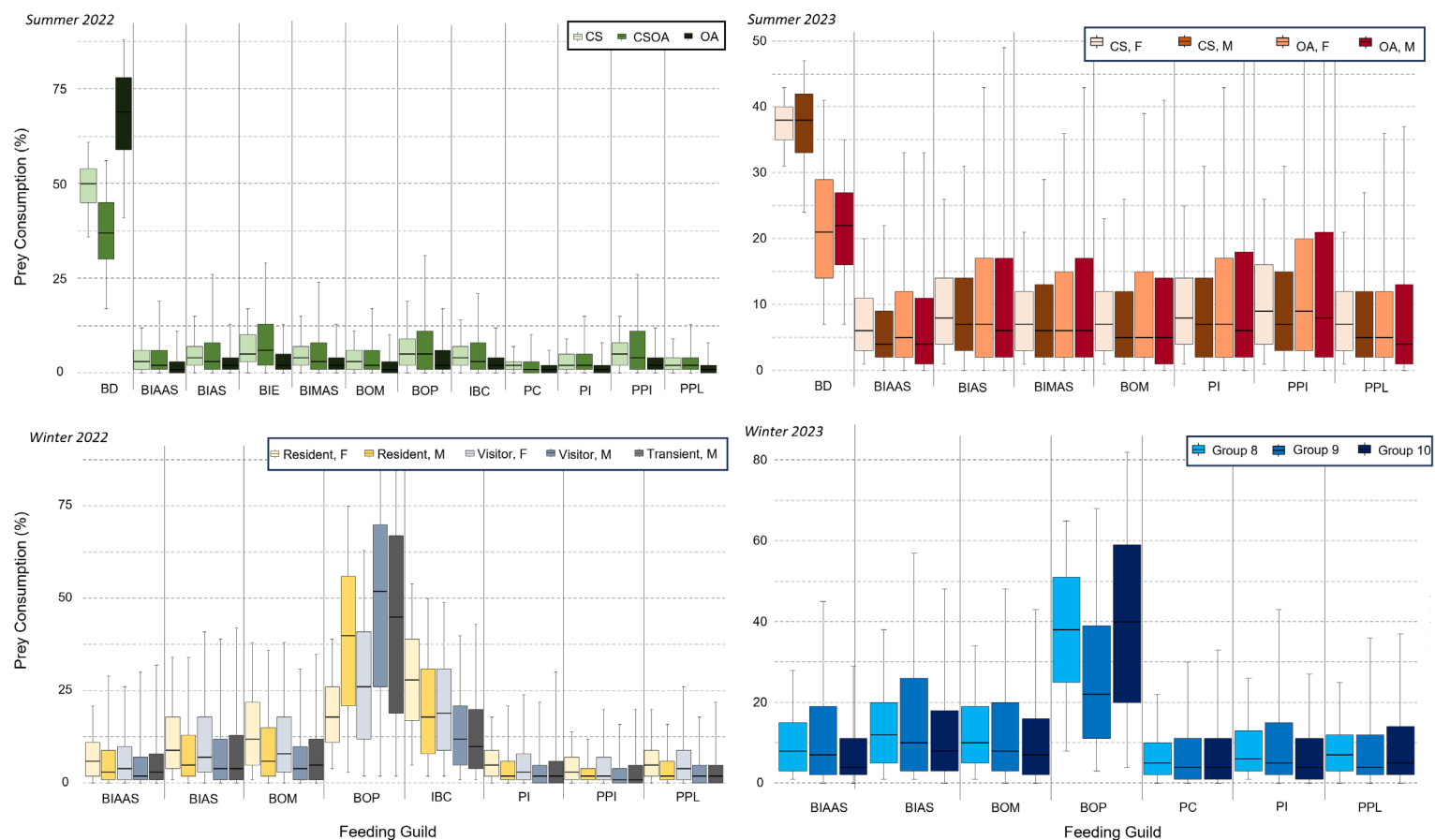


**Figure 5.** Carbon and nitrogen stable isotope ratios (mean  $\pm$  SD, ‰) measured in the skin of Indo-Pacific bottlenose dolphins sampled across Owen Anchorage (OA) and Cockburn Sound (CS) and in the muscle of potential prey species aggregated by feeding guild. Important covariates for each seasonal mixing model varied and were the results of comparison between different combinations of covariates models that converged. Summer 2022 best model (**Area used**) included the covariate area used (CS - Cockburn Sound; OA - Owen Anchorage; CSOA – both areas); summer 2023 best model (**Area used + Sex**) had area used with sex nested (F-female; M-male); winter 2022 best model (**Residency status + Sex**) included the residency status (resident, visitor, transient) with sex nested; and winter 2023 best model (**Group**) used grouping provided by the hierarchical cluster analysis (three groups). Feeding guilds represented were: Benthic Detritivore (BD), Benthic Invertivore annalid/arthropod specialist (BIAAS), Benthic Invertivore arthropod specialist (BIAS), Benthic Invertivore Elasmobranch (BIE), Benthic Invertivore Mollusk/arthropod specialist (BIMAS), Benthic Omnivore (BOM), Benthic Opportunist (BOP), Invertebrate – Benthic Carnivore (IBC), Pelagic Carnivore (PC), Pelagic Invertivore (PI), Pelagic Piscivore (PPI), and Pelagic Planktivore (PPL).

Despite variation in the importance of covariates for inferring the diet of dolphins, the results of feeding guild contributions among the various categories did not differ. Specifically, for summer 2022, benthic detritivores contributed, on average, between 37% for individuals using both Cockburn Sound and Owen Anchorage and 68% for individuals using mainly Owen Anchorage to the dolphins' diet (**Figure 6**). The other feeding guilds always contributed less than 9% to the diet. Similarly, considering the second-best model, benthic detritivores contributed, on average, between 37% for visitors and 50% for residents to the dolphins' diet (Figure not presented), while all other feeding guilds had less than 9% contribution. For summer 2023, benthic detritivores contributed, on average, between 21% and 38% to the diet, with the highest contribution being for females and males primarily using Cockburn Sound (**Figure 6**). In both winters, no benthic detritivore prey was sourced

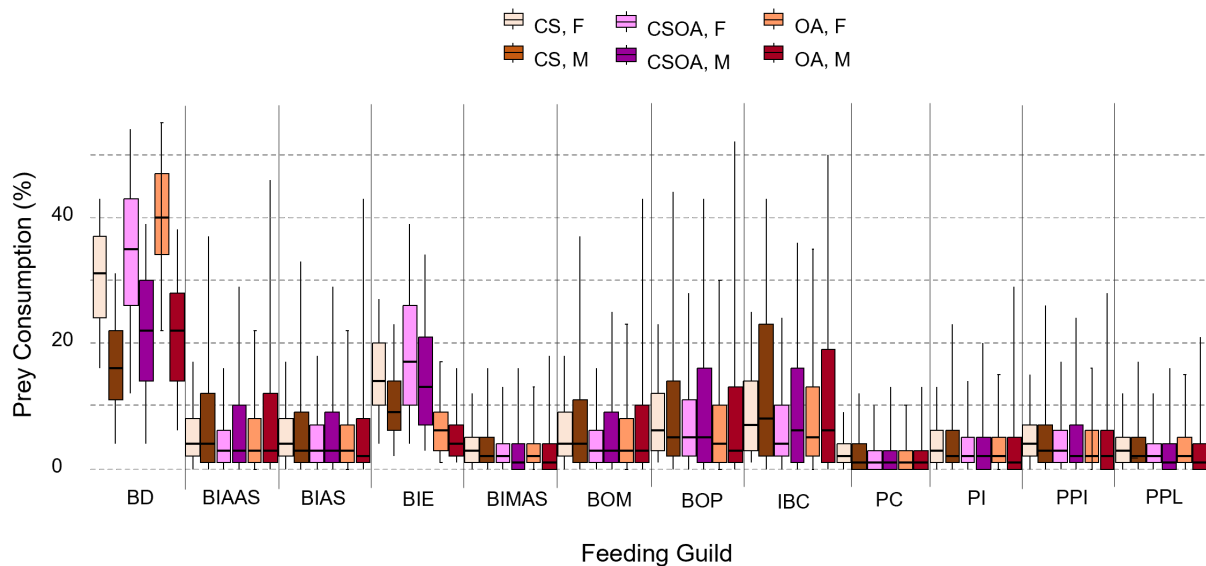


and therefore could not be included in the analysis. For winter 2022, prey described as benthic opportunistic contributed the most to the diet of male dolphins, whether they were residents, visitors, or transients (ranging from 39% to 48%, **Figure 6**). This was also the case for female visitors (28%). Invertebrate benthic carnivores also contributed to all (second most presented group with contributions ranging from 0.14 to 0.28%). Resident females had the highest contribution of the latter group. For winter 2023, prey described as benthic opportunistic contributed the most to the diet of all three identified groups, ranging from 27% to 40%. Other benthic prey contributed to more than 10% of the diet for all (**Figure 6**).



**Figure 6.** Seasonal mixing models of the relative contributions of prey (%) by feeding guild of the Indo-Pacific bottlenose dolphin sampled in Owen Anchorage and Cockburn Sound. Important covariates for each seasonal mixing model varied and were the results of comparison between different combinations of covariates models that converged. The summer 2022 model included the covariate area used (CS - Cockburn Sound; OA - Owen Anchorage; CSOA – both areas); summer 2023 model had area used with sex nested (F-female; M-male); winter 2022 model included the residency status (Resident, Visitor, Transient) with sex nested; and winter 2023 model was best when using grouping provided by the hierarchical cluster analysis (three groups). Feeding guilds represented were: Benthic Detritivore (BD), Benthic Invertivore annalid/arthropod specialist (BIAAS), Benthic Invertivore arthropod specialist (BIAS), Benthic Invertivore Elasmobranch (BIE), Benthic Invertivore Mollusk/arthropod specialist (BIMAS), Benthic Omnivore (BOM), Benthic Opportunist (BOP), Invertebrate – Benthic Carnivore (IBC), Pelagic Carnivore (PC), Pelagic Invertivore (PI), Pelagic Piscivore (PPI), and Pelagic Planktivore (PPL).

When considering the data pooled into one mixing model without the temporal scale, only the model with area used and sex as covariates converged (but see results with other models in **Appendix 4**). In this scenario, the diet of the females and males from Cockburn Sound, Owen Anchorage or both areas (CSOA) were primarily composed of benthic detritivores (ranging from 17% for CS males to 40% for females in OA), with a larger contribution observed in females (**Figure 7**). Other feeding guilds contributed to their respective diet (>10%) and were generally of benthic fish (BIE and BOP) and cephalopods, while no pelagic prey contributed more than 5% (except 6% of PPI for males from OA) (**Figure 7**).



**Figure 7.** Overall mixing modelling of the relative contributions of prey (%) grouped by feeding guild of the Indo-Pacific bottlenose dolphin sampled in Owen Anchorage and Cockburn Sound. Only model covariates area used (CS - Cockburn Sound; OA - Owen Anchorage; CSOA – both areas) and sex (nested, F-female; M-male) converged. Feeding guilds were: Benthic Detritivore (BD), Benthic Invertivore annalid/arthropod specialist (BIAAS), Benthic Invertivore arthropod specialist (BIAS), Benthic Invertivore Elasmobranch (BIE), Benthic Invertivore Mollusk/arthropod specialist (BIMAS), Benthic Omnivore (BOM), Benthic Opportunist (BOP), Invertebrate – Benthic Carnivore (IBC), Pelagic Carnivore (PC), Pelagic Invertivore (PI), Pelagic Piscivore (PPI), and Pelagic Planktivore (PPL).

## 4 Discussion

This study investigated the trophic ecology of Indo-Pacific bottlenose dolphins in Cockburn Sound and Owen Anchorage using carbon and nitrogen stable isotope signatures from samples collected from dolphins and selected potential prey species. The finding revealed several key insights:

- 1) Dolphins exhibited seasonal foraging behaviour, with a summer diet primarily consisting of benthic detritivores (e.g. sea mullet) and a winter diet dominated by benthic opportunistic species such as butterflyfish and invertebrate benthic carnivores like octopus, cuttlefish, and squid;
- 2) Complex niche partitioning was observed, as dolphins identified as resident and/or females exhibited higher carbon isotope ( $\delta^{13}\text{C}$ ) values compared to transient and/or male dolphins. This suggests that resident female dolphins spend more time foraging in the same benthic near-shore environments (i.e. exhibiting a degree of site fidelity) and therefore be more specialised in prey selection, while other dolphins may feed on a wider range of species;
- 3) Variations in nitrogen signatures were also noted, with dolphins identified as resident showing lower nitrogen isotope ( $\delta^{15}\text{N}$ ) values than dolphins identified as transient. This reflects a diet comprising of lower trophic level prey for the resident compared to the transient;
- 4) Finally, further analyses revealed a complex interplay of dietary preferences among individuals.

### 4.1 Seasonal feeding guild contributions to the dolphins' diet

Covariates associated with the best mixing models varied depending on the season and year, thus indicating potential complex intraspecific niche partitioning among the dolphins using Owen Anchorage and Cockburn Sound. This means that different individual dolphins might have unique feeding habits or preferred prey that change with the seasons and/or years. Overall, benthic detritivores were the largest (21% to 68%) dietary sources for all dolphins sampled in the summer season. Sea mullets of size ranging from 52 mm to 204 mm were found with a mean calorific value of 81.34 KJ/fish along the coastline of Bunbury (McCluskey et al. 2016). In this study, sea mullets (*Mugil cephalus*) were of small-size, averaging 67 mm in total length, solely represented the benthic detritivore feeding guild, although originally targeted as prey for little penguins (*Eudyptula minor*). Sea mullets are known to frequent seagrass areas, suggesting an overlap between dolphin habitat use and seagrass habitats. No sea mullets were caught during the winter months. It is worth noting that while sea mullet is a more estuarine-dependent species (Chubb et al. 1981), indicative of low abundance, catches of sea mullet in Cockburn Sound have also seen a decrease over the last two decades (Duffy et al. 2022).

In the winter season, benthic invertivore elasmobranchs (e.g. rays, gummy sharks), benthic opportunistic species (e.g. western butterflyfish), and invertebrate benthic carnivores (e.g. New Holland cuttlefish, western rock octopuses, southern squids) contributed the most to the diet of the dolphins sampled during that season. However, the lack of seasonal variation among the stable isotope signatures of dolphins suggests that benthic detritivore species may still have an important contribution in enabling consistent signatures across seasons. Another explanation for not observing a seasonal variation in stable isotopes despite a potential shift in the diet could be that benthic invertivore elasmobranch, benthic opportunistic species, and invertebrate benthic carnivores fall within a similar trophic range (see the report of WAMSI Westport Marine Science Program project *Trophic pathways and food web structure of Cockburn Sound and Owen Anchorage*, Hyndes et al. (2024)). This latter explanation may indeed be more likely, especially given the sulphur ( $\delta^{34}\text{S}$ ) signatures were found to be significantly smaller in summer (15.46‰) than in winter (16.98‰), indicating a change in diet (Connolly et al. 2004). The lower  $\delta^{34}\text{S}$  obtained in samples collected in the summer suggests that consumed prey were at a trophic level closer to primary benthic producers than prey consumed in winter. Such seasonal variation may be associated with the state of seagrass

during this season. Indeed, in winter and swell-influenced environments such as Owen Anchorage, recruiting seedlings can be completely lost depending on the frequency and duration of the winter storms. Additionally, species such as *Halophila ovalis* generally die back over winter (Kendrick et al. 2023), thus potentially reducing productivity for consumers.

Among the prey species important for dolphins in Cockburn Sound and Owen Anchorage, the western butterflyfish (*Pentapodus vitta*), the only benthic opportunistic species available for this study, has significantly increased in abundance in Cockburn Sound over the last 15 years and persists prominently in Owen Anchorage (Daviot 2017, Bowe 2023). Invertebrate benthic carnivores, such as cephalopods like western rock octopus (*Octopus djinda*), New Holland cuttlefish (*Sepia novaehollandiae*) and southern squid (*Sepioteuthis australis*), also constitute significant portions of the dolphins' diet. A diet study conducted in Bunbury waters also identified octopus and cuttlefish as significant prey species for dolphins (McCluskey et al. 2021), and are known for their high energy values (Clarke et al. 2009, Schaafsma et al. 2018). Although no samples were available to confirm seasonal variations in the occurrence of these species in the diet of dolphins in Cockburn Sound and Owen Anchorage, observations of Bunbury dolphins interacting with these prey were predominantly made in the cooler months (Smith & Sprogis 2016, Sprogis et al. 2017), indicating a feeding specialisation likely to occur during the cooler months in Perth as well. In McCluskey et al. (2021) study, the authors concluded that prey quantity is lower but of higher quality during winter, potentially ideal for sustaining lactating mothers and their calves staying in local waters given the high energy costs of lactation and growth (Malinowski & Herzing 2015). Coupled with recent observations supporting the frequent use of the Kwinana Shelf by female dolphins and calves, cephalopods would likely assume an even more pivotal role in the diet of females to meet their daily nutritional needs while ensuring the production of nutrient-rich milk for the rapid growth and development of their offspring.

The results suggested that pelagic prey did not contribute significantly to the dolphins' diet, despite opportunistic observations of them catching Australian anchovies (*Engrolis australis*). This may reflect the rarity of observing dolphins catching prey, especially underwater (Finn & Calver 2008). Additionally, Australian anchovies were found with a low calorific value (1.81 KJ/fish) along the coastline of Bunbury (McCluskey et al. 2016) suggesting a low contribution to their diet given the potential effort invested in catching them. Although bottlenose dolphins have traditionally been described as opportunistic generalists (Shane et al. 1986), our findings were consistent with those of other populations of dolphins from Bunbury (McCluskey et al. 2021), as well as other populations of *Tursiops* sp. inhabiting estuary and bay environments (e.g. Rossman et al. 2015a), which documented seagrass as an important habitat for dolphins, as the majority of their prey depend on it.

#### 4.2 Niche partitioning among dolphins using Cockburn Sound and Owen Anchorage

The average  $\delta^{13}\text{C}$  values for resident dolphins (-15.9‰) were higher than those for transient dolphins (17.3‰), consistent with the ranges and differences observed between inshore and offshore dolphins reported in Bunbury (McCluskey et al. 2021), as well as between the estuarine community in the Peel-Harvey estuary and coastal dolphins in Mandurah (Nicholson et al. 2021b). The lower  $\delta^{15}\text{N}$  values observed for resident dolphins (11.95‰) compared to transient dolphins (12.52‰) also indicate variations in their diet, suggesting that transients may feed on prey at a higher trophic level or on primary producers with differing carbon ratios fuelling the food web in their primary area (Troina et al. 2020). Indeed, a minority of dolphins observed in Owen Anchorage have been sighted in Shoalwater Bay (*personal comment by Dr Krista Nicholson*). The rare observations of those individuals in Cockburn Sound, however, raise the possibility for individuals travelling between the two areas via the west side of Garden Island, where fewer environmental barriers occur, allowing for the presence of more marine and/or larger species. Further studies on the diet of the dolphins using

Shoalwater Bay would be necessary to understand whether dolphins from Cockburn Sound and Owen Anchorage are ecologically different.

The carbon signature for resident females (-15.5‰) was found to be higher than for males (-16.6‰), a trend further supported by the significantly lower sulphur signatures in females (15.92‰) compared to males (17.48‰). These findings suggest a higher level of site fidelity among females than males. Such behaviour aligns with existing literature, which commonly describes females as having a smaller home range than males (e.g. Sprogis et al. 2016b). This is also reinforced by a greater frequency of female sightings across the Kwinana Shelf during the data collection period from 2022 to 2023 (see the report of WAMSI Westport Marine Science Program project *Fine-scale understanding of the use of the Kwinana Shelf by the resident dolphins in Cockburn Sound and evaluate the importance of specific habitats present in the area*, Chabanne (2024)), as well as observations of males visiting neighbouring communities for reproduction purposes (Chabanne et al 2021).

The Kwinana Shelf in Cockburn Sound is recognised as both feeding and nursery grounds for dolphins (Finn 2005, Finn & Calver 2008) and for the early life stages of certain fish species, rendering it an essential habitat for female dolphins. Here, females have the opportunity to teach their calves how to handle and ingest prey without the challenge of dealing with larger prey items, which dolphins must fragment before ingestion as they cannot masticate. Given that younger (i.e. smaller) fish tend to exhibit lower  $\delta^{15}\text{N}$  values compared to adults (Nagata et al. 2015), it is plausible that resident adult females, with their lower  $\delta^{15}\text{N}$  values, may target juvenile fish with similarly reduced  $\delta^{15}\text{N}$  values for teaching purposes, and in addition to other prey items. In other populations,  $\delta^{15}\text{N}$  values for calves have been observed to show minimum values before increasing as they mature (Rossman et al. 2015b). Further exploration into the dietary variations of females based on their reproductive status would provide valuable insights into crucial prey items during the nursing stage, thereby enhancing the survival prospects of their calves as they transition to independence.

Further individual specialization may occur among dolphins. Variations in available prey species for the mixing models, as well as other unknown individual characteristics not examined here, may lead to additional ecological units. However, it is unlikely that these variations are related to genetics, given the species' general panmictic behaviour (Chabanne et al. 2021). In Sarasota Bay, Florida, specialization was documented among females using only a subset of habitat types and employing different foraging strategies, likely to minimise intraspecific competition (Rossman et al. 2015b). Although no tools have been observed in dolphins foraging in Perth metropolitan waters, differentiation in diet has been well documented among dolphins in Shark Bay between those that use sponges and those that do not (Krützen et al. 2014). Observations during the study period included female dolphins following rays or engaging in 'snack parties' using an imitation of 'kerplunking'-forcefully slapping the water surface with their tails-while in water deeper than 6 m. This behaviour, observed in a different environment from than described by Connor et al. (2006) (which involved very shallow waters above seagrass patches), suggests some degree of specialisation among individual females. These specialisations may indeed involve prey such as cuttlefish (Smith & Sprogis 2016), octopus (Sprogis et al. 2017), as well as estuary cobblers (see below).

### 4.3 Limitations

The accuracy of the individual characteristics used in this study to infer any niche partitioning can be debated for some individuals. Indeed, due to the concentrated sampling efforts in the Kwinana Shelf area and the time gap since the last systematic surveys (2011-2015), targeting well-known individuals using Owen Anchorage and Cockburn Sound was not feasible. In response to this limitation, residency status, primary area used as well as sex of sampled individuals not known from prior studies were inferred based on behaviour and sighting locations limited to surveys conducted in 2022

and 2023, the majority being conducted across the Kwinana Shelf and representing only a portion of the dolphins home ranges.

Despite not meeting the formal conditions imposed by the Geweke diagnostic for convergence of the majority of the mixing models, longer simulations are unlikely to provide different results. Indeed regardless of the covariates, benthic detritivores dominated the diet of the dolphins, followed by benthic omnivores and invertebrate benthic carnivores (e.g. cephalopods and octopus). Mixing models are sensitive to missing sources but also to the number of sources provided (Phillips et al. 2014, Hunt et al. 2023). This can therefore prove to be challenging when working with a species such as the Indo-Pacific bottlenose dolphin, which is generally known to be an opportunistic generalist (Shane et al. 1986).

The representation of the stable isotope signatures for all sources (i.e. feeding guilds) and consumers (i.e. dolphins) in the summers, for example, suggests a gap in available sources for this study (see also mixing polygons). Specifically, the absence of species representing benthic detritivores and/or one trophic level higher suggests the importance of non-targeted fish prey. An example of this is the estuary cobbler (*Cnidoglanis macrocephalus*) with a stable isotope signature found to be within the range for dolphins' prey ( $\delta^{13}\text{C} = -15.9$ ;  $\delta^{15}\text{N} = 11.5$ , Hyndes & Lavery 2005). Between 2022 and 2023, 35 estuary cobbles were caught along the eastern side of Garden Island and Mangles Bay (*personal comment Dr James Tweedley*). While this number was much smaller than any other fish species found in Owen Anchorage and Cockburn Sound, opportunistic observations of dolphins and an Australian sea lion tossing estuary cobbles, suggest a larger importance of the species for dolphins and Australian sea lions. Similar conclusions for dolphins in Bunbury were made by McCluskey et al. (2021), who indicated that otoliths would not be detected in the stomach contents of dolphins due to how dolphins handle the prey before ingesting it (i.e. breaking the head containing the venomous spines). Similar observations have been made in Mandurah and among dolphins in Shoalwater Bay (*personal comment by Dr Krista Nicholson*). Estuary cobbler has a large caloric value (1952 KJ/fish, McCluskey et al. 2016), thus providing an important daily intake for dolphins. It is also worth noting that while further research will be required, there is some evidence that females may specialize in estuary cobbler as previously observed with the cuttlefish and octopus (Smith & Sprogis 2016, Sprogis et al. 2017). Opportunistic observations conducted in Owen Anchorage during the study and others from Shoalwater Bay suggested that the majority of the dolphins observed tossing estuarine cobbles were females, many of them having interacted with rays before the capture (*personal comment by Dr Krista Nicholson*). While the relationship between dolphins and rays and the catch of estuarine cobbler is currently being assessed by Dr Krista Nicholson, these observations again underscore the importance of the seagrass habitat for many species and their interactions in the ecosystem.

Other prey likely to be present in the diet of dolphins, albeit more seasonal, include Western Australian salmon (*Arripis truttaceus*) and pink snapper (*Chrysophrys auratus*). While direct observations of catches were made of both species in autumn and spring seasons, respectively (see section 3.3), dolphin samples were collected late summer/autumn or late winter/spring and therefore would not detect the signatures of those prey due to a half-life turnover rate of one to two months (Giménez et al. 2016). Nonetheless, it is worth noting that Australian salmon has a large caloric value (2073 KJ/fish, McCluskey et al. 2016) and would therefore contribute significantly to the daily intake of dolphins during the autumn season. Although no stranded dolphins were available during the study and they have generally been rare to sight in Cockburn Sound or Owen Anchorage, obtaining stomach contents from stranded dolphins would significantly increase knowledge and accurately infer key fish species for dolphins. More potential prey species can be identified in the report of WAMSI Westport Marine Science Program project *Trophic pathways and food web structure of Cockburn Sound and Owen Anchorage* (Hyndes et al. 2024).

Given that only two years of data were available for this study, natural stochastic processes of fish species, events associated with the El Niño-Southern Oscillation (ENSO), and climate change may not

be fully accounted for in the findings regarding key prey species. These factors contribute to interannual variation in prey availability (Fletcher et al. 2017).

## 5 Conclusions/Recommendations

The findings of this study provide valuable insights into the trophic ecology of Indo-Pacific bottlenose dolphins in Cockburn Sound and Owen Anchorage, emphasizing the significance of distinct feeding guilds (benthic versus pelagic) and identifying key prey species, such as small-sized sea mullets, octopus, and cuttlefish, along with seasonal variations. These findings underscore the crucial role of seagrass habitats in Cockburn Sound and Owen Anchorage, emphasizing the need for their conservation efforts.

This study strongly suggests complex niche partitioning among dolphins, particularly among resident females exhibiting isotopic signatures indicative of higher levels of site fidelity and specialized foraging behaviours, potentially linked to nursing needs. By consistently employing specific foraging strategies, dolphins likely enhance their efficiency at capturing prey and pass on these skills to their offspring, ensuring their survival in their natal habitat. However, transitioning to a new habitat may require significant time and effort, increasing risks for the dolphins, especially if alternative habitats are limited or already in use. To help ensure a stable food source, it is crucial to minimise modifications to seagrass habitats.



## 6 References

- Bivand R, Keitt T, Rowlingson B (2022) sp: Classes and methods for spatial data. R package version 1.4-7.
- Bowe BE (2023) Biology of Western Butterfish (*Pentapodus vitta*) in Cockburn Sound, Western Australia. Honours thesis, Murdoch University, Perth, Western Australia.
- Braulik G, Natoli A, Kiszka J, Parra G, Plön S, Smith BD (2019) *Tursiops aduncus*, Indo-Pacific Bottlenose Dolphin. The IUCN Red List of Threatened Species:e.T41714A50381127.
- Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer-Verlag, New York, USA.
- Chabanne D, Finn H, Salgado-Kent C, Bejder L (2012) Identification of a resident community of bottlenose dolphins (*Tursiops aduncus*) in the Swan Canning Riverpark, Western Australia, using behavioural information. *Pac Conserv Biol* 18:247-262.
- Chabanne DBH (2023) Broad-scale distribution and habitat modelling of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) in Owen Anchorage and Cockburn Sound using boat-based survey data from 2011-2015. Prepared for the WAMSI Westport Marine Science Program. Western Australian Marine Science Institution, Perth, Western Australia.
- Chabanne DBH (2024) Fine-scale understanding of the use of the Kwinana Shelf by the resident dolphins in Cockburn Sound and evaluate the importance of specific habitats present in the area. Prepared for the WAMSI Westport Marine Science Program. Western Australian Marine Science Institution, Perth, Western Australia.
- Chabanne DBH, Allen SJ, Sherwin WB, Finn H, Krützen M (2021) Inconsistency between socio-spatial and genetic structure in a coastal dolphin population. *Front Mar Sci* 7:1217.
- Chabanne DBH, Finn H, Bejder L (2017a) Identifying the relevant local population for environmental impact assessments of mobile marine fauna. *Front Mar Sci* 4:148.
- Chabanne DBH, Krützen M, Finn H, Allen SJ (2022) Evidence of male alliance formation in a small dolphin community. *Mammal Biol* 102:1285–1298.
- Chabanne DBH, Pollock KH, Finn H, Bejder L (2017b) Applying the multistate capture-recapture robust design to characterize metapopulation structure. *Methods Ecol Evol* 8:1547-1557.
- Chubb CF, Potter IC, Grant CJ, Lenanton RCJ, Wallace J (1981) Age structure, growth rates and movements of sea mullet, *Mugil cephalus* L., and yellow-eye mullet, *Aldrichetta forsteri* (Valenciennes), in the Swan-Avon river system, Western Australia. *Aust J Mar Freshw Res* 32:605-628.
- Clarke A, Clarke MR, Holmes LJ, Waters TD (2009) Calorific Values and Elemental Analysis of Eleven Species of Oceanic Squids (Mollusca:Cephalopoda). *J Mar Biol Assoc UK* 65:983-986.
- Connolly RM, Guest MA, Melville AJ, Oakes JM (2004) Sulfur stable isotopes separate producers in marine food-web analysis. *Oecologia* 138:161-167.
- Connor RC, Heithaus MR, Berggren P, Miksis JL (2006) “Kerplunking”: Surface Fluke-Splashes during Shallow-Water Bottom Foraging by Bottlenose Dolphins. *Mar Mammal Sci* 16:646-653.
- Daviot JR (2017) The potential for Baited Remote Underwater Video to detect changes in fish communities caused by natural and human-induced events in Cockburn Sound. Honours thesis, Murdoch University, Perth, Western Australia.
- de Lecea AM, de Charmoy L (2015) Chemical lipid extraction or mathematical isotope correction models: should mathematical models be widely applied to marine species? *Rapid Commun Mass Spectrom* 29:2013-2025.
- Duffy R, Harris D, Fisher E, Smith K, Johnston D (2022) South-West estuarine and nearshore finfish resource Part1: Sea mullet and Yellowfin whiting. Fisheries Research Report No. 322. Western Australia.
- Finn H (2005) Conservation biology of bottlenose dolphins (*Tursiops* sp.) in Perth metropolitan waters. PhD thesis, Murdoch University, Perth.
- Finn H, Calver MC (2008) Feeding aggregations of bottlenose dolphins and seabirds in Cockburn Sound, Western Australia. *W Austral* 26:157-172.

- Fletcher WJ, Mumme MD, Webster FJ (2017) Status Reports of the Fisheries and Aquatic Resources of Western Australia 2015/16: The State of the Fisheries. Department of Fisheries, Western Australia. Western Australia.
- Giménez J, Louis M, Barón E, Ramírez F, Verborgh P, Gauffier P, Esteban R, Eljarrat E, Barceló D, G. Forero M, de Stephanis R (2018) Towards the identification of ecological management units: A multidisciplinary approach for the effective management of bottlenose dolphins in the southern Iberian Peninsula. *Aquat Conserv: Mar Freshw Ecosyst* 28:205-215.
- Giménez J, Ramírez F, Almunia J, G. Forero M, de Stephanis R (2016) From the pool to the sea: Applicable isotope turnover rates and diet to skin discrimination factors for bottlenose dolphins (*Tursiops truncatus*). *J Exp Mar Biol Ecol* 475:54-61.
- Hastie T (1990) Generalized Additive Models. Routledge, New York.
- Hohn AA, Thomas L, Carmichael RH, Litz J, Clemons-Chevis C, Shippee SF, Sinclair C, Smith S, Speakman TR, Tumlin MC, Zolman ES (2017) Assigning stranded bottlenose dolphins to source stocks using stable isotope ratios following the Deepwater Horizon oil spill. *Endanger Species Res* 33:235-252.
- Holyoake C, Finn H, Stephens N, Duignan P, Salgado C, Smith H, Bejder L, Linke T, Daniel C, Lo HN, Ham GS, Moiler K, Allen S, Bryant K, McElligott D (2010) Technical report on the bottlenose dolphin (*Tursiops aduncus*) unusual mortality event within the Swan Canning Riverpark, June-October 2009. Perth, WA.
- Hunt GL, Cobain MRD, McGill RAR, Pinnegar JK, Engelhard GH, Wigham BD, Polunin NVC (2023) Quantifying spatio-temporal consistency in the trophic ecology of two sympatric flatfishes. *J Fish Biol* 103:378-392.
- Hyndes GA, Lavery PS (2005) Does transported seagrass provide an important trophic link in unvegetated, nearshore areas? *Estuar Coast Shelf Sci* 63:633-643.
- Hyndes GA, McCallum R, Beltran L, Billingham J, Tweedley JR, Cox D, Haywood M, Krispyn KN, Johnston D (2024) Trophic pathways and food web structure of Cockburn Sound and Owen Anchorage. Prepared for the WAMSI Westport Marine Science Program. Western Australian Marine Science Institution, Perth, Western Australia.
- Kendrick G, Austin R, Ferretto G, van Keulen M, Verduin J (2023) Stage 1: Review of past seagrass restoration projects and guidelines for restoration in Cockburn Sound. Prepared for the WAMSI Westport Marine Science Program. . Perth, Western Australia.
- Krützen M, Barre LM, Moller LM, Heithaus MR, Simms C, Sherwin WB (2002) A biopsy system for small cetaceans: darting success and wound healing in *Tursiops* spp. *Mar Mammal Sci* 18:863-878.
- Krützen M, Kreicker S, MacLeod CD, Learmonth J, Kopps AM, Walsham P, Allen SJ (2014) Cultural transmission of tool use by Indo-Pacific bottlenose dolphins (*Tursiops* sp.) provides access to a novel foraging niche. *Proc Biol Sci* 281:20140374.
- Logan JM, Jardine TD, Miller TJ, Bunn SE, Cunjak RA, Lutcavage ME (2008) Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *J Anim Ecol* 77:838-846.
- Louis M, Simon-Bouhet B, Viricel A, Lucas T, Gally F, Cherel Y, Guinet C (2018) Evaluating the influence of ecology, sex and kinship on the social structure of resident coastal bottlenose dolphins. *Mar Biol* 165:80.
- Malinowski CR, Herzing DL (2015) Prey use and nutritional differences between reproductive states and age classes in Atlantic spotted dolphins (*Stenella frontalis*) in the Bahamas. *Mar Mammal Sci* 31:1471-1493.
- McCluskey SM, Bejder L, Loneragan NR (2016) Dolphin prey availability and calorific value in an estuarine and coastal environment. *Front Mar Sci* 3:30.
- McCluskey SM, Sprogis KR, London JM, Bejder L, Loneragan NR (2021) Foraging preferences of an apex marine predator revealed through stomach content and stable isotope analyses. *Glob Ecol Conserv* 25:e01396.
- Nagata RM, Moreira MZ, Pimentel CR, Morandini AC (2015) Food web characterization based on  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  reveals isotopic niche partitioning between fish and jellyfish in a relatively pristine

- ecosystem. *MEPS* 519:13-27.
- Newsome SD, Chivers SJ, Berman Kowalewski M (2018) The influence of lipid-extraction and long-term DMSO preservation on carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope values in cetacean skin. *Mar Mammal Sci* 34:277-293.
- Newsome SD, Koch PL, Etnier MA, Aurióles-Gamboa D (2006) Using carbon and nitrogen isotope values to investigate maternal strategies in Northeast Pacific otariids. *Mar Mammal Sci* 22:556-572.
- Nicholson K, Bejder L, Loneragan N (2021a) Niche partitioning among social clusters of a resident estuarine apex predator. *Behav Ecol Sociobiol* 75:160.
- Nicholson K, Loneragan N, Finn H, Bejder L (2021b) Social, spatial and isotopic niche partitioning identify an estuarine community of bottlenose dolphins as a discrete management unit. *Aquat Conserv* 31:3526-3542.
- Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. *Annu Rev Ecol Evol Syst* 18:293-320.
- Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW, Parnell AC, Semmens BX, Ward EJ (2014) Best practices for use of stable isotope mixing models in food-web studies. *Can J Zool* 92:823-835.
- Rossmann S, Berens McCabe E, Barros NB, Gandhi H, Ostrom PH, Stricker CA, Wells RS (2015a) Foraging habits in a generalist predator: Sex and age influence habitat selection and resource use among bottlenose dolphins (*Tursiops truncatus*). *Mar Mammal Sci* 31:155-168.
- Rossmann S, Ostrom PH, Stolen M, Barros NB, Gandhi H, Stricker CA, Wells RS (2015b) Individual specialization in the foraging habits of female bottlenose dolphins living in a trophically diverse and habitat rich estuary. *Oecologia* 178:415-425.
- Rowlingson B, Diggle P (2020) *splancs: Spatial and space-time point pattern analysis*. R package version 2.01-41.
- Schaafsma FL, Chérel Y, Flores H, van Franeker JA, Lea MA, Raymond B, van de Putte AP (2018) Review: the energetic value of zooplankton and nekton species of the Southern Ocean. *Mar Biol* 165:129.
- Shane SH, Wells RS, Wursig B (1986) Ecology, behavior, and social organization of the bottlenose dolphin: a review. *Mar Mammal Sci* 2:34-63.
- Smith HC, Sprogis KR (2016) Seasonal feeding on giant cuttlefish (*Sepia apama*) by Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) in south-western Australia. *Aust J Zool* 64
- Smith JA, Mazumder D, Suthers IM, Taylor MD, Bowen G (2013) To fit or not to fit: evaluating stable isotope mixing models using simulated mixing polygons. *Methods Ecol Evol* 4:612-618.
- Sprogis KR, Pollock KH, Raudino HC, Allen SJ, Kopps AM, Manlik O, Tyne JA, Bejder L (2016a) Sex-specific patterns in abundance, temporary emigration and survival of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) in coastal and estuarine waters. *Front Mar Sci* 3
- Sprogis KR, Raudino HC, Hocking D, Bejder L (2017) Complex prey handling of octopus by bottlenose dolphins (*Tursiops aduncus*). *Mar Mammal Sci* 33:934-945.
- Sprogis KR, Raudino HC, Rankin R, MacLeod CD, Bejder L (2016b) Home range size of adult Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) in a coastal and estuarine system is habitat and sex-specific. *Mar Mammal Sci* 32:287-308.
- Stock BC, Jackson AL, Ward EJ, Parnell AC, Phillips DL, Semmens BX (2018) Analyzing mixing systems using a new generation of Bayesian tracer mixing models. *PeerJ* 6:e5096.
- Stock BC, Semmens BX (2016) Unifying error structures in commonly used biotracer mixing models. *ECO* 97:2562-2569.
- team P (2023a) *RStudio: Integrated development environment for R*. Posit Software, PBC, Boston, MA.
- Team RC (2023b) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Teixeira CR, Troina GC, Daura-Jorge FG, Simões-Lopes PC, Botta S (2022) A practical guide on stable isotope analysis for cetacean research. *Mar Mammal Sci* 38:1200-1228.
- Troina GC, Dehairs F, Botta S, Tullio JCD, Elskens M, Secchi ER (2020) Zooplankton-based  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isoscapes from the outer continental shelf and slope in the subtropical western South

- Atlantic. Deep Sea Res 159:103235.
- Vehtari A, Gelman A, Gabry J (2016) Practical Bayesian model evaluation using leave-one-out cross-validation and WAIC. Stat Comput 27:1413-1432.
- Wood SN (2011) Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. J R Stat Soc Series B Stat Methodol 73:3-36.
- Yeoh D, Mitchell P, Tweedley J, Johnston D, Fairclough D, West L, Krispyn K, Seymour J, Scouling B, Nilsen J, Fourie S, Norris J, McIlwain J, Middelfart P, Jackson G (2024) Spatial distribution and temporal variability in life stages of key fish species in Cockburn Sound. Prepared for the WAMSI Westport Marine Science Program. Western Australian Marine Science Institution, Perth, Western Australia

## 7 Acknowledgements

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I would also like to extend my thanks to members of the Science Program Leadership Team and other experts who reviewed the draft report.

## 8 Appendices

8.1 Appendix 1. Summary of dolphin sample characteristics and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope ratios (n = 49 samples).  $\delta^{34}\text{S}$  values for a subset of samples are also provided (n = 29 samples).

**Table 5.** Summary of dolphin samples (individual characteristics and stable isotope signatures <sup>( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$ )</sup>)

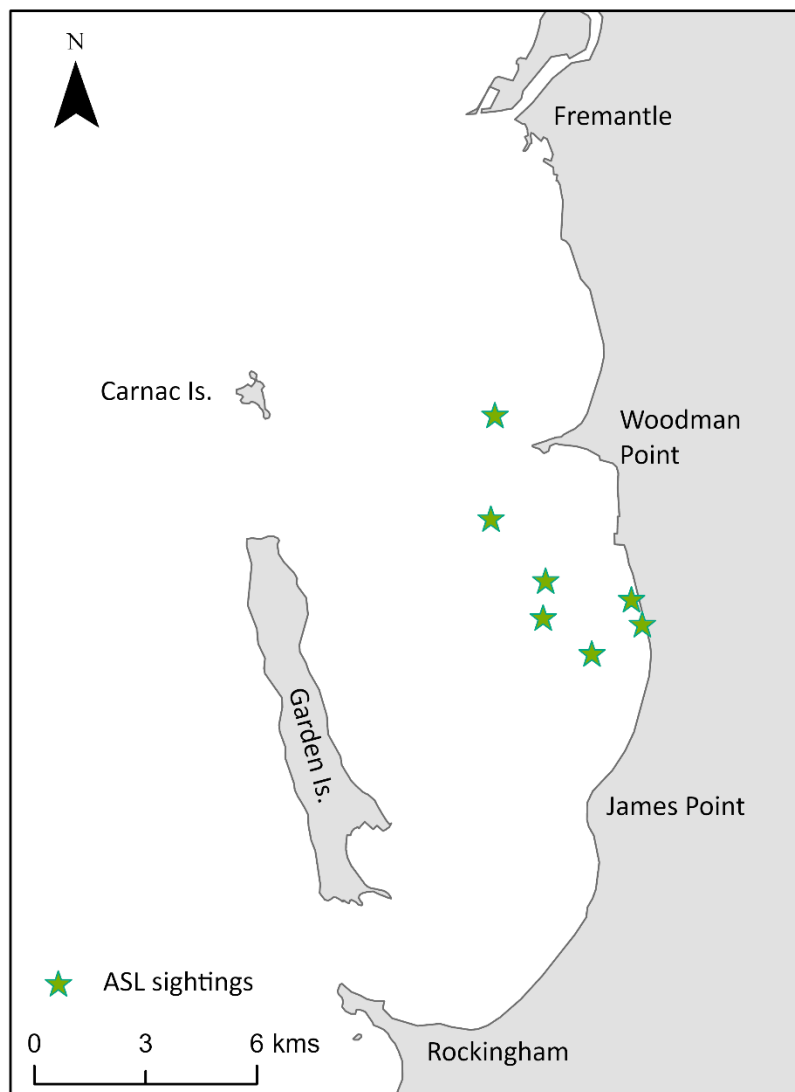
ID sample	Season	Year	Sex	Status	Area	Group <sup>1</sup>	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{34}\text{S}$
5	Summer	Y2022	F	resident	OA	3	-14.57	10.75	
7	Summer	Y2023	F	visitor	OA	4	-15.76	10.23	
10	Summer	Y2022	M*	resident	CS	1	-16.30	11.91	
16	Winter	Y2023	M*	resident	CS	10	-17.55	11.12	18.50
23	Winter	Y2023	M*	resident	CS	9	-18.26	12.56	
31	Summer	Y2022	M	resident	CS	1	-17.11	11.79	
32	Winter	Y2023	M*	resident	CS	10	-17.73	11.19	18.90
34	Summer	Y2022	F*	resident	CS	3	-14.67	10.89	15.00
35	Summer	Y2023	F*	resident	CS	4	-15.44	12.47	15.80
40	Winter	Y2022	F*	visitor	CS	7	-16.39	11.92	19.20
43	Winter	Y2023	F*	visitor	CS	88	-16.15	11.83	16.30
45	Summer	Y2022	F*	resident	CS	3	-14.67	10.89	
50	Winter	Y2023	F*	resident	CS	11	-14.99	12.64	16.40
52	Summer	Y2023	F	resident	CS	4	-15.79	11.91	17.40
54	Winter	Y2023	M*	resident	CS	8	-16.67	12.20	16.90
62	Winter	Y2022	M*	visitor	CS	7	-16.44	11.75	18.80
75	Winter	Y2022	M*	transient	OA	7	-17.44	12.75	
77	Winter	Y2023	M*	visitor	CS	8	-17.35	11.51	17.70
78	Winter	Y2022	M*	visitor	CS	7	-16.84	13.13	
85	Summer	Y2023	M*	transient	OA	5	-17.51	12.98	
86	Winter	Y2023	F	transient	OA	8	-16.87	11.83	17.90
ACA	Summer	Y2023	F	resident	CS	5	-16.50	12.45	16.00
ARC	Summer	Y2023	M	resident	CS	4	-16.14	11.73	
BLW	Winter	Y2022	M	resident	CSOA	6	-15.66	12.29	16.50
BRV	Summer	Y2022	M	visitor	CSOA	1	-16.62	12.71	17.10
BUS	Summer	Y2022	F	resident	CS	2	-15.34	12.60	13.10
CHA	Summer	Y2022	F	resident	CS	2	-15.29	12.62	12.70
CLR	Winter	Y2023	F	visitor	CS	8	-17.25	11.89	
FLK	Winter	Y2022	F*	resident	CS	7	-16.12	12.03	17.20
HAM	Summer	Y2022	F	resident	CS	1	-15.79	11.97	15.30
INF	Summer	Y2023	F	visitor	OA	4	-15.54	12.12	
INJ	Winter	Y2022	F	resident	OA	6	-14.55	10.81	14.20
KIN	Summer	Y2022	F	resident	CS	2	-15.68	12.33	13.00
LOL	Winter	Y2022	F	resident	CS	6	-15.08	11.27	
MIM	Summer	Y2022	M	resident	CS	1	-16.06	11.70	
MON	Winter	Y2022	M	resident	CS	7	-16.19	13.07	
OVA	Winter	Y2022	M	resident	CSOA	7	-16.32	11.62	15.4
POE	Summer	Y2022	M	visitor	CSOA	1	-16.27	12.34	
PMP	Winter	Y2023	F*	resident	CS	11	-15.10	12.35	
RHO	Summer	Y2022	F	resident	CS	2	-15.50	12.38	
ROF	Winter	Y2022	M*	resident	CS	6	-14.93	13.07	
SCW	Summer	Y2023	M	visitor	OA	4	-15.86	11.48	
SCL	Winter	Y2023	F	visitor	OA	8	-16.36	11.32	14.7
SOF	Winter	Y2022	F	resident	CSOA	6	-15.13	12.11	17.6
SPF	Summer	Y2023	F	resident	CS	4	-15.86	11.48	16
STR	Winter	Y2022	F	resident	CS	7	-16.59	11.86	18.7
TBO	Winter	Y2023	F	visitor	CSOA	11	-15.02	12.21	13.7
WAV	Summer	Y2023	F	resident	CS	5	-17.17	12.32	18.3
WHI	Summer	Y2022	F	resident	CS	1	-16.31	12.10	15.8

\* Sex determined through observations of behaviour and social structure based on data collected in 2022-2023. <sup>1</sup> Group obtained via a hierarchical cluster analysis based on the means of the stable isotope signatures.

8.2 Appendix 2. Opportunistic observations of Australian sea lion (*Neophoca cinerea*) in Cockburn Sound, including feeding events.

During the course of the study (January 2022–November 2023), seven sightings of Australian sea lions (*Neophoca cinerea*) were recorded (Figure 8), with four of them involving individual observed feeding. The identified prey included cobbler, ray, and cuttlefish (Figure 9). In the fourth observation, only scraps of the prey were left, making it impossible to identify.

None of the Australian sea lions could be matched with the selected marked or tagged individuals from Project 8.2.

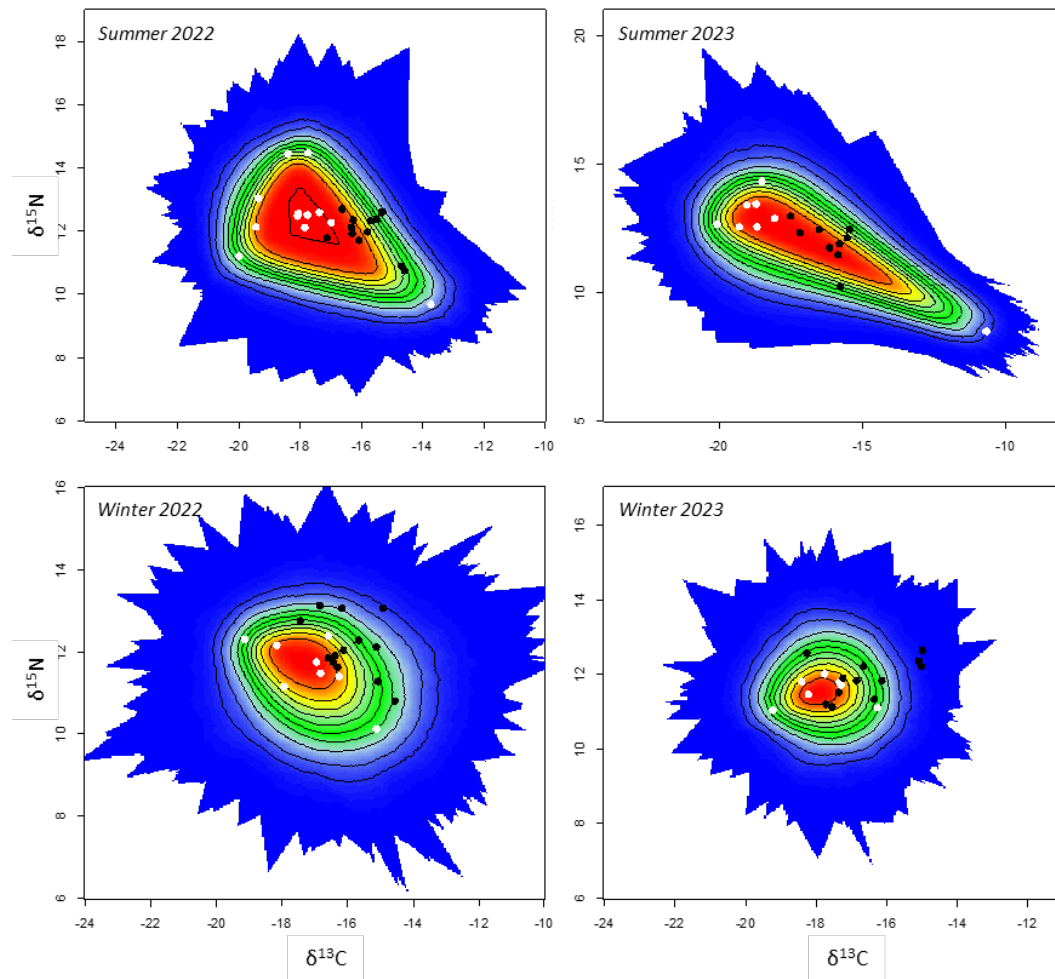


**Figure 8.** Opportunistic observations of Australian sea lions (‘ASL’, *Neophoca cinerea*) made during the boat-based surveys conducted for Indo-Pacific bottlenose dolphins between January 2022 and November 2023. Note that no stars were added on Carnac Island although ASLs were present (see report of the WAMSI Westport Marine Science Program project report *Fine-scale understanding of the use of the Kwinana Shelf by the resident dolphins in Cockburn Sound and evaluate the importance of specific habitats present in the area*, Chabanne (2024)).



**Figure 9.** Opportunistic observations of Australian sea lion catching prey in Kwinana Shelf and Woodman Point areas: (a) eagle ray; (b) cuttlefish; and (c) cobbler.

### 8.3 Appendix 3. Seasonal and yearly simulated mixing polygons.



**Figure 10.** Simulated mixing regions for summer 2022, summer 2023, winter 2022, and winter 2023. Individual dolphins (consumers) are represented as black dots, and potential prey grouped by feeding guilds are in white dots. The black lines represent probability contours at 10% levels. In winter 2023, the isotope signatures of three individual dolphins fell outside the 95% contour.



#### 8.4 Appendix 4. Result of all mixing models (regardless of convergence) for the overall dataset and each season for the Indo-Pacific bottlenose dolphins sampled in Owen Anchorage and Cockburn Sound in 2022 and 2023.

Following the Gelman–Rubin diagnostic, all models converged successfully, with no parameters having a Gelman-Rubin diagnostic of  $>1.05$ . However, based on the Geweke test, the majority of the models ( $n = 20$  out of 32 models) did not converge adequately in at least one chain (i.e.  $p > 0.05$ , **Table 5**). Although most traces showed convergence toward similar values, longer MCMC runs may improve the ratio of model convergence based on the Geweke test. Below, results comparing all models regardless of their convergence are described.

Model comparison for each season/year dataset indicated that the model with a fixed effect of groups provided by the hierarchical cluster analysis had the lowest LOOic and received between 37% of the Akaike weight for winter 2023 and 100% for summer 2022 (refer to **Table 6**). In summer 2022 and winter 2023, three groups were identified while two were identified in summer 2022 and winter 2022. When examining the diet without consideration of the temporal scale, the best mixing model included a fixed effect of groups obtained from the independent hierarchical clusters (by season/year) with an Akaike weight of 100%.

Feeding guild contributions remained consistent with previous reports, irrespective of the groups. In the summers of 2022 and 2023, benthic detritivore prey constituted the primary component of the dolphin diet, ranging from 28 to 78% across all identified groups. During winter, benthic omnivore (BOM) prey and invertebrate benthic carnivore (IBC), particularly cephalopod species, dominated the diet (BOM ranging from 18 to 40%; IBC ranging from 21 to 36%). However, while the former had a greater contribution for group 7, the latter was more important for group 6 in winter 2022 (**Figure 11**).

The overall mixing model yielded consistent results, with benthic detritivore prey emerging as either the primary or secondary most important feeding guild, accounting for contributions ranging from 6% for the diet of group 9 to 55% for group 3 (**Figure 12**). Contrasting the results of temporal-scaled models, the overall mixing model highlighted that pelagic carnivore feeding guild contributed more than 15% (up to 22%) to the diet of the three groups identified in winter 2023. However, the latest communication by Prof Glenn Hyndes (Project 4.2.3) indicated that scaly mackerel (*Sardinella lemurus*) were best described as Pelagic Planktivore and would therefore suggest this feeding guild to be more important than previously thought. Future research and modelling would be required to confirm it.

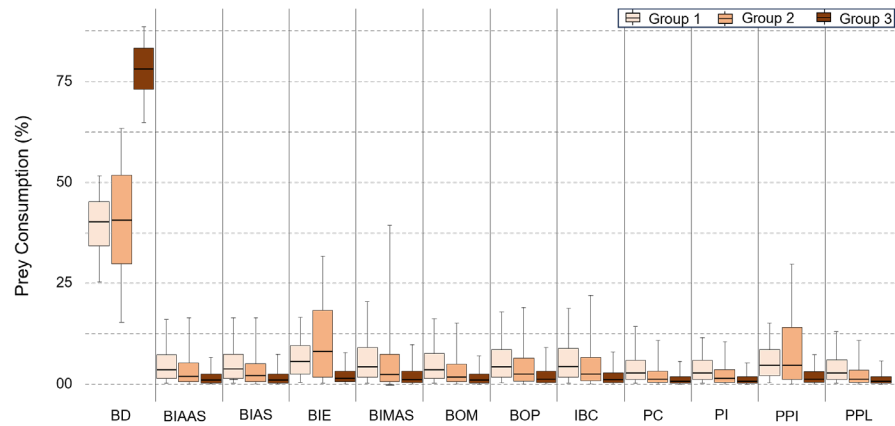
During the summer and winter of 2023, no prey from the invertebrates-benthic carnivore feeding guild (i.e. cephalopods) was collected, rendering insight into their contribution to the dolphins' diet during this period. However, the overall mixing model indicated that group 4, represented by samples collected in summer 2023, also exhibited a moderate contribution of cephalopods to their diet (16%), mirroring the findings of group 6 in its respective season/year mixing model. Additionally, benthic invertebrates such as gummy sharks (*Mustelus antarcticus*) were found to moderately contribute to the diet of dolphins, particularly in groups 1, 2, 5, 6, 7 and 11 (**Figure 12**). However, only two specimens of the same species were available for summer 2022, highlighting the need for a more extensive dataset across multiple seasons to fully comprehend the contribution of elasmobranchs to the dolphins' diet. Apart from the pelagic carnivore mentioned above, although most likely currently represented by a pelagic planktivore species (scaly mackerel), other pelagic prey, including invertivores, piscivores, and planktivores, made negligible contributions to the dolphins' diet. This suggests either suboptimal species selection by the dolphins associated with unfavourable calorific value relative to the energy expended in capturing them, or seasonal variations in the dolphins' targeting of these prey species.

**Table 6.** Comparison of the mixing models fitted with MixSIAR on Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) data for the overall and per temporal set: summer 2022 (n = 14 samples); summer 2023 (n = 10 samples); winter 2022 (n = 13 samples); winter 2023 (n = 9 samples, excluding three that fell outside the 95% contour of the mixing polygon). Single covariates were included as fixed effects. A nested effect was added for the second covariate when paired. Pass: the chain has converged with  $\leq 5\%$  of variables outside of  $\pm 1.96$  (Stock and Semmens 2016).

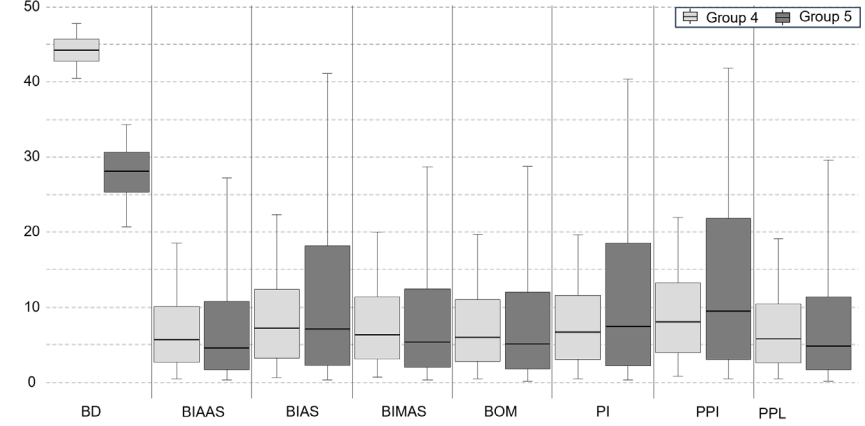
#	Model covariate	Geweke test chain1 / chain2 / chain3	LOOic	SE (LOOic)	dLOOic	SE (LOOic)	Akaike weight
<i>Overall</i>							
<b>8</b>	<b>Group</b>	<b>8% / 9% / Pass</b>	<b>65</b>	<b>14.9</b>	<b>0</b>	<b>NA</b>	<b>1.00</b>
7	Area used + Residency status	Pass / 7% / 14%	127.9	14.4	62.9	17.9	0.00
4	Residency status + Sex	Pass / 11% / Pass	133.5	13.5	68.5	15.4	0.00
6	Area used + Sex	Pass / Pass / Pass	134.4	13.2	69.4	14.7	0.00
2	Sex	Pass / 20% / Pass	135.5	12.9	70.5	13.7	0.00
3	Residency status	7% / Pass / 15%	142.2	14.2	77.2	15.9	0.00
1	Null	Pass / Pass / Pass	146.5	13.6	81.5	13.8	0.00
5	Area used	Pass / 8% / 10%	147	13.4	82	14.4	0.00
<i>Summer 2022</i>							
<b>8</b>	<b>Group</b>	<b>Pass / 9% / Pass</b>	<b>11</b>	<b>7.7</b>	<b>0</b>	<b>NA</b>	<b>1.00</b>
2	Sex	7% / Pass / Pass	37.6	4.9	26.6	9	0.00
6	Area used + Sex	Pass / 7% / Pass	40.1	5.5	29.1	9.6	0.00
4	Residency status + Sex	8% / Pass / 9%	41.2	4.6	30.2	8.8	0.00
5	Area used	Pass / Pass / Pass	43.1	6.3	32.1	10	0.00
7	Area used + Residency status	7% / 8% / Pass	44.1	6.4	33.1	10.2	0.00
3	Residency status	Pass / Pass / Pass	44.6	5.7	33.6	9.6	0.00
1	Null	17% / 7% / Pass	45.7	6	34.7	9.8	0.00

#	Model covariate	Geweke test chain1 / chain2 / chain3	LOOic	SE (LOOic)	dLOOic	SE (LOOic)	Akaike weight
<i>Summer 2023</i>							
<b>8</b>	<b>Group</b>	<b>12% / Pass / Pass</b>	<b>10</b>	<b>6.4</b>	<b>0</b>	<b>NA</b>	<b>0.99</b>
3	Residency status	6% / 13% / Pass	20.9	5.3	10.9	5.4	0.00
6	Area used + Sex	Pass / Pass / Pass	23.6	4.2	13.6	5	0.00
4	Residency status + Sex	Pass / Pass / 6%	24.1	4.2	14.1	5.1	0.00
7	Area used + Residency status	-	24.4	4.3	14.4	5.1	0.00
1	Null	Pass / Pass / Pass	27.7	6.7	17.7	4.8	0.00
2	Sex	Pass / Pass / Pass	29.9	5.7	19.9	5.1	0.00
5	Area used	Pass / 14% / 6%	30.1	7.5	20.1	5.9	0.00
<i>Winter 2022</i>							
<b>8</b>	<b>Group</b>	<b>6% / Pass / Pass</b>	<b>49.1</b>	<b>5.8</b>	<b>0</b>	<b>NA</b>	<b>0.77</b>
4	Residency status + Sex	Pass / Pass / Pass	54.1	5.7	5	1.5	0.06
2	Sex	Pass / Pass / 9%	54.1	5.9	5	1.7	0.06
3	Residency status	6% / Pass / Pass	54.6	6	5.5	1.4	0.05
6	Area used + Sex	Pass / Pass / 6%	56.3	6.2	7.2	1.5	0.02
1	Null	Pass / Pass / Pass	57	6.1	7.9	1.4	0.02
7	Area used + Residency status	Pass / Pass / Pass	57.5	6.1	8.4	1	0.01
5	Area used	6% / Pass / Pass	59.1	6.5	10	1.3	0.01
<i>Winter 2023</i>							
<b>8</b>	<b>Group</b>	<b>Pass / Pass / Pass</b>	<b>36.3</b>	<b>2.8</b>	<b>0</b>	<b>NA</b>	<b>0.37</b>
1	Null	11% / Pass / Pass	38.3	2.6	2	0.4	0.14
3	Residency status	Pass / Pass / Pass	38.8	2.1	2.5	1.3	0.11
2	Sex	7% / Pass / 7%	39.2	2.7	2.9	0.3	0.09
5	Area used	Pass / 9% / Pass	39.2	2.8	2.9	0.9	0.09
4	Residency status + Sex	Pass / Pass / Pass	39.5	2.1	3.2	1.1	0.08
7	Area used + Residency status	Pass / Pass / Pass	39.6	2.3	3.3	1.3	0.08
6	Area used + Sex	Pass / 20% / Pass	39.9	2.9	3.6	0.7	0.06

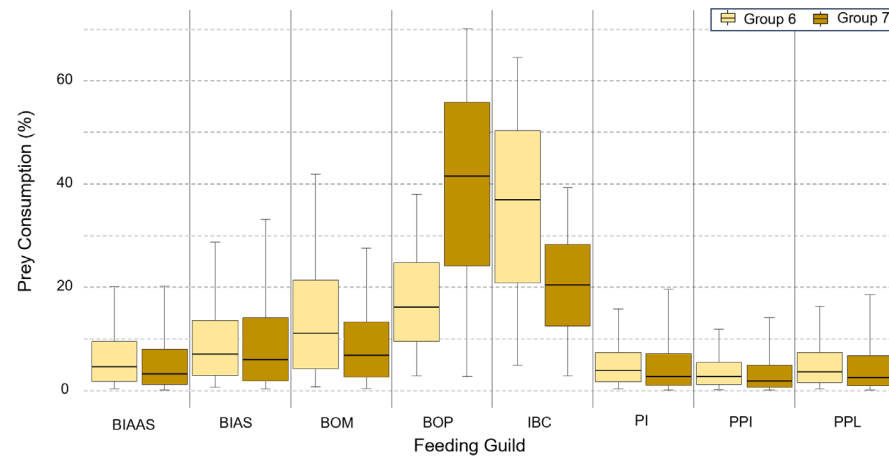
Summer 2022



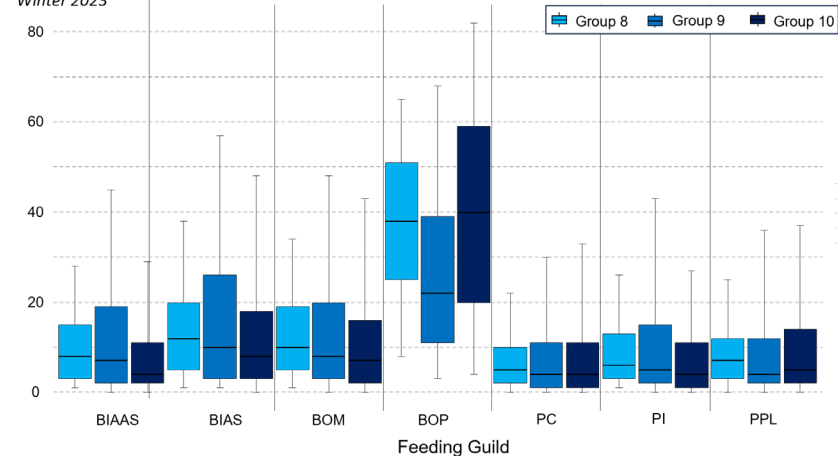
Summer 2023



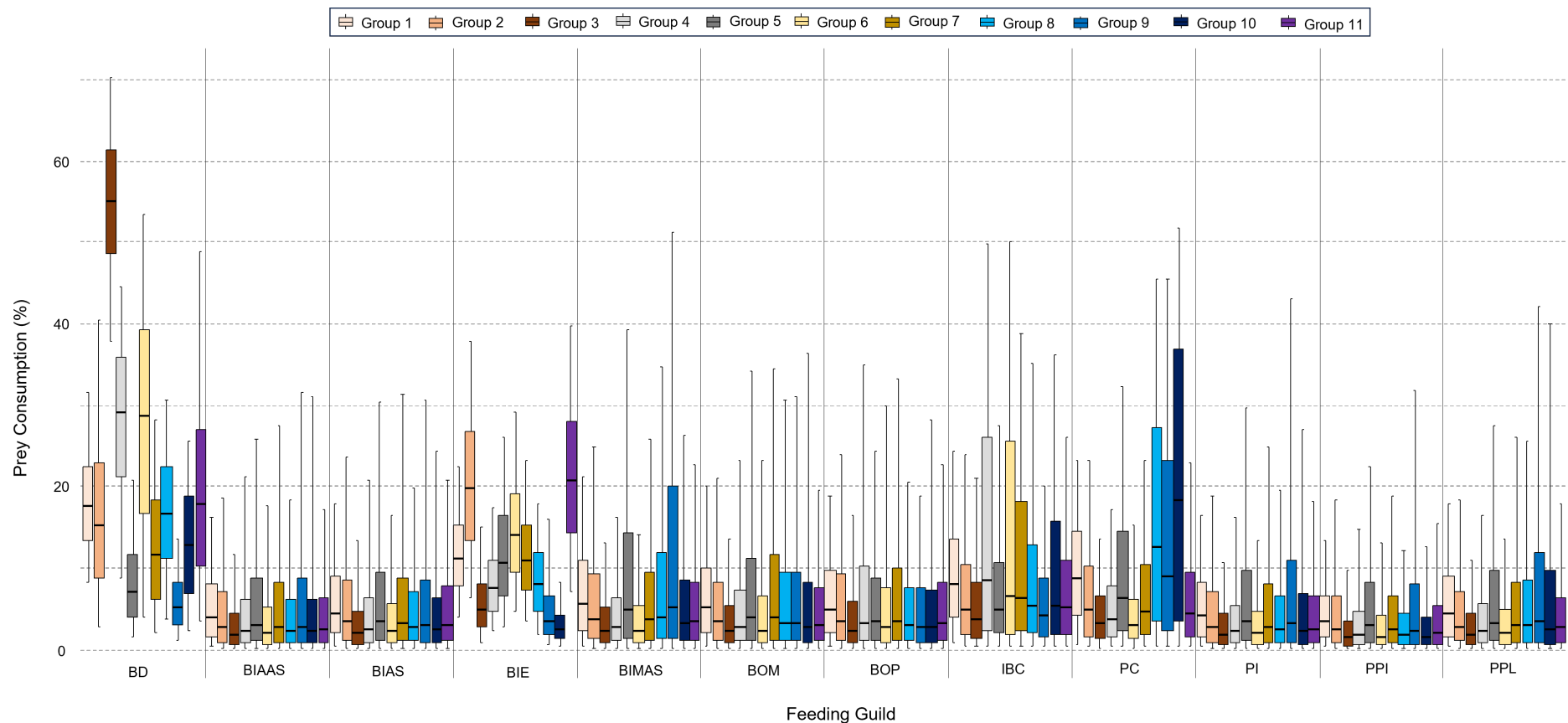
Winter 2022



Winter 2023



**Figure 11.** Seasonal best mixing modelling (regardless of convergence) depicting the relative contributions of prey (%) by feeding guild of Indo-Pacific bottlenose dolphins sampled in Owen Anchorage and Cockburn Sound. The covariate best explained the data was groupings obtained by independent hierarchical cluster analyses. Feeding guilds represented include Benthic Detritivore (BD), Benthic Invertivore annalid/arthropod specialist (BIAAS), Benthic Invertivore arthropod specialist (BIAS), Benthic Invertivore Elasmobranch (BIE), Benthic Invertivore Mollusk/arthropod specialist (BIMAS), Benthic Omnivore (BOM), Benthic Opportunist (BOP), Invertebrate – Benthic Carnivore (IBC), Pelagic Carnivore (PC), Pelagic Invertivore (PI), Pelagic Piscivore (PPI), and Pelagic Planktivore (PPL).

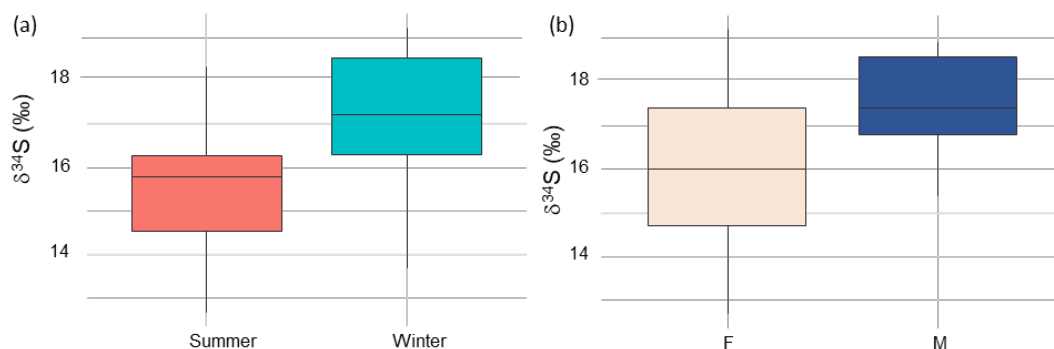


**Figure 12.** Overall best mixing modelling (regardless of convergence) of the relative contributions of prey (%) grouped by feeding guild of the Indo-Pacific bottlenose dolphin sampled in Owen Anchorage and Cockburn Sound. Only model covariates area used (CS - Cockburn Sound; OA - Owen Anchorage: CSOA – both areas) and sex (nested, F-female; M-male) converged. Feeding guilds include Benthic Detritivore (BD), Benthic Invertivore annalid/arthropod specialist (BIAAS), Benthic Invertivore arthropod specialist (BIAS), Benthic Invertivore Elasmobranch (BIE), Benthic Invertivore Mollusk/arthropod specialist (BIMAS), Benthic Omnivore (BOM), Benthic Opportunist (BOP), Invertebrate – Benthic Carnivore (IBC), Pelagic Carnivore (PC), Pelagic Invertivore (PI), Pelagic Piscivore (PPI), and Pelagic Planktivore (PPL).

## 8.5 Appendix 5. Sulphur stable isotopes

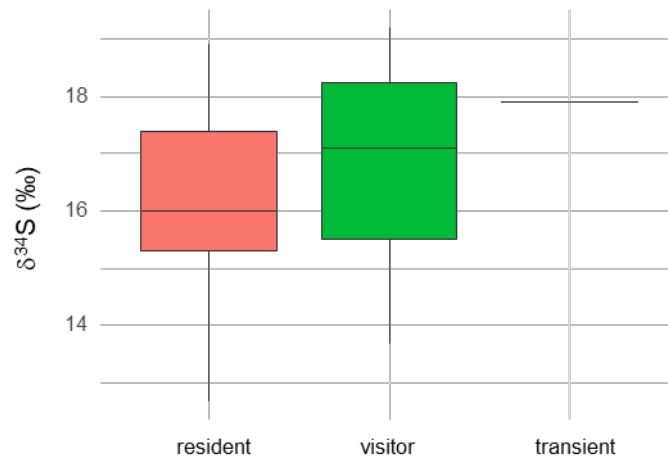
Among the 49 samples analysed, 29 underwent  $\delta^{34}\text{S}$  analysis, revealing values ranging from 12.7 to 19.2‰ (Figure 8).  $\delta^{34}\text{S}$  values are known to gradually increase from terrestrial (2-6‰) to marine habitats (21‰, Peterson & Fry 1987). The observed  $\delta^{34}\text{S}$  values in this study closely aligned with those documented in dolphins inhabiting intermediate environments between estuarine and marine habitats (Hohn et al. 2017). While two-dimensional isotopic niche modelling has shown limitations in distinguishing the feeding habits and ecological niches of distinct social clusters within populations (e.g. Louis et al. 2018), the inclusion of  $\delta^{34}\text{S}$  for a three-dimensional analysis holds promise in providing additional insights beyond those captured by  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  alone.

The mean  $\delta^{34}\text{S}$  values did not exhibit significant differences among the primary area being used ( $F = 0.44$ , p-value = 0.64), across years ( $T_{24.98} = -1.14$ , p-value = 0.26), or residency status ( $T_{9.48} = -0.76$ , p-value = 0.47), although the transient category could not be tested due to low sample size ( $n = 1$  sample). However, significant variations were observed in the mean  $\delta^{34}\text{S}$  values between seasons ( $T_{23.14} = -2.31$ , p-value = 0.03) and across sexes ( $T_{19.82} = -2.58$ , p-value = 0.02) (Figure 13). Specifically, the mean  $\delta^{34}\text{S}$  for individuals sampled during winter ( $\delta^{34}\text{S}_{\text{winter}} = 16.98\text{‰}$ , SE 0.41) was significantly higher than those sampled during summer ( $\delta^{34}\text{S}_{\text{summer}} = 15.46\text{‰}$ , SE 0.51). Furthermore, it was significantly higher for males ( $\delta^{34}\text{S}_{\text{Male}} = 17.48\text{‰}$ , SE 0.44) than for females ( $\delta^{34}\text{S}_{\text{Female}} = 15.92\text{‰}$ , SE 0.42). These findings highlight notable seasonal and sex-related differences in  $\delta^{34}\text{S}$  values, suggesting potential variations in the dolphins' habitat use and dietary preferences across these factors.



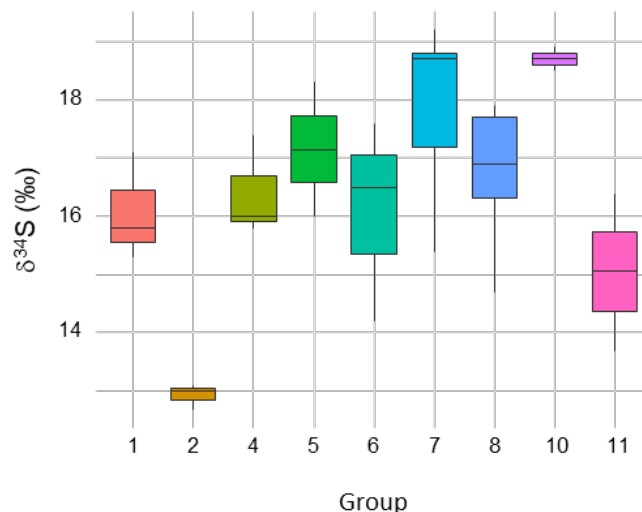
**Figure 13.** Sulphur ( $\delta^{34}\text{S}$ ) stable isotope ratios (mean  $\pm$  SD, ‰) measured in the skin of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) by (a) season (summer vs. winter) and (b) sex (Female vs. Male). Both covariates were significantly different (t-test, p-value  $\leq$  0.03 respectively).

When comparing individuals of different residency statuses, no significant difference was detected ( $T_{7.24} = -1.24$ , p-value = 0.25). However, it is important to note that 'transient' individuals could not be adequately tested due to a small sample size ( $n = 1$ ,  $\delta^{34}\text{S}_{\text{transient}} = 17.13\text{‰}$ ). The mean  $\delta^{34}\text{S}$  for the visitors ( $\delta^{34}\text{S}_{\text{visitor}} = 17.13\text{‰}$ , SE 0.81) also appeared higher than that of the residents ( $\delta^{34}\text{S}_{\text{resident}} = 16.02\text{‰}$ , SE 0.37) (Figure 14). These findings suggest that dolphins, particularly males or individuals with a lower residency level, may increase their home range during winter or have their home range overlapping with a more marine environment, where foraging occurs. Such behaviour is known to occur in some neighbored populations, with a potential shift in distribution between inshore and offshore areas during the winter season (e.g. Bunbury, Sprogis et al. 2016a).



**Figure 14.** Sulphur ( $\delta^{34}\text{S}$ ) stable isotope ratios (mean  $\pm$  SD, ‰) measured in the skin of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) by residency status (resident; visitor; transient). Although, the test indicated no significant results between resident and visitor, a tendency for  $\delta^{34}\text{S}$  to increase as the status changes from resident to transient can be observed. Note: Only one sample was available for transient and could not be included in the statistical test.

In addition to individual covariates, the samples were grouped for each respective season/year period, a total of 11 groups, although only nine groups were represented with  $\delta^{34}\text{S}$ . Not all groups could be included for testing due to low sample size ( $n < 3$  for three groups), significant differences in  $\delta^{34}\text{S}$  values were found with all others ( $F = 7.59$ ,  $p\text{-value} = 0.01$ ) (Figure 15). Specifically, the  $\delta^{34}\text{S}$  value of group 2 was significantly different to groups 7 and 8 (Pairwise t test with Bonferroni correction,  $p\text{-values} \leq 0.03$ ). Visuals would also indicate a significant difference between the groups 2 and 10.



**Figure 15.** Sulphur ( $\delta^{34}\text{S}$ ) stable isotope ratios (mean  $\pm$  SD, ‰) measured in the skin of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) by group obtained through a hierarchical cluster analysis.

This result suggests that individual variation in diet occurred with complexity in providing an overall understanding. Here, individuals from group 2 (n = 3) were sampled during the summer of 2022 and are all female residents in CS known from 2011-2015. On the opposite, dolphins in group 10 (n = 2) were sampled in winter 2023 and are two males described as residents in CS, supporting the general observations in  $\delta^{34}\text{S}$  values between females and males. Similarly, groups 7 and 8 were individual samples in winter, thus also supporting the general difference in  $\delta^{34}\text{S}$  values found between seasons.



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