The ecology of *Octopus* aff. *O. tetricus* and prevalence of anisakid nematodes in near-coastal waters of North Fremantle, Western Australia

Sleeping *Octopus* aff. *O. tetricus* in Coogee, Western Australia. Image by J. Claybrook

Submitted by

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This Thesis is presented for the degree of Bachelor of Science Honours in Marine Science  
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Declaration

I declare that this Thesis is my own account of my research and contains its main content work, which has not been previously submitted for a degree at any tertiary education institute

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Abstract

In contrast to most fished groups, global landings for cephalopod have been increasing over the last two decades. This and their fast-growth rate and short lifecycles, responsiveness to environmental change, and their function as mesopredators within the marine environment have led to an increased focus on their ecology. Within Western Australia, the common octopus, known currently as Octopus aff. O. tetricus is the target species of the commercial fishery which operates in Cockburn Sound. This Thesis focused on the population of O. tetricus inhabiting near-coastal waters of North Fremantle, ~7 km north of the current commercial operations and assessed any inter and intra-population variability between the two sites. Samples of octopus were collected by commercial fishers setting four lines of shelter pots, two in shallow (≤10 m) and two in deeper water (15-17 m) on seven occasions between March and June 2020 in North Fremantle. One sample of octopus was collected from Cockburn Sound on 1st April. These data were used to examine if the distribution, abundance, and sex ratio differed across the sampling period and between depths.

A total of 701 octopus were caught ranging in size from 35 to 218 mm mantle length (ML) and 5.13 to 691.62 g mantle weight. Over the duration of the study, the mean number of octopuses caught decreased. Males were more abundant in March and May, while females were more prevalent in April. The mean size of female octopus fluctuated in the first four hauls the trap lines and then declined from late-April to June, indicating a possible migration out of the area by larger females. Females caught in Cockburn Sound over a wider range of habitats were larger than those from North Fremantle at the same time. The mean size of male octopus increased gradually over the duration of the study with the highest mean ML recorded in June. Male octopus matured during the study with the number of immature males decreasing from March to June, in both depth regions.

Initially, this Thesis aimed to include a detailed analysis of the dietary composition of Octopus aff. O. tetricus, however due to time constraints only a preliminary analysis is presented in this dissertation. The contents of the gastric tract (crop and stomach) of 701 octopus were examined. A total of 12 taxa were identified in the gastric contents, with crustaceans identified as the most common prey, followed by teleosts and non-cephalopod molluscs. Cannibalism was observed in 51 octopus (10.8% of gastric contents with dietary items).
During the dissection of octopus mantles, parasites, tentatively identified as anisakid nematodes, were recorded on the internal organs and this became an additional focus of the research. The incidence of parasites was nearly 60% in North Fremantle and when present, always occurred in the gastric tract but were also found in the mantle muscles, digestive gland, posterior salivary glands and gonads. Binomial logistic models with a logit link function showed that the probability of parasite incidence was nearly ten times higher in the North Fremantle population compared with Cockburn Sound. The probability of parasite occurrence was also significantly greater on medium (80-160 mm ML) and large octopus (>160 mm ML) than small octopus.

The results from this study show that the nearshore waters of North Fremantle are likely a nursery habitat for juvenile and maturing male octopus’ but females probably migrate to deeper waters to mate and brood their eggs. The prevalence of parasites and location suggests that nematodes are being ingested within North Fremantle at a much higher rate than in Cockburn Sound.
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1. Introduction

Interest in cephalopod ecology has increased exponentially over the past few decades, with a spotlight being focused on their importance in not only within the marine ecosystem but also as valuable food resource, especially with growing concerns of the sustainability of traditionally fished species (O'Brien et al., 2018). Coeloid cephalopods (*i.e.* those lacking an external shell) are considered keystone species within many marine ecosystems. In many food webs, cephalopods are mesopredators which establish the link between apex predators and lower trophic levels (Boyle & Rodhouse, 2005; Heery et al., 2018; Yick et al., 2012). Being mesopredators, cephalopods have the potential to influence the structure and dynamics of their ecosystem (Yick et al., 2012). Due to their sensitivity to environmental change, cephalopods can act as ecological indicators and exhibit top-down control over their prey (Boyle & Rodhouse, 2005; Rodhouse et al., 1997). Furthermore, their short-lived, fast-growing life history means they can rapidly respond to fluctuations within their environment, which can cause detrimental consequences for upper and lower trophic levels within their ecosystem (Tian, 2009).

In recent years, considerable focus has been directed to the increasing economic importance of cephalopods as traditionally targeted finfish species continue to decline due to anthropogenic stresses such overfishing and climate change (Caddy & Rodhouse, 1998; Rosa et al., 2019; Watson & Pauly, 2001). This thesis focuses on examining the distribution and abundance of the target species of the Western Australian Octopus fishery, *Octopus aff. O. tetricus*, in near-coastal waters of Western Australia. It aims to build upon current knowledge of cephalopods and their importance in commercial and ecological settings.

1.1.1 Cephalopod Fisheries

Scientific advances throughout the past 50 years have led to a massive increase in the comprehension of marine ecosystem functions (FAO, 2020). Commercial fish production from capture fisheries and aquaculture are estimated to have reached 179 million tonnes in 2018 worldwide, with an estimated value of 401 billion USD (FAO, 2020). Consumption of seafood has increased by 3.1% annually between 1961 to 2017, which is almost twice the rate of population increase (1.6%), and higher than other protein foods (2.1% annual increase) (FAO, 2020). Fisheries can, directly and indirectly, affect marine ecosystem predator-prey relationships, which if overexploited may lead to the shift in trophic relationships and
potentially loss biodiversity or collapse of fish stocks (Coleman & Williams, 2002; Thrush et al., 2016).

Cephalopods have been an increasingly important resource in fisheries worldwide and contribute 2–5% of global landings from capture fisheries. Unlike finfish fisheries, which have plateaued or declined, cephalopod fisheries have gradually increased from 0.5 million tonnes in 1950 to a peak of 4.85 million tonnes in 2014, this number decreased to 3.5 million in 2018 but has continued to stay at relatively high levels (FAO, 2020).

Worldwide, octopus’ are fished using passive shelter pots, which are low cost, easy to operate and cause minimal impact on the surrounding environment. However, the pots often cannot be deployed in deep waters or areas of high energy (Hart et al., 2016; Leporati et al., 2015; Sauer et al., 2019). Alternative trapping methods used by commercial octopus fisheries include the frame traps, which utilise a netting that allows the octopus to push in but not back out (Hernández-García et al., 1998). More recently, the use of octopus traps with an active closing mechanism, triggered by a tripwire, and bait have been trialled in commercial fisheries (Carreira & Gonçalves, 2009; Sauer et al., 2019). The utilisation of active traps is prohibited in many octopus’ fisheries worldwide. However, the Department of Primary Industries and Resource Development in Western Australia has recently conducted a trigger trap trial and allows recreational fisherman to use up to six active traps (Hart et al., 2019; Leporati et al., 2015; Sauer et al., 2019).

The management and assessment of commercial cephalopod fisheries are challenging (FAO, 2020) because of the short life-cycle, fast growth rates and the variability in recruitment and catch rates of cephalopods with environmental fluctuations (Boyle & Boletzky, 1996). These challenges are highlighted by recent declines in three of the main commercial squid species; jumbo flying squid (Dosidicus gigas), Argentine shortfin squid (Illex argentinus) and Japanese flying squid (Todarodes pacificus) (FAO, 2020). In recent years, there has been a decline in commercial octopus’ suppliers due to an increasing scarcity of octopus, as some important fisheries have become less productive, requiring urgent and strict management strategies (FAO, 2020).
1.2.1 Australian Cephalopod Fisheries

Commercial cephalopod fisheries are a relatively new development in Australia. Initially, international chartered vessels discovered significant resources of arrow squid *Nototodarus goudi* off the southern coast of Australia in the 1970s (Kailola et al., 1993). However, it was not until the mid-1980s that cephalopod stocks were exploited commercially (Kailola et al., 1993; Nottage et al., 2007). Most cephalopod landings in Australia are taken as a by-product from other fisheries, particularly lobster fisheries. Australian cephalopod commercial landings primarily consist of squid, followed by octopus. Cuttlefish are caught only by-catch of other fishing efforts (Kailola et al., 1993; Nottage et al., 2007).

The southern squid jig fishery (SSJF) operates between Fraser Island in Queensland to the South Australian and Western Australian border and includes waters around Tasmania (Nowara & Walker, 1998; Trinder & McKinley, 2003). The target species of the fishery is arrow squid (*Nototodarus gouldi*) and has incidences of *Sepioteuthis australis* (southern calamari), *Todarodes filippovae* (Southern Ocean arrow squid) and *Omnastrephes bartramii* (red ocean squid) as bycatch. Incidental catches of *N. gouldi* are also caught in the Southern and Eastern Scalefish and Shark Fishery. SSJF uses up to 10 automatic jig machines on each vessel, each with 2 spools of heavy line, with ~ 25 jigs attached to each line (Furlani et al., 2007).

At present, Australia has two dedicated octopus’ fisheries in the Eastern Indian Ocean, the *Octopus aff. O. tetricus* fishery in Western Australia (for further detail see 1.4.1 below) and the *O. pallidus* fishery in Tasmania (Sauer et al., 2019). The Tasmanian octopus’ fishery was established in 1980, is operated by a single company with two full-time vessels which fish in the Bass Strait (Leporati et al., 2009). The fishery uses passive shelter pots on demersal longlines ~4 km in length, each line contains up to 1000 pots per line and the lines are deployed in depths from 18–85 m (Leporati et al., 2009).

1.3 Ecology of coastal cephalopods

Coleoid cephalopods (*i.e.* those without an external shell) are present in practically all types of coastal habitats worldwide (Boyle & Rodhouse, 2005). They are not known to inhabit marine environments with low salinity, and no cephalopods are endemic to estuarine environments. Coastal species exhibit demersal lifestyles (*i.e.* strong association to the seafloor) (Boyle & Rodhouse, 2005).
1.3.1 Adaptations to the coastal environment

Demersal cephalopods utilise sophisticated organs to solve problems they encounter within the natural environment (Hanlon & Messenger, 2018). Cephalopods have practical locomotive adaptations (i.e. strong mantle, funnel, and fin muscles) and highly evolved sensory organs. The sensory organs of cephalopods are the most evolved of all invertebrates and rival the sophistication of vertebrate systems (Boyle & Rodhouse, 2005). Coleoid cephalopods possess a ‘camera-like’ eye similar to vertebrates, containing a pupil, lens and rhabdomeric photoreceptors which form the retina (Serb & Eernisse, 2008). Cephalopods are colour-blind (Marshall & Messenger, 1996) but are sensitive to polarisation (Moody & Parriss, 1960; Pignatelli et al., 2011; Shashar et al., 2000).

Most coleoid cephalopods have a complex nervous system, with a multi-lobed brain. The brain to body ratio of cephalopods is akin to those of lower vertebrates (Mather & Kuba, 2013). The complexity of the nervous systems allows cephalopods to adapt to a wide range of learning (Mather & Kuba, 2013) including; associative (Cole & Adamo, 2005), spatial (Alves et al., 2006; Scatà et al., 2016), social (Tricarico et al., 2011) and problem-solving (Richter et al., 2016). Cephalopods exhibit remarkable behavioural plasticity (Alves et al., 2006; Brown & Piscopo, 2013; Hanlon & Messenger, 2018).

Coastal species actively use the benthic environment. They are predominantly bottom feeders, feeding on the rich diversity of invertebrates and small fish available (Boyle & Rodhouse, 2005). Octopuses have adapted sophisticated hunting and prey-handling (Boyle & Rodhouse, 2005; Hanlon & Messenger, 2018). Demersal cephalopod eggs are encapsulated in tough protective sheaths and fixed to the hard substrate, kelp plants and artificial surfaces such as buoys or fishing traps. Moreover, octopuses are highly dependent on crevices, rock, and reefs within the coastal environment for protection from predation (Anderson, 1997; Boyle & Rodhouse, 2005; Mather, 1994; Mather & O'Dor, 1991).

Feeding ecology

Feeding and foraging of coleoid cephalopods have been comprehensively studied and reviewed (Boyle & Rodhouse, 2005; Villanueva et al., 2017). Coleoid cephalopods have a voracious, exclusively carnivorous feeding regime, functioning as mesopredators within the
marine environment (Boyle & Rodhouse, 2005; Greenwell et al., 2019a; Hanlon & Messenger, 2018). Cephalopods possess a range of physiological, morphological, and behavioural adaptations that help them be highly successful predators within the marine environment, which can exhibit significant predatory pressure on benthic communities.

Cephalopods use versatile feeding apparatus (arms and tentacles) to feed on a diverse group of prey (Boyle & Rodhouse, 2005). All cephalopods exclusively feed by dismembering prey with their beak, consequently, diet analysis can be exceptionally challenging. Demersal cephalopods primarily feed on crustaceans, fish and other cephalopods (Boyle & Rodhouse, 2005; Greenwell et al., 2019a; Greenwell et al., 2019b; Villanueva et al., 2017) As cephalopods are opportunistic, their prey selection varies with prey availability which varies with depth and habitat type (Smith, 2003). Variations in diets of octopus are also related to size and maturation stage (Smith, 2003).

The main predators of coastal cephalopods are seabirds, marine mammals, and larger fishes. Animals which predate on fish will also take cephalopods (Boyle & Rodhouse, 2005). Except for a few elasmobranch species, no animals are known to exclusively predate on cephalopods (Boyle & Rodhouse, 2005).

**Habitat utilisation**

Coeloid cephalopods have lost the external shell to increase mobility, and as a consequence, they are very vulnerable to predation (Cigliano, 1993). Demersal species often use dens or naturally occurring crevices for protection (Aronson, 1986; Hanlon & Messenger, 2018). The characteristics of the substrate where benthic species reside are thought to be a key factor in influencing their distribution (Hanlon & Messenger, 2018). Studies investigating habitat preference of octopus species found den availability and preference for the reef edges were key factors affecting octopus’ distribution (Anderson, 1997; Mather, 1994).

Den availability has also been described as a limiting factor for octopus’ density (Hartwick et al., 1984; Katsanevakis & Verriopoulos, 2004; Mather, 1994). Demersal cephalopods routinely compete for shelters; with size and sex influencing the success of shelter occupancy (Mather, 1982; Narvarte et al., 2013).

Sandy substrates often have limited shelter available for cephalopods which can impact the density of cephalopods and the capacity for females to brood their eggs in these areas. Molluscan shells are often used by an octopus to build suitable dens on sandy substrate.
Narvarte et al. (2013) found that juvenile Patagonian octopus used barnacle shells and adults utilised larger oyster and clamshells to create dens.

**Camouflage**

Crypsis and cryptic behaviour is the primary defence mechanism of coastal cephalopods (Boyle & Rodhouse, 2005; Hanlon et al., 2010; Marshall & Messenger, 1996). Cephalopod camouflage abilities exploit surrounding environmental features and allow them to ‘blend in’ to their surroundings (Boyle & Rodhouse, 2005). Hanlon and Messenger (2018) summarised the different camouflage techniques of cephalopods, including background resemblance, countershading, disruptive colouration, and deceptive resemblance (Doubleday et al., 2016; Villanueva et al., 2016).

**1.3.2 Distribution and abundance**

Cephalopod distribution varies considerably depending on species (Doubleday et al., 2016). Demersal cephalopods exhibit the lowest dispersal capacity in shelf waters (within 10's km). Pelagic cephalopods inhabit open oceanic waters and have the highest capacity for dispersal (thousands of km), due to having a paralarvae stage (duration ~5-115 d) and a highly motile adult stage

Octopus and cuttlefish tend to be concentrated in demersal habitats in coastal environments (Boyle & Rodhouse, 2005; Quetglas et al., 2000), while the squids from the order Myopsida are restricted to the neritic zone, likely due to their dependence on the benthos for egg-laying and feeding opportunities (Pierce et al., 2008). Within shallow coastal habitats, benthic octopuses dominate rocky reef habitats whereas, cuttlefish and squid species are more commonly found in soft substrate habitats. True squids are predominantly pelagic, and their distribution and depth of occurrence vary widely among species. Most studies examining distribution and abundance of coastal cephalopods focus on north Atlantic and Mediterranean populations (Arechavala-Lopez et al., 2019; Belcari et al., 2002; Quetglas et al., 2000; Vargas-Yáñez et al., 2009) with far less published data on those in Southern Hemisphere (Ramos et al., 2014).

**Impact of urbanisation**

Understanding the impact of urbanisation on cephalopod distribution and abundance is still in its infancy. As marine urbanisation changes natural habitats, this is likely to alter
cephalopod interactions with these environments. Artificial structures are known to support distinct assemblages of marine fauna, especially mobile species such as octopus (Mather, 1994). At present, there is one published study investigating the impact of urbanisation on distribution and abundance of octopus. Heery et al. (2018) observed urbanisation-related patterns of *Enteroctopus dofleini*. Unlike terrestrial animals in urban environments which benefit directly or indirectly from enhanced food resources from humans, there is no evidence to suggest that octopus’ diets are subsidised by urbanisation (Heery et al., 2018). However, octopuses do frequently utilise structures as dens and were more likely to occur in areas with large amounts of anthropogenic debris (Heery et al., 2018). The use of artificial structures for dens has been established in several studies (Katsanevakis & Verriopoulos, 2004; Mather, 1994). Urbanisation is likely to affect octopus abundance more in populations which occur on sandy environments, where dens are scarce (Katsanevakis & Verriopoulos, 2004).

Historically, octopuses were identified as habitually solitary animals, only interacting with one another in mating periods, with other encounters being hostile. Recently, a few studies have identified more social behaviours (*i.e.* aggregation and interaction) in certain species including *O. tetricus* (Scheel et al., 2017) and the undescribed Larger Pacific Striped Octopus (Caldwell et al., 2015). Scheel et al. (2017) identified *O. tetricus* residing in high densities in two locations in Jervis Bay, on the east coast of Australia. Typically, *O. tetricus* has been identified as a solitary species but within these locations exhibit complex social behaviours. It has been hypothesised that these large aggregations form in response to limited den availability but an abundant food supply (Scheel et al., 2017). It has been suggested that clustered shelters heighten social interactions of octopus (Scheel et al., 2017).

**Environmental influences on distribution and abundance**

Environmental conditions and the strength of recruitment have a significant impact on abundance (Sobrino et al., 2002). The size of cephalopod populations is variable at annual timescales with rapid expansion occurring when conditions are favourable and rapid decline when conditions become unfavourable (Rodhouse, 2010). In recent years there has been growing speculation that cephalopod populations may be proliferating in response to changing conditions, as evidenced by the increasing trends in cephalopod catch rates (Doubleday et al., 2016). It is well established that cephalopods are highly sensitive to shifts in environmental conditions. Cephalopods respond to changes in ecological conditions either actively (*i.e.* moving with changing conditions to areas more favourable for spawning or feeding) or
passively (*i.e.* effects on growth and paralarvae survival) (Pierce et al., 2008). Due to their sensitivity to changing conditions, cephalopods could potentially act as environmental indicators (Pierce et al., 2008).

Octopus distribution shows a significant relationship between size and depth, with an ontogenetic shift to deeper waters with increasing size (Leite et al., 2009). The ontogenetic distribution also appears to be linked with environmental conditions such as temperature. Smaller individuals of *O. insularis* are known to occupy warmer waters than larger individuals, likely due to the high productivity attributed to warm waters, which promotes faster growth and therefore, shortens the period in which they are most vulnerable to predation (Leite et al., 2009). Migration to brooding habitats has been documented for several species of octopus including *Octopus* aff. *O. tetricus* (Leporati et al., 2015), *O. magnificus* (Smith et al., 2006), *O. tetricus* (Anderson, 1997), *O. insularis* (Leite et al., 2009; Lima et al., 2014) and, *O. vulgaris* (Belcari et al., 2002; Katsanevakis & Verriopoulos, 2004).

Many coastal octopus species exhibit interannual variability and cyclical patterns of abundance (Regueira et al., 2014). The relationship of the abundance of *O. vulgaris* and the variability of SST were investigated in the Alboran Sea (Vargas-Yáñez et al., 2009). Results showed that *O. vulgaris* abundance was adversely affected by warm water anomalies occurring in the previous year (Vargas-Yáñez et al., 2009). These findings indicated that *O. vulgaris* landings could be negatively affected by long-term warming of the Western Mediterranean. Similarly, Sobrino et al. (2002) identified a beneficial influence from low winter temperature for the next years landings. They found that cephalopod landings in the Gulf of Cadiz reached a maximum in winter before decreasing to their minimum in September. Other studies in the Mediterranean Sea found similar results, with minimum abundance occurring between August and September (Vargas-Yáñez et al., 2009). The influence of temperature is intrinsically linked to other environmental and biological variables (*i.e.* upwelling intensity, enhanced primary production, embryonic and paralarvae growth) (Sobrino et al., 2002; Vargas-Yáñez et al., 2009).

Abundance patterns of tropical cephalopod species do not exhibit the same seasonal variations as those seen in temperate and sub-tropical areas (*i.e.* *O. vulgaris*, Katsanevakis and Verriopoulos, 2004; *Entereoctopus dofleini*, Hartwick et al., 1984). The abundance of tropical species appears to be more influenced by habitat characteristics such as depth and substrate than temperature (Leite et al., 2009).
There are growing concerns about the impact of long-term warming and climate change on cephalopod populations. Warming temperatures are known to increase growth rates which subsequently accelerates their life cycles (Doubleday et al., 2016) and could cause a faster population turnover. Warmer temperatures are also known to cause premature hatchings, leading to decreased chances of survival during the crucial first few days (Uriarte et al., 2016). The rapid life cycle of cephalopods will likely aid in their adaptations to changing oceanic conditions, and some cephalopod species may benefit from changing conditions (Doubleday et al., 2016).

1.3.3 Reproduction and Brooding

Nearly all cephalopods are semelparous (i.e. one reproductive episode) (Boyle & Boletzky, 1996; Boyle & Rodhouse, 2005). Unlike other molluscs, the sexes are separate, and there is no evidence of hermaphroditism or sex change within cephalopods.

Assessment of maturity stages is an important aspect of both ecological studies and fishery management. The temporal and spatial distributions of breeding aggregations, the size at maturity and the sex ratio all require an assessment to ensure population levels are sustained. The mating strategy of cephalopods is uncommon among other molluscs, reproduction always takes place through individual mating. Octopus males possess a hectocotylus (i.e. modified arm) which is used to transfer spermatophores to females during copulation. Spermatophores can be stored within the mantle cavity, which means that a significant time may pass between mating and fertilisation. Males are likely to mate with several females. Unlike other cephalopods that may spawn in batches over an extended period, octopuses tend to only lay eggs over a few days (Boyle & Rodhouse, 2005)

Locating a suitable egg-laying habitat is important to demersal cephalopod reproductive success (Pratasik et al., 2017). Habitat selection for other demersal cephalopods, especially for mature females, is influenced by suitable environments to brood their eggs (Guerra et al., 2015; Narvarte et al., 2013; Pratasik et al., 2017). The quality of cephalopod shelters is known to affect reproductive output (Iribarne, 1990; Narvarte et al., 2013).
All female octopuses studied are known to protect and clean their eggs throughout the entire duration of embryonic development (Robin et al., 2014). Female benthic octopuses brood their eggs in rocky crevices, coralline overhangs, or confined spaces (Boyle & Rodhouse, 2005). Eggs are deposited into the den using two methods: (i) the eggs are individually encrusted into the hard substrate or; (ii) cemented into the hard substrate in clusters where individual eggs are intertwined with one another (Hanlon & Messenger, 2018). Some genera of octopus that occupy habitats without suitable brooding shelters carry their developing eggs within the webbing of their arms, e.g. Hapaloclaena (Norman et al., 2000) and Amphioctopus (Guerrero-Kommritz et al., 2016; Guerrero-Kommritz & Rodriguez-Bermudez, 2018).

Narvarte et al. (2013) investigated the effect of egg predation and competition on brooding shelter selection of O. tehuelchus in San Antonio Bay, Patagonia Argentina. They found that shelter availability was a constraint on brooding and affected the level of parental care for the fertilised eggs. Furthermore, Iribarne (1990) found that the fecundity of O. tehuelchus was higher in large-volume shelters than smaller shelters. If octopuses of both sexes at a similar size compete for a single shelter, the female will win occupancy of the shelter (Narvarte et al., 2013). Contest over shelters between sexes has been observed for several species including O. tehuelchus (Narvarte et al. 2013), O. dofleini (Hartwick et al. 1984) and O. briareus (Aronson, 1986). Competition for shelters is more apparent during brooding periods and a female octopus may inhabit a single shelter for more than two months (Narvarte et al., 2013).

The final life stage for octopuses is a period of senescence (Anderson et al., 2002). Usually, males undergo senescence after mating, while females senesce while brooding their eggs. Typically, symptoms of senescence include lack of feeding, retraction of skin around the eyes, decreased physical abilities and lesions on the skin (Anderson et al., 2002; Boyle & Rodhouse, 2005; Wodinsky, 1977). Many aspects of octopus senescence are still poorly understood (Anderson et al., 2002).

After spawning, female octopus’ decrease and then stop feeding during the incubation of the eggs, focusing all energy entirely on egg care until the hatchlings emerge (Boyle & Rodhouse, 2005; Hanlon & Messenger, 2018; Robin et al., 2014; Roumbedakis et al., 2018b). Maternal care from female octopuses usually involves protecting eggs from predators, increasing ventilation by flushing water over the eggs, cleaning eggs and removing any dead embryos (Roumbedakis et al., 2018b). The physiological condition of female brooding octopus declines over time. The starvation behaviour of female octopuses during the brooding stage
is well established throughout literature. In a study of *O. hummelincki*, Wodinsky (1977) suggested that the biological trigger for this behaviour could be linked to secretions of the optical glands. Experiments involving the removal of the optical gland after spawning revealed that *O. hummelincki* females returned to feeding, gained weight and the lifespan was extended by four months or more (Wodinsky, 1977).

1.4 *Octopus aff. O. tetricus*

*Octopus aff. O. tetricus* is a merobenthic (*i.e.* has a paralarval stage) species endemic to temperate coastal waters (*≤* 70 m deep) of Western Australia. This species is relatively short-lived (*≤* 18 months), medium-sized (< 4 kg) and is highly fecund (125 000 – 150 000) (Joll, 1976). Once considered synonymous with the Common Sydney Octopus, *Octopus tetricus*, taxonomic studies suggest that *Octopus aff. O. tetricus* (not yet formally described) is a separate species, with a geographically distinct population that extends from Shark Bay to the South Australian Border (Amor et al., 2014). *Octopus aff. O. tetricus* and *O. tetricus* are closely related to the cosmopolitan species *O. vulgaris*.

Similar to many other octopus’ species, *Octopus aff. O. tetricus* (hereafter *O. tetricus*) is semelparous. Both sexes of *O. tetricus* are known to have similar life expectancies, with males reaching maturity at an earlier age than females (Leporati & Hart, 2015). The maximum size for females (~4 kg) is considerably larger than males (~2.5 kg). This species inhabits rocky reefs, sandy substrates and seagrass meadows. Recent studies have provided evidence of mature females migrating to deep waters to find appropriate dens to brood their eggs, followed by mature males seeking mates (Leporati et al., 2015).

1.4.1 *Western Australian Octopus Fishery*

The first commercial fishing operation for octopus in Western Australia was instigated by Japanese scientists during 1979 – 1981 as a mitigation strategy for the extensive octopus’ predation and continual bycatch within the Western Australian rock lobster (*Panulirus cygnus*) fishery (Joll, 1977). At present, commercial octopus fishing yields approximately 204 tonnes from three primary fisheries; the Western Australian Developmental Octopus Fishery, the West Coastal Rock Lobster Fishery and the Cockburn Sound Pot and Line Fishery (Hart et al., 2016; Sauer et al., 2019). Since the establishment of the Western Australian Octopus Fishery in 2001, the primary trap used has been passive shelter pots; a light, open-ended plastic pot which is attached to a
demersal long line and deployed in water < 30 m. Due to the tendency for the shelter pots to be buried or lost in high energy areas, the fishery operations have been limited to shallow areas around the Perth coastline. The Perth fishery consistently yielded 30 tonnes per year while exclusively using passive shelter pots (Sauer et al., 2019). In 2010, active trigger traps, consisting of a rectangular trap baited with a plastic crab attached to a tripwire that activates a trap door when seized by an octopus (Figure 1.1), were introduced into the fishery (Leporati et al., 2015). These traps are considerably heavier and require less soak time than passive pots and can be used in deeper waters around Perth. The introduction of trigger traps in Perth saw the catches increase substantially to 170 tonnes in the first year of their use. Research conducted by Leporati et al. (2015) suggested that the catch composition of each trap type also differed significantly, with passive traps predominantly catching smaller, maturing individuals of both sexes and trigger traps catching predominantly large (>1 kg), mature males with very few mature females. However, the authors state that there was no overlap of depths in which each trap type was deployed, therefore differences in catch compositions between traps cannot be determined conclusively.

The target species in the Western Australian Octopus Fishery is *Octopus aff. O. tetricus* although other species are also caught occasionally, such as *O. cyanea* off the northern coast and *Macroctopus maorum* in southern waters (Hart et al., 2016).
1.5 Rationale and study aims

The overall aim of this Honours project is to investigate the spatial and temporal distribution patterns of *Octopus* aff. *O. tetricus* in the nearshore, coastal waters in North Fremantle, Western Australia (Figure 2.1). This region is located ~7 km north of the traditional commercial fishing grounds. The North Fremantle site was selected following discussions with commercial octopus fisher (P. Jasinski) about catches of small juvenile octopuses in the area, indicating that this area is potentially a spawning or nursery area for *O. tetricus*. The specific aims of the thesis are to:

(i) Investigate how octopus abundance varies with depth and sex;
(ii) Determine the size distribution and growth of octopus within the area; and
(iii) Compare the size distribution of octopus from this area with that on the fishing grounds in Cockburn Sound, about 7 km south of the North Fremantle study site.

Detailed studies of octopus diet in shallow and deep waters of the area north of Fremantle were also planned. Although gastric tracts were removed during dissection and all dietary items in the gastric mill and stomach identified, it was not possible to analyse these data comprehensively within the time constraints of Honours. However, preliminary findings are presented from this component of the research. During the dissection, high numbers of parasites were identified, and an additional aim was introduced:

(iv) Provide a preliminary description of the diet of octopus in coastal waters, and
(v) Determine the incidence, distribution, and abundance of parasites in octopus in the coastal waters north of Fremantle and compare this (a) size classes of octopus, (b) between shallow and deep water and (c) with that in Cockburn Sound.

2. Methods

2.1 Study site

Octopus’ were collected within the near coastal waters between North Fremantle and Cottesloe, Perth, Western Australia during contracted commercial fishing in the area (31°58 S 115°49 E) (Fig. 2.1). This area is north of the main fishing grounds of the Octopus Fishery, which is conducted within the Cockburn Sound (DoF, 2015). The study site is located approximately 1.5 km from the nearest land, where depths range from ~8 to 22 m and the
benthic habitat consists of a sandy substrate with seagrass and macroalgal beds dispersed irregularly throughout (P. Jasinski, Octopus fisher, pers. comm.).

2.2 Collection of Octopus aff. O. tetricus

Octopus were collected using open-ended, weighted, passive shelter pots (Figure. 2.2), that rely on the octopuses entering the pots for shelter. Shelter pots have a 16.9 cm² opening, are 38 cm long and have a volume of 6 L. A commercial fishing vessel, chartered by the Department of Primary Industries and Regional Development (DPIRD), deployed four demersal long lines (1 – 1.4 km in length), each comprising 150 pots placed < 10 m apart, and left in the water for soak times of ~7 to 22 days on each sampling occasions. A total of seven separate sampling trips were conducted between the first haul on 23rd of March and ending with the final retrieval of the lines on 15th of June 2020. Two lines (A and B) were set in shallow water depths of 8 to 10 m and the other two lines (C and D) were set in water > 10 m deep (Figure. 2.1, see Appendix 1.1 for coordinates of each line). The lines were reset in the same location each time after being hauled following the normal commercial octopus fishing operation (Figure. 2.1). The lines were separated by distances of ~500–1500 m and covered an area of ~7.8 km². One sample of octopus mantles was obtained from the commercial octopus' fishery which operates within Cockburn Sound and, in surrounding waters of Carnac and Garden Island on 1st of April 2020. Data on size distribution, sex ratios, gonad condition and parasites from this sample were compared with those from the main study area at the same time.

After collection, octopus' mantles were severed from their arms, following the standard commercial practice, and the mantles placed immediately placed on ice. The arms of the octopus of commercial size i.e. > ~300 g wet weight, were kept by the commercial fisherman for sale. Whole small octopus’ i.e. < ~300 g were retained on the sampling trips between 14th April and 15th June and placed on ice. Samples were frozen within 2 h of capture.
Figure 2.1  Map showing the location of the four octopus trap lines set between March and June 2020 in the coastal waters north of Fremantle Harbour, Western Australia. Each line had 150 traps and was ~1 to 1.5 km in length.

Figure 2.2  Passive shelter pots used in the Western Australian developmental octopus’ fishery (from Leporati et al. 2015). Trap dimensions are 38 x 14 x 17 cm.
2.3 Biological processing of *Octopus aff. O. tetricus*

After defrosting the mantles, the mantle weight (MW; to 0.1g) and mantle length (ML; mm) were recorded for each octopus. The small octopuses were weighed whole (TW).

The total weight (TW) was estimated from ML using the empirical relationships in Hart et al. (2019):

- **Females:** \( TW = 0.00054 \times ML^{2.94} \) \((r^2 = 0.90, \ n = 345)\)
- **Males:** \( TW = 0.0005 \times ML^{2.86} \) \((r^2 = 0.90, \ n = 372)\)

The mantle weight of small juvenile octopus collected was estimated using the empirical relationships in Hart et al. (2019):

- **Females:** \( MW = 0.0004 \times ML^{2.94} \) \((r^2 = 0.90, \ n = 10)\)
- **Males:** \( MW = 0.0005 \times ML^{2.86} \) \((r^2 = 0.90, \ n = 2)\)

2.3.1 Maturation

The mantles were then dissected, and the sex of the octopus determined from a macroscopic inspection of the gonads. The gonads were removed and weighed to the nearest 0.01 g, and the maturity stage was determined by visual examination using the classification scheme outlined in Leporati et al. (2015) and summarised in Table 2.1. The gastric tract, including the gastric mill and stomach, was also removed from the mantle for dietary studies. Although all samples have been analysed to identify and quantify dietary items, these data are not presented as part of the Thesis due to time constraints. However, details of the processing of samples are provided below.
Table 2.1  Summary of female and male gonad maturation stages of Octopus aff. O. tetricus from Leporati et al. (2015). Note, Stage IV males were not described by Leporati et al.

<table>
<thead>
<tr>
<th>Gonad stage</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Immature</td>
<td>Ovary whitish, very small, no signs of granulation.</td>
<td>Spermatophoric organ transparent and whitish.</td>
</tr>
<tr>
<td>II Developing</td>
<td>Ovary yellowish with signs of granulation.</td>
<td>Spermatophoric organ with white streaks of sperm</td>
</tr>
<tr>
<td>III Mature</td>
<td>Ovary very large, yellow/ orange in colour, or clear if in the process of laying eggs. Oviducts and oviducal glands enlarged</td>
<td>Needhams sack full of spermatophores</td>
</tr>
<tr>
<td>IV Spent</td>
<td>Ovary purple in colour and flaccid, with few to no eggs</td>
<td></td>
</tr>
</tbody>
</table>

2.3.4 Parasites

During preliminary stages of dissecting octopus’ mantles, several parasites were observed within the viscera. Consequently, the internal organs of the mantle *i.e.* the crop, digestive gland, alimentary canal, gonads and tissues, were examined for parasites under a dissecting microscope. Parasites were removed with tweezers, counted, with their location recorded and preserved in 70% ethanol.

The parasites found in the mantle and its associated organs were tentatively identified as anisakid nematodes by Professor Alan Lymbery (Murdoch University). The identification of nematodes to greater resolutions requires the use of molecular methods which was not possible within the scope of this Thesis. Therefore, their identification was only possible to family level (Anisakidae).

2.4 Data analyses

All data exploration and analyses were carried out using R version 4.0.2 (R Core Team, 2020) in the integrated development RStudio, version 1.3.1093 (RStudio Team 2020). All figures were created using Microsoft Excel™.

Prior to all parametric statistical analysis, the assumptions of normality and homogeneous variance were assessed by using Q-Q plot and Levene’s test, respectively. When violations of assumptions were detected, the data were transformed.

2.4.1 Environmental parameters

Data on the monthly mean minimum and maximum sea surface temperatures for the period from July 2019 to June 2020 were obtained from seatemperature.org that summarises
NOAA daily satellite readings (http://www.seatemperature.org, 30th October 2020). This covers the sampling period and the months before sampling commenced.

2.4.2 Distribution and abundance of octopus

A three-way analysis of variance (ANOVA) was used to test whether the numbers of octopus differed between sexes (Sex), water depths (Depth) and sampling times (Haul). A post hoc Tukey’s Honest Significance Difference (HSD) was used to investigate any significant difference between levels of factors. Abundances were compared without adjusting for soak times.

2.4.3 Octopus size structure

The overall mean (± 1 SE) of the mantle weight (MW) and mantle length (ML) of Octopus aff. O. tetricus males and females were calculated. Preliminary diagnostic plots established that no variables violated the assumptions of the T-test and so the data were not transformed. A two-sample independent T-test was used to test whether mean values for MW and ML differed significantly between male and female octopuses.

A two-way analysis of variance (ANOVA) was performed to test whether ML differed significantly between the North Fremantle and Cockburn Sound sites and between sexes.

2.4.4 Mantle length distributions

Octopuses were grouped into 10 mm mantle length (ML) classes and length percentage frequency histograms were constructed for each sex, both depth regions and each sampling trip, including octopus collected from the Cockburn Sound site. Descriptive statistics were used to summarise the size distribution of the mantle length (ML).

The mean size of octopus’ from North Fremantle and Cockburn Sound on 1 April was compared using an independent T-test.

2.4.5 Length-weight relationships

The relationships between mantle length (ML) and mantle weight (MW) for Octopus aff. O. tetricus were estimated using a power function in Microsoft Excel for each sex. The equation for the length-weight relationship was MW = aML^b where a and b are constant.

In R, the ML and MW data were transformed (Log_{10}) and a linear equation was fitted to the data:
\[
\text{Log}_{10}(MW) = a\text{Log}_{10}(ML) + b
\]

Analyses of covariance (ANCOVA) was used to determine if the relationship between MW and ML differed between the sexes (\(\alpha = 0.05\)) using ML as the covariate.

### 2.4.6 Gonadosomatic Index

The gonadosomatic index (GSI) was calculated as a percentage for each octopus as the gonad weight (GW) divided by the mantle weight (MW) minus the gonad weight (GW):

\[
\text{GSI} = \left(\frac{GW}{MW - GW}\right) \times 100
\]

The mean GSI (± 1 SE) was calculated for each month for males and females separately, for both North Fremantle and Cockburn Sound samples. The monthly proportion of *O. tetricus* at each stage of gonad development was plotted separately for males and females in North Fremantle.

### 2.4.7 Parasites

The intensity (*i.e.* the number of parasites per octopus) and prevalence (*i.e.* the number of octopuses with at least one parasite) was calculated following the definitions given in Bush et al. (1997). Generalised linear models (GLMs) were used to test whether the presence of parasites was influenced by depth, month, sex and size of an octopus. Octopuses were assigned to three size groups based on mantle length classes; *i.e.*, small = < 80 mm, medium = 80-150 mm and large = ≥160 mm, in an attempt to measure the influence of octopus size on parasite infection.

Three binomial GLMs were used to test the presence of at least one parasite per octopus. For each GLM the binomial response variable was the presence of nematodes (presence = 1, absence = 0), which was realised through the logit link function. The binomial model was:

\[
G(y) = \text{logit} \left( \frac{p}{1-p} \right) = x^T \beta
\]

where, \(p\) is the probability of at least one parasite being present, and \(x^T\) denotes the vector of predictor variables and \(\beta\) is the vector of coefficients.

The first model aimed to assess the predictor variables of time (month), size and sex for the North Fremantle *O. tetricus* population. The second model used size and sex as predictor variables for the population from Cockburn Sound and the third model, tested for differences
between North Fremantle and Cockburn Sound, using only samples from North Fremantle collected on the 1st April 2020

2.5 Diet Analysis

During dissection, the gastric tract was removed, with the crop being clamped to avoid stomach and crop contents mixing. The stomach fullness was estimated by eye on a scale 0 to 10, with 10 being the equivalent to 100% full. The contents from the gastric tract were rinsed under water through a 500 µm sieve to remove silt to ease in the identification of prey components. Contents were examined under a dissecting microscope and identified to the lowest taxonomic level possible, based mainly on an examination of hard parts such as crustacean exoskeletons, teleost vertebrae, radulae of molluscs and the byssus strands of bivalves. Cephalopods could be identified by flesh with distinct appearance of chromophores and suckers, remnants of beaks and presence of black ink throughout the gastric tract. The contribution of each prey item was estimated by eye and expressed as a percentage. Any prey items which were heavily masticated or in an advanced stage of digestion was recorded as ‘unidentified’. The percentage frequency of occurrence was calculated and the %volume of each item was estimated. Only preliminary findings from the dietary studies are presented in the results.

2.6 Permits and approval of ethics committees

All necessary permits were obtained for this study. Octopus were collected by the commercial fisher for this research under Murdoch Animal Ethics – Cadaver and/or Tissue Notification, Permit No. 744 “Cadaver application for octopus’ ecology”.

3. Results

3.1 Water temperature and storms

The mean maximum water temperature derived from NOAA data on SST during the period from March to June 2020 ranged from 25.1 ºC in March to 23 ºC in June (Figure 3.1). In the 12 months before this, the lowest mean maximum water temperature was in September 2019 (19.9 ºC) and the highest mean (25.3 ºC) was in February 2020.
During the soaking period of the fourth and fifth haul, considerable storm activity in Perth caused the loss of several octopus’ pots and moved large amounts of sand into the traps, resulting in the suffocation of at least one octopus.

![Temperature graph](image)

**Figure 3.1** Mean sea surface temperature in North Fremantle, Western Australia from daily satellite readings extracted from http://www.seatemperature.org (30th October 2020). Arrow on the X-axis shows the duration of sampling octopus in the region.

### 3.2 Changes in numbers with depth and month

A total of 713 octopuses were collected during the seven sampling trips between 23rd March and 16th June 2020. These comprised 344 females, 358 males and 12 individuals whose sex could not be determined. The highest number of octopus were caught from Line B (n = 194), followed by Line A (n = 172), both in waters < 10 m deep. A similar number were caught on Line C (n = 163) and the lowest numbers from Line D (n =140) in the deepest water (> 10 m). A total of 49 octopuses (30 females, 19 males) were collected from the Cockburn Sound on 1st April 2020. This compares with the second haul, caught in the waters of North Fremantle on 1st April 2020 of 90 octopus, with 43 males and 47 females.

The third haul on 14th April 2020 yielded the highest number of octopuses (135) when 54 males and 78 females were caught (Figure 3.2). The last haul yielded the lowest number of octopus (70), with equal numbers of males and females. The lowest number of males (6) per line was collected on 2nd June and the lowest number of females (5) per line on 11th May, both
on Line D. The biggest discrepancies between male and female catches were recorded was on
the 23rd March on Line B (9 females, and 23 males) and on 14th April on Line D (21 females,
and 7 males).

A two-way analysis of variance (ANOVA) showed that the mean number of octopuses
collected per haul differed significantly ($P = 0.01$) among hauls but not between depths or sexes
and that none of the interaction terms were significant ($P >0.05$, Table 3.1). The Tukey’s *post
hoc* test showed that only the third and seventh haul differed significantly ($P = <0.05$), with a
significantly higher mean catch on the third haul (14th April, 16.5±0.49) than on the 7th haul
(15th June, 8.4±0.20).
Figure 3.2  The number of female (black) and male (grey) Octopus aff, O. tetricus collected on each line from North Fremantle, Western Australia for each sampling trip between 23rd March and 15th June 2020. a) Line A, b) Line B, c) Line C and d) Line D. Mean depths of lines were: A = 9.29 m; B = 9.64 m; C = 16.79 m; D = 15.34 m.
Table 3.1  Summary of results for the three-way analysis of variance (ANOVA) to test differences in the abundance of Octopus aff. O. tetricus between depth regions, sex, and sampling haul. Significant factors are in bold. df = degrees of freedom, MS = mean squares.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
<th>% MS</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haul</td>
<td>6</td>
<td>49.03</td>
<td>26.94</td>
<td>0.01</td>
</tr>
<tr>
<td>Depth</td>
<td>1</td>
<td>27.16</td>
<td>14.92</td>
<td>0.18</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>17.16</td>
<td>9.43</td>
<td>0.29</td>
</tr>
<tr>
<td>Haul*Depth</td>
<td>6</td>
<td>27.41</td>
<td>15.06</td>
<td>0.12</td>
</tr>
<tr>
<td>Haul*Sex</td>
<td>6</td>
<td>32.99</td>
<td>18.13</td>
<td>0.07</td>
</tr>
<tr>
<td>Depth*Sex</td>
<td>1</td>
<td>0.16</td>
<td>0.09</td>
<td>0.92</td>
</tr>
<tr>
<td>Haul<em>Depth</em>Sex</td>
<td>6</td>
<td>13.49</td>
<td>7.41</td>
<td>0.49</td>
</tr>
<tr>
<td>Residuals</td>
<td>28</td>
<td>14.59</td>
<td>8.02</td>
<td></td>
</tr>
</tbody>
</table>

3.3 Sex ratio

Overall, the sex ratio for female and male Octopus aff. O. tetricus sampled from the North Fremantle site was 1.08:1 (339 males, 313 females), which does not differ significantly from 1:1 ($\chi^2_1 = 0.959, P = 0.328$) (Table 3.2). Neither did the overall sex ratio differ significantly between shallow and deep locations ($\chi^2_1 = 0.041, P = 0.8$). Comparisons of the sex ratios across the study region (i.e. pooled for shallow and deep water) for each haul showed a significant dominance of males in the catches in May (1.89:1) ($\chi^2_1 = 7.440, P < 0.01$) but that sex ratios did not differ significantly from 1:1 for any other haul. (Table 3.2).

Within the shallow region, the only haul in which the sex ratio differed significantly from 1:1 was the first haul (23 March, $\chi^2_1 = 4.063, P = 0.04$), with a male to female ratio of 1.73:1. In the deeper region, sex ratios differed from 1:1 in two hauls: on the third haul (14 April) significantly more females than males were caught (0.58:1, $\chi^2_1 = 3.750, P = 0.05$), and on the fifth haul, significantly more males than females were present (1.89:1, $\chi^2_1 = 5.357, P = 0.02$) (Table 3.2).

The sex ratio for octopuses collected from Cockburn Sound (0.60:1) did not differ significantly from that for octopuses sampled from North Fremantle on 1st April 2020 (0.91:1) ($\chi^2_1 = 0.956, P = 0.328$).
Table 3.2 The observed sex ratio (male: female), sample sizes (n), P-values (P) and chi-squared values (χ²), presented for overall ratio, by month and the two depth regions for Octopus aff. O. tetricus collected from North Fremantle near-coastal waters between March and June 2020. Bold values = Significant differences from 1:1

<table>
<thead>
<tr>
<th>Haul</th>
<th>&lt;10 m</th>
<th>n</th>
<th>χ²</th>
<th>P</th>
<th>&gt;10 m</th>
<th>n</th>
<th>χ²</th>
<th>P</th>
<th>Overall</th>
<th>n</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>23-Mar</td>
<td>1.73:1</td>
<td>63</td>
<td>4.063</td>
<td>0.04</td>
<td>1.05:1</td>
<td>37</td>
<td>~0</td>
<td>~1</td>
<td>1.43:1</td>
<td>100</td>
<td>2.890</td>
<td>0.09</td>
</tr>
<tr>
<td>1-Apr</td>
<td>0.72:1</td>
<td>55</td>
<td>1.163</td>
<td>0.28</td>
<td>1.34:1</td>
<td>35</td>
<td>0.457</td>
<td>0.50</td>
<td>0.91:1</td>
<td>90</td>
<td>0.100</td>
<td>0.75</td>
</tr>
<tr>
<td>14-Apr</td>
<td>0.80:1</td>
<td>72</td>
<td>0.681</td>
<td>0.41</td>
<td>0.58:1</td>
<td>60</td>
<td>3.750</td>
<td>0.05</td>
<td>0.69:1</td>
<td>132</td>
<td>4.008</td>
<td>0.05</td>
</tr>
<tr>
<td>24-Apr</td>
<td>1.35:1</td>
<td>56</td>
<td>0.446</td>
<td>0.50</td>
<td>0.89:1</td>
<td>34</td>
<td>0.029</td>
<td>0.86</td>
<td>1.09:1</td>
<td>90</td>
<td>0.100</td>
<td>0.75</td>
</tr>
<tr>
<td>11-May</td>
<td>1.63:1</td>
<td>42</td>
<td>1.929</td>
<td>0.17</td>
<td>2.23:1</td>
<td>42</td>
<td>5.357</td>
<td>0.02</td>
<td>1.89:1</td>
<td>84</td>
<td>7.440</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2-Jun</td>
<td>1.05:1</td>
<td>39</td>
<td>~0</td>
<td>~1</td>
<td>1.32:1</td>
<td>51</td>
<td>0.706</td>
<td>0.40</td>
<td>1.20:1</td>
<td>90</td>
<td>0.544</td>
<td>0.45</td>
</tr>
<tr>
<td>15-Jun</td>
<td>1.12:1</td>
<td>36</td>
<td>0.028</td>
<td>0.868</td>
<td>31</td>
<td>0.129</td>
<td>0.72</td>
<td>0.97:1</td>
<td>67</td>
<td>~0</td>
<td>~1</td>
<td></td>
</tr>
</tbody>
</table>

Overall | 1.10:1| 362| 0.798| 0.371   | 1.06:1| 290| 0.169| 0.681   | 1.08:1  | 652| 0.959| 0.327   |

3.4 Size distribution

3.4.1 Overall size distribution

A total of 709 octopuses were measured and weighed. The smallest female was measured at 35 mm ML, 31.94 g total weight (TW) and as the octopus was taken whole the mantle weight was estimated at 5.13 g. The largest female was 211 mm ML, 687.72 MW and an estimated TW of 2397.96 g (Table 3.3). The smallest male was 14 mm ML larger than the smallest female (mm), but the largest male was a similar size to the largest female (Table 3.3). The mean estimated TW for females was 882.38 g which was significantly lighter than that of males (1253.01 g) (T = -7.926, df = 633.56, P <0.001). The mean ML and MW did not differ significantly between females (141.49 mm, 262.52 g) and males (144.83 mm, 264.44 g) (P > 0.5, Table 3.3).

Eleven of the small juvenile O. tetricus were too small to determine their sex. The smallest of which was 14 mm ML and 2.75 g TW. The average TW for undetermined individuals was 3.70±1.70 g and average ML was 35.3±6.82 mm.

Females collected from the Cockburn Sound site ranged from 102 to 217 mm ML and 71.3 to 588.8 g MW, with an estimated TW range of 299.9 to 2598.2 g. Males collected from Cockburn Sound ranged from 99 to 191 mm ML, 80.5 to 441.74 g MW and an estimated total weight range of 368.2 to 2542.2 g.
Table 3.3: Summary of the mean sizes (± 1 SE) and range of mantle length (ML), mantle weight (MW) and total weight (TW), for Octopus aff. O. tetricus caught in waters north of Fremantle, Western Australia. The t-statistic (T) and significance (P) of the Welch two-sample t-test between sexes also shown. df = degrees of freedom

<table>
<thead>
<tr>
<th>Sex</th>
<th>n</th>
<th>Mean±SE</th>
<th>Range</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mantle length (mm) (df = 611.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>313</td>
<td>141.49±1.95</td>
<td>35-211</td>
<td>-1.333</td>
<td>0.18</td>
</tr>
<tr>
<td>M</td>
<td>339</td>
<td>144.83±1.57</td>
<td>49-218</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mantle weight (g) (df = 604.99)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>313</td>
<td>262.52±8.35</td>
<td>5.13-687.72</td>
<td>-1.333</td>
<td>0.18</td>
</tr>
<tr>
<td>M</td>
<td>339</td>
<td>266.86±6.59</td>
<td>15.22-691.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estimated total weight (g) (df = 633.56)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>313</td>
<td>882.38±28.59</td>
<td>31.94-2598.18</td>
<td>-7.926</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>M</td>
<td>329</td>
<td>1253.01±34.75</td>
<td>29.95-3750.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The total weight (TW) estimated from the equation in Hart et al. 2019

The mean MLs in the shallow and deep waters was almost identical (< 10: 143.21 mm, > 10: 143.25 mm, P ~ 1). The mean MW for the shallower region was 268.52 g, slightly, but not differ significantly greater than that for the deeper region of 260.11 g.

3.4.2 Mantle Length-weight relationship

An analysis of covariance (ANCOVA) found that the slopes of the relationship between Log mantle length (ML) and the Log mantle weight (MW) (Figure 3.3) did not differ significantly between males and females, therefore the data were pooled. The equations for the relationship was:

Log(MW) = 2.483Log(ML) – 6.849 (n = 696, r² = 0.92)

Table 3.4 Summary of results from the analysis of covariance (ANCOVA) to test whether the relationship between mantle weight (MW) and mantle length (ML) differed significantly between sexes in Octopus aff. O. tetricus. Both MW and ML were log-transformed.

<table>
<thead>
<tr>
<th>Variable</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LogML</td>
<td>1</td>
<td>259.38</td>
<td>5137.46</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>0.002</td>
<td>0.03</td>
<td>0.853</td>
</tr>
<tr>
<td>LogML*Sex</td>
<td>1</td>
<td>0.184</td>
<td>3.65</td>
<td>0.057</td>
</tr>
<tr>
<td>Residuals</td>
<td>646</td>
<td>0.050</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.3.3 **Size distribution in deep and shallow water**

The mantle length (ML) size distribution pooled over all sampling times and depths showed that most male and female octopus were between 120 and 170 mm long with modal sizes of 150 mm ML for both males and females (Figure 3.4a). The number of females in each size class declined more sharply than the number of males between 160 and 190 mm and few male or female octopus >200 mm ML were caught.

The modal ML class for females in the <10 m depth region was 160 mm and 150 mm for the >10 m region. The modal ML class for males was the same for both depth regions (150 mm). The largest individuals for both sexes were collected from the shallow region. The smallest males were found in the shallow waters (Figure 3.4b) in comparison, the smallest females were found in the deeper region (Figure 3.4c). There is a sharp decline in the number of females after the 150 to 160 mm ML classes for both shallow and deep regions.
Figure 3.4 Mantle length-frequency distributions for female and male *Octopus aff. O. tetricus* for pooled (a), ≤10 m (b) and > 10 m (c) depth areas in North Fremantle, Western Australia. Collected between March and June 2020.
3.4.4 Temporal variation in size

One small juvenile male was collected during the first sampling trip on 23rd March (0-9.9 mm ML class). Except for this individual, small octopus (< 80 mm ML class) were not seen until the third sampling trip, hauled on 1st April. Individuals in the smallest length classes (< 80 ML mm) were the least common across the sampling period, with small females (1.84%) being more common than small males (0.61%). Small juveniles of indeterminate sex were collected on the 14th April (n = 6, mean ML 48.6 mm), 11th May (n = 2, mean ML 22.5 mm) and 2nd June (n = 3, mean ML 18.34) trip but are not shown on the ML frequency figure (Figure 3.5).

Female length distribution varied across the sampling period but tended to decrease from March to June, which contrasts with males, who increased in size across the sampling periods (Figure 3.5). Throughout the majority of the sampling period, the modal ML classes for females stayed between 150 to 160 mm but was more variable in the third sampling trip (Figure 3.5a). From the fifth sampling trip, a second modal ML class can be identified for females at 100 mm and remains consistent between 100 to 130 mm for the until the conclusion of the hauls. The ML size distribution for males varied over the four months of sampling. Initially, the ML modal length for males was 130 mm, over the duration of the sampling period the modal length for males increased to 160 – 170 mm. From 14th April onwards most males were above 140 mm.

The mean ML for females above 80 mm ML was highest in March (158.3 mm) and lowest in May (133.7 mm). The mean ML for males gradually increased during the study, with the lowest mean recorded on 1st April (140.7 mm) and the highest mean on 15th June (153.6 mm) (Figure 3.6). The mean ML of male and female octopus >80 mm ML was almost identical on April 1 and 24 (~140–142 mm, Figure 3.6).

A two-way analysis of variance (ANOVA) showed that the mean ML differed significantly among hauls (P < 0.001) and that the haul x sex interaction was significant (P = 0.01) (Table 3.5). The factor Haul accounted for 46.1% of the total mean squares and the interaction for 30.5%. A Tukey’s post hoc test showed that the mean ML for females collected on the 23rd March (156.44 mm) was significantly longer than that on 14th April (131.95 mm) (P = 0.003). The mean ML for female octopuses collected on 11th May (125.72 mm) were significantly smaller than those collected on March 23rd (Tukey post hoc P = 0.004). In contrast
to females, the mean ML of males did not differ significantly between any of the sampling trips ($P > 0.05$), which explains the significant sex x haul interaction.
Figure 3.5  Mantle length frequencies for female and male *Octopus* aff. *O. tetricus* for each sampling trip collected from North Fremantle, Western Australia between March and June 2020. a) = 23rd March, b) = 1st April, c) = 14th April, d) = 24th April, e) = 11th May, f) = 2nd June, g) = 15th June.

Figure 3.6  Mean (± 1SE) mantle length (ML) of *Octopus* aff. *O. tetricus* larger than 80 mm for each haul in North Fremantle, collected between March and June 2020.
Summary of the results from the two-way analysis of variance (ANOVA) to test if the mean mantle length (ML) of Octopus aff. O. tetricus differed significantly between sexes and among each haul from March to June, in North Fremantle, Western Australia. Bold = significant terms

<table>
<thead>
<tr>
<th>Variable</th>
<th>df</th>
<th>MS</th>
<th>MS%</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haul</td>
<td>6</td>
<td>4014</td>
<td>46.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>1067</td>
<td>12.3</td>
<td>0.293</td>
</tr>
<tr>
<td>Haul*Sex</td>
<td>6</td>
<td>2654</td>
<td>30.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Residuals</td>
<td>636</td>
<td>965</td>
<td>11.1</td>
<td></td>
</tr>
</tbody>
</table>

3.4.5 Cockburn Sound size distribution

Octopus collected from the Cockburn Sound in April 2020 had a modal ML of 120 mm for males and 160 mm for females (Figure 3.7a). This is smaller than the modal sizes for males (140 to 150 mm) and larger for females (150 mm) caught in North Fremantle (Figure 3.7b).

A two-way analysis of variance (ANOVA) showed the mean ML for octopuses collected on April 1 differed significantly between North Fremantle and Cockburn Sound ($P = 0.003$) and sexes ($P = 0.02$) and that the site and sex interaction was significant ($P = 0.03$) (Table 3.6). The mean ML for male (139.78 mm) and females (162.27 mm) collected from the Cockburn Sound differed significantly ($P = 0.02$). The mean ML of female octopus sampled from Cockburn Sound was 162.27 mm which was significantly longer than that from North Fremantle (140.36 mm). Moreover, the males collected from North Fremantle were significantly smaller than the females collected from Cockburn Sound on the same date (Tukey post hoc test $P = < 0.05$). The mean ML for males did not differ significantly between the sites (Table 3.6).
Mantle length frequencies for male and female *Octopus aff. O. tetricus* for samples collected from the Cockburn Sound (a) and North Fremantle (b), collected from 1st April 2020.

Summary of results for the two-way analysis of variance (ANOVA) to test differences in mean mantle length (ML) of *Octopus aff. O. tetricus* collected between the two sites (North Fremantle and Cockburn Sound) and sex on 1st April 2020, df = degrees of freedom, MS = mean squares.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
<th>% MS</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>1</td>
<td>2788</td>
<td>21.22</td>
<td>0.04</td>
</tr>
<tr>
<td>Site</td>
<td>1</td>
<td>6683</td>
<td>50.86</td>
<td>0.002</td>
</tr>
<tr>
<td>Sex*Site</td>
<td>1</td>
<td>3029</td>
<td>23.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Residuals</td>
<td>134</td>
<td>640</td>
<td>4.87</td>
<td></td>
</tr>
</tbody>
</table>
3.5 Reproduction and Maturity

3.5.1. Gonad maturity stages

The majority (74.4%) of females sampled from North Fremantle between March and June 2020 were immature, 23.72% were maturing, 1.60% mature and 0.32% spent (Figure 3.8). Catches of immature females were lowest in March and peaked in May before declining again in June in both depth regions. Mature females were only caught during April in both depth regions. The only spent female was collected from the deep region in April (Figure 3.8).

Maturing males dominated catches at 46.38% overall, with immature males at 26.25% and mature 25.39%. All male maturity stages were found each month. The rate of immature males being caught decreased from March to June, with a more notable decline in shallower waters. The lowest proportion of mature males was recorded in March in shallow waters in April for the deep.

More immature females (232) were caught between March and June than males (89). Most males (165) collected from North Fremantle were in the second maturity stage (maturing) (Table 3.5). The mean mantle length and mantle weight for females were larger than males in each maturity stage except for Stage III (mature) ML, in which the mean ML was ~171 mm for both sexes. The mean gonad weight (GW) was considerably heavier for mature female octopus (41.1 g) than that for mature males (17.8 g) (Table 3.5). One spent (Stage IV) female was collected during this study on 14th April, an individual with a ML of 132 mm, weighing 233.4 g MW and 23 g GW (GSI = 10.92%) (not included in Table 3.5).

On 1st April, most females were classed as immature in samples from both North Fremantle and Cockburn Sound (Figure 3.9). Cockburn Sound had considerably more maturing females (36.67%) than North Fremantle (17.02%), but no mature females were recorded in the Sound at this time. Males collected from both sites on the 1st April were predominantly at the maturing stage, with 48.4% maturing males at North Fremantle and 72.2% from Cockburn Sound. North Fremantle had a higher frequency for immature and mature males when compared with Cockburn Sound (Figure 3.9).
Figure 3.8  Percentage frequency of gonad stages for females (n = 318) (a) and males (n = 331) (b) Octopus aff. O. tetricus collected between 23rd March and 15th June 2020 from North Fremantle, Western Australia.
Table 3.7  Summary of the mean sizes (± 1SE) for mantle length (ML), mantle weight (MW) and gonad weight (GW) for each maturity stage of *Octopus* aff. *O. tetricus* sampled from North Fremantle, collected between March to June 2020.

<table>
<thead>
<tr>
<th>Sex</th>
<th>n</th>
<th>ML (mm)</th>
<th>MW (g)</th>
<th>GW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage I (Immature)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>232</td>
<td>131.23(±2.09)</td>
<td>209.59(±7.62)</td>
<td>2.58(±0.16)</td>
</tr>
<tr>
<td>M</td>
<td>89</td>
<td>110.24(±2.11)</td>
<td>124.60(±6.31)</td>
<td>3.23(±0.28)</td>
</tr>
<tr>
<td><strong>Stage II (Maturing)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>74</td>
<td>171.49(±2.56)</td>
<td>415.45(±13.4)</td>
<td>11.81(±0.85)</td>
</tr>
<tr>
<td>M</td>
<td>165</td>
<td>149.54(±1.45)</td>
<td>275.99(±5.82)</td>
<td>10.96(±0.26)</td>
</tr>
<tr>
<td><strong>Stage III (Mature)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>5</td>
<td>171.6(±13.3)</td>
<td>460.17(±86.1)</td>
<td>49.14(±9.49)</td>
</tr>
<tr>
<td>M</td>
<td>86</td>
<td>171.71(±1.72)</td>
<td>396.68(±8.20)</td>
<td>17.80(±0.31)</td>
</tr>
</tbody>
</table>

Figure 3.8  Percentage frequency of gonad stages for *Octopus* aff. *O. tetricus* collected from North Fremantle and Cockburn Sound on 1st April 2020, a) Females, b) Males.
3.5.2 Gonadosomatic Indices

The maximum GSI recorded for females from North Fremantle was 20.33% and 7.64% for males. The monthly mean gonadosomatic index (GSI) for males increased from 3.35% in March to 4.17% in June (Figure 3.7). In contrast, the mean GSI for females, which was always lower than that for males, ranged from only 2.08% in March to 1.48% in June and did not show an increasing trend (Figure 3.7).

The mean GSI for female octopuses collected from Cockburn Sound was marginally smaller (1.30%) than those collected from North Fremantle on 1st April (1.4%). Similarly, males collected from Cockburn Sound had a smaller mean GSI (3.40%) than those from North Fremantle (3.78%) at this time.

![Gonadosomatic Index Chart]

**Figure 3.9** Monthly mean gonadosomatic index (±1SE) for male (n = 312) and female (n = 291) *Octopus aff. O. tetricus* for specimens collected in North Fremantle, Western Australia between March and June 2020.

3.6 Preliminary diet results

In total, 701 octopus’ gastric tracts were examined and 67% contained dietary items. Preliminary analysis of the dietary contents found that the dominant categories in both male and female gastric tracts by frequency of occurrence were brachyurans (>60), teleosts (30 and 56) and non-cephalopod molluscs (~20) (Figure 3.10). The frequency of brachyurans and
teleosts was higher for males than females. A total of 50 octopus contained cephalopods in their stomachs (Figure 3.10).

![Graph showing prey occurrence by gender](image)

**Figure 3.10** The frequency of each prey item of *Octopus* aff. *O. tetricus* collected from North Fremantle and Cockburn Sound between March and June 2020.

### 3.7 Parasites

#### 3.7.1 Parasite presence

Nearly 60% of all octopus in North Fremantle (58.19%) had anisakid nematodes (hereafter nematodes) present in at least one internal organ. Only one parasite taxon was found during the dissection of all octopus. In octopus with at least one parasite, it was always found in the crop (100% prevalence), 58.5% in the mantle muscles, 9.56% in the digestive gland, 2.82% in posterior salivary glands, 0.25% in the gonads (Figure 3.11). Across all samples, 7 octopuses (1.53%) had eggs of parasites present in the crop.

The mean intensity (± 1 SE) of nematode infection in North Fremantle was 18.31 ± 0.68 per octopus and ranged from 1 to 75 nematodes per octopus. Of the infected octopus, 59.9% had nematodes in one internal organ, 32.2% in two and 8.0% in three or more organs. The prevalence of nematodes was markedly higher in octopus from North Fremantle on April 1 (75.6%) than those from Cockburn Sound (25%) on the same date (Figure 3.11). The GLM of nematode presence found that the odds of octopus collected from North Fremantle having at
least one nematode present were 9.27 ($= e^{2.227}$) times greater ($P < 0.001$) than those from Cockburn Sound (Table 3.8c).

Figure 3.11 Examples of Anisakidae infestation in *Octopus* aff. *O. tetricus* in crop (a) and digestive gland (b) from samples collected in Perth, Western Australia between March and June 2020 (Photo: Jorja Claybrook).
3.7.2 Parasite presence modelling

Binomial logistic regression with a logit link function was used to examining the relationship between on the presence of at least one nematode and depth, haul and size of octopus, using March and the size group 'Small' as the intercept. The odds of nematode infestation for octopus were significantly greater for both larger classes of octopus than small octopus: by 2.35 times (\(= e^{0.855}\)) for medium (80-150 ML), and 2.69 times (\(= e^{0.991}\)) for large octopus (\(\geq 160\) mm ML) than the small individuals (<80 mm ML) (Table 3.8a). The odds of an octopus having a nematode present were significantly lower in June (0.58) than March (1.0) (\(= e^{-0.549}\); Table 3.8a) The presence of nematodes did not differ significantly between sexes (\(P = 0.9\)).

Nematode presence also differed significantly with octopus size in Cockburn Sound, with the odds of nematode infestation being significantly higher for the medium (2.09, \(= e^{0.738}\)) and large (2.16, \(= e^{0.771}\)) than small octopus (Table 3.6b). Like North Fremantle, the presence of nematodes did not differ significantly between sexes in Cockburn Sound (\(P = 0.65\), Table 3.8b).
Table 3.8 Coefficients of binomial Generalised Linear Models (GLMs) analysis with logit link function for the presence of anisakid nematodes in Octopus aff. O. tetricus caught in North Fremantle and Cockburn Sound and the influence of octopus size, month and sex.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>SE</th>
<th>Z-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) North Fremantle (size + month + sex)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept (Size: Small, March, Females)</td>
<td>-0.253</td>
<td>0.077</td>
<td>-0.776</td>
<td>0.438</td>
</tr>
<tr>
<td>Size: Medium</td>
<td>0.855</td>
<td>0.065</td>
<td>3.314</td>
<td>0.002</td>
</tr>
<tr>
<td>Size: Large</td>
<td>0.991</td>
<td>0.068</td>
<td>3.426</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>April</td>
<td>0.066</td>
<td>0.243</td>
<td>-0.271</td>
<td>0.787</td>
</tr>
<tr>
<td>May</td>
<td>-0.505</td>
<td>0.306</td>
<td>-1.650</td>
<td>0.099</td>
</tr>
<tr>
<td>June</td>
<td>-0.549</td>
<td>0.265</td>
<td>-2.070</td>
<td>0.038</td>
</tr>
<tr>
<td>Males</td>
<td>0.022</td>
<td>0.163</td>
<td>0.135</td>
<td>0.893</td>
</tr>
<tr>
<td>b) Cockburn Sound (size + sex)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept (Size: Small, Females)</td>
<td>-0.471</td>
<td>0.255</td>
<td>-1.847</td>
<td>0.064</td>
</tr>
<tr>
<td>Medium</td>
<td>0.738</td>
<td>0.267</td>
<td>2.767</td>
<td>0.006</td>
</tr>
<tr>
<td>Large</td>
<td>0.771</td>
<td>0.280</td>
<td>2.753</td>
<td>0.006</td>
</tr>
<tr>
<td>Males</td>
<td>0.070</td>
<td>0.154</td>
<td>0.453</td>
<td>0.650</td>
</tr>
<tr>
<td>c) North Fremantle vs. Cockburn Sound</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept (Site: Cockburn Sound)</td>
<td>-1.099</td>
<td>0.333</td>
<td>-3.296</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Site: North Fremantle</td>
<td>2.227</td>
<td>0.412</td>
<td>5.381</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

4. Discussion

This study evaluated the spatial and temporal variability of the North Fremantle population of Octopus aff. O. tetricus. The data used for these analyses were collected by commercial fishing operation setting four lines of traps, two in shallow (≤ 10 m) and two in deeper water (>15-17 m) on seven occasions between March and June 2020. Differences in size distribution, gonad stage and body size were examined to test whether they differed between the sexes, depths of water and sampling time. Preliminary investigations of octopus’ diet in this region have also been made. During the study, an anisakid parasite was found infesting octopus’ and data on their incidence and intensity of infection were collected to investigate the site of infection. Comparisons of the biology and parasite infestation have been made between octopus in North Fremantle and those collected at one time from octopus fishing grounds in Cockburn Sound, ~7 km south of the main study.
4.1 Abundance and sex ratio

The abundance of octopus varied between sampling times but not between depths or sexes of octopus. The major difference between sampling times was the significantly greater numbers caught in early April compared with those in mid-June. The overall decrease in the abundance of octopus in the North Fremantle site in June coincided with the decrease in SST. Octopuses exhibit large fluctuations in abundance, mainly due to their susceptibility to environmental factors during their paralarval stage (Pierce et al., 2008).

Significant storm activity in the region during the fifth and sixth hauls in late April and May could have contributed to the lower catches at this time. The increased wave action associated with the storms resulted in several octopus’ pots becoming submerged in sand and at least one octopus was suffocated by sand (P. Jasinski, pers. comm.). Potentially, any small juvenile octopus’, which are at mercy to the currents, may have swept out to other areas during these weather events. Decreases in octopuses per haul from within the North Fremantle study may also be the result of the targeted removal of octopuses during the study period. Decreases in abundance due to targeted fishing pressure were examined in San Matas Gulf, northern Patagonia. The relative abundance of *O. tehuelchus* decreased during the fishing season on commercial fishing grounds compared with no change in abundance within a Marine Protected Area (Narvarte et al., 2013)

The abundance of octopus was not significantly affected by depth (<10 m and ≥10 m) within the waters of North Fremantle. While there was not any statistically significant difference, Line C and D continually yielded the least number of octopus per haul. The soak time of shelter pots was considered an important variable for the number of octopuses collected per haul; it was expected that catches would increase with increasing soak time. However, the number of octopus did not appear to be affected by the soak time of traps, as the hauls that were submerged for the shortest amount of time and the haul which was submerged for longest had very similar catches.

**Sex ratio**

The sex ratio did not differ significantly from 1:1, except on one occasion in the shallow waters and twice in the deeper waters. On the 23rd March, nearly twice as many males were caught in the shallow lines than females, while in the deep, females were significantly more prevalent than males on the 14th April, but this was reversed in early May when more than double the number of males to females were caught. The overall sex ratio did not differ for
either depth region or the Cockburn Sound population and did not differ between Cockburn Sound and North Fremantle in early April.

The sex ratio for April is consistent with the known composition of *O. tetricus* in the Western Australian fishery, where males tend to dominate the catches but have the lowest frequency of capture in April and the highest in October (Hart et al., 2016; Hart et al., 2019; Leporati et al., 2015). Until senescence, males will actively hunt and move around their environment seeking potential mates, which explains why males typically dominate catches (Leporati et al., 2015). Aside from the octopus’ collected in April, males consistently dominated the catches over females. This is quite unusual to other octopus’ population in which fluctuation between male and female abundance occur throughout the year, usually coinciding with peaks in the GSI (Alonso-Fernández et al., 2017; Guard & Mgaya, 2002). Similar patterns of male dominance have been observed in the Mexican *O. maya* fishery (Duarte et al., 2018). Currently, female coastal octopuses are hypothesised to cease feeding around the time they lay their eggs, seeking refuge in dens to care for their eggs until they hatch (Hart et al., 2019). During this time the females are unlikely to be actively hunting and would be less likely to enter traps (Leporati et al., 2015). It is not known precisely why the Western Australian octopus’ fishery has mostly continuous male dominance, but it is likely attributed to the use of active trigger trap use in the deep regions of the fishery (>30 m) which is considerably more likely to capture actively hunting octopus’ (Hart et al., 2019; Leporati et al., 2015).

### 4.2 Size distribution and growth

The mean mantle length (ML) of octopus from North Fremantle did not differ significantly between the sexes. Although the overall modal ML over the four months was the same for both males and females, the frequency of males in the larger size classes (>160 mm) was higher than females. The largest individual collected over the sampling period was a male caught in June, with a ML of 218 mm and MW of 691.62 g from the shallow region. This octopus was larger than those in previous studies of *O. tetricus* from within the commercial fishery where the largest males was 210 mm (Larsen, 2008). The maximum ML recorded for females in Larson (2008) was 250 mm, substantially longer than at the maximum of 211 mm recorded in the current study.

Small juveniles were first observed in the third haul in mid-April, with the smallest individual (sex undetermined) being observed at the end of April. As *Octopus aff. O. tetricus* has a two-month paralarval stage, these individuals were potentially hatched between January
and February. *Octopus* aff. *O. tetricus* have been identified as recruitment pulses every six months, with a peak spawning event occurring in September (Joll, 1978; Larsen, 2008; Leporati et al., 2015) and a secondary one in the summer months (Larsen, 2008; Leporati et al., 2015). The two modal length classes that become prominent from the mid-April to the end of June indicate that two cohorts are present in the population.

The monthly mean ML of male octopus increased steadily between March and June, with the largest mean ML (152.27 mm) recorded in June. In contrast to males, the mean ML of Females fluctuated between months, with octopus captured in March being significantly larger than those in April and May. This coincides with the influx of juveniles on the third haul in mid-April. Male and female octopuses typically exhibit similar growth rates until the onset of maturation, however males reach maturity earlier, and females reach a significantly larger maximum size (Leporati et al., 2015). Leporati et al. (2015) estimated the ML at 50% maturity for male *Octopus* aff. *O. tetricus* in the WA commercial fishery was 128 mm and reached at 243 d of age, 150 d faster and 54 mm smaller than the size at maturity for females (182 mm ML at 379 d), (Leporati et al., 2015). These findings are consistent with octopus’ collected in North Fremantle and demonstrate that two cohorts are likely to be present, one that spawned the previous year in August or September of 2019, and the second from spawning in January or February 2020.

During the current study, growth from the North Fremantle region did not appear to be very rapid for either sex. This may be due to two main reasons: (1) larger individuals migrate from the shallow waters and move outside the study region where the deepest line samples were 16.79 m, and move into waters deeper than 20 m, as was found previously for octopus in the WA fishery (Larsen, 2008; Leporati et al., 2015), and (2) growth slowed with the decreasing SST from March to June, which is consistent with the Larson (2008), who examined samples from the developing octopus fishery in the Perth region between June and August in water <20 m deep and in deeper water (30–40 m) from May to September. She found slower growth between April and August. Temperature is a major influence on the growth of octopus, especially in the early life stages. Modelled projections of temperature-dependent growth of *O. ocellatus* and *O. pallidus* suggests that an increase of 1 °C (from 22 to 23°C) could lead to a 62.6% greater octopus body mass after 100 days at the higher temperature (André et al., 2009). Temperature related growth is also intrinsically linked with an increase in metabolism, food intake and food availability (Rigby & Sakurai, 2004). The food intake of *O. briareus* doubled when water temperatures increased from 20 to 30 °C (Borer & Lane, 1971).
4.3 Maturity and reproduction

Most females collected during the sampling period were immature. The percentage of immature females increased over the sampling period reaching a maximum in May, with more than 90% of the females being immature in both depth regions. This is consistent with the findings of Larson (2008), who found that virtually all females in waters < 20 m deep were immature.

The data on gonad stages and gonadosomatic indices (GSIs) collected during this study are not sufficient to define the spawning season for octopus in this region. However, in a longer-term study of octopus based on samples from the commercial fishery in Cockburn Sound, Larsen (2008) suggested that some spawning may occur in late autumn (April-May) and early spring (September-October). Moreover, based on laboratory studies, Joll (1978) proposed that the peak spawning for *O. tetricus* wild populations may occur in spring.

The maturity stages recorded for *O. tetricus* in Cockburn Sound were very similar to those collected in North Fremantle on the same day in early April. North Fremantle had a higher frequency of immature females than Cockburn Sound, however, North Fremantle also recorded at least one female in each maturity stage, and no mature females were collected from the Cockburn Sound site. Both sites had a high proportion of maturing males and recorded at least one male in each maturity stage. Despite Cockburn Sound females having significantly larger body sizes, the mean GSI was slightly lower than that for North Fremantle. The mean GSI differed significantly for males and females in both North Fremantle and Cockburn Sound sites, with males having a higher GSI for both areas. Results from this study provide strong evidence of males maturing earlier than females in both sites, which is consistent with the findings from previous studies (Joll, 1976; Leporati et al., 2015).

Only five mature females were recorded during the four months of this study which may have been due to a migration out of the study area by maturing females or a change in female catchability as they mature; mature females actively brood eggs, do not feed and are not actively hunting at this time, and are thus not adventuring into traps (Leporati et al., 2015). The migration of female octopuses to find mates and suitable dens for brooding eggs during the breeding season has been suggested for several species, including the east coast *O. tetricus* (Anderson, 1997), *O. insularis* in Brazil (Lima et al., 2014) and *O. vulgaris* in South Africa (Oosthuizen & Smale, 2003).
Both sexes exhibited very similar monthly maturity proportions in the shallow and deeper water of North Fremantle. The remarkable similarity of the maturity stages indicates that the difference between the depth region is not large enough to demonstrate the migration of mature individuals. The study design divided the two depth areas into \( \leq 10 \) and \( > 10 \) m to a maximum of 20 m. In Larson (2008) the two depth regions examined were a shallow inshore habitat (<20 m) and an offshore deeper habitat (30-40 m). The findings in this study are consistent with Larson (2008) results for the inshore habitat, and the distinct lack of mature females within the North Fremantle site demonstrates that the depth range is not adequate to observe the size-related offshore migration that females undertake while maturing.

Mature males were observed in every haul in North Fremantle, with the highest number being recorded in May. The presence of mature males for much of the year suggests that they have adapted an opportunistic reproductive strategy. Octopus aff. *O. tetricus* mates all year round, with two distinct pulses every six months (Larsen, 2008; Leporati et al., 2015) similar reproductive patterns are seen with *O. vulgaris* (Katsanevakis & Verriopoulos, 2004).

Moreover, the storm incidents in May and June 2020 in the North Fremantle study area may have accelerated the maturation of *O. tetricus*. Turbidity caused by high wind speeds and wave action reduces light penetration within the water. In studies of the sexual maturation of *O. vulgaris* it was found that light inhibits the release of the gonadotrophin hormone from the optic gland (Mangold, 1983) which delays the onset of maturing gonads.

Males reach maturity earlier than females and are typically present within the population throughout most of the year (Leporati et al., 2015). Female *O. tetricus* reach maturity at ~12 months, as they have a semelparous life history and longevity of ~ 18 months there is a ~6-month spawning window (Joll, 1976; Leporati et al., 2015). However, females may mate at an earlier maturity stage and are able to store the spermatophores in the oviducts for up to 16 weeks (Joll, 1976). Laboratory mating trials at the University of Western Australia of *O. tetricus* revealed that multiple paternity is common among broods of *O. tetricus* (Morse, 2008). Theories behind female promiscuity are that either, females mate with multiple partners to ensure phenotypic diversity among their offspring, or females may be sperm limited, and as they only have a singular opportunity to reproduce, mating with multiple partners ensures the fertilisation of larger broods (Joll, 1976; Morse, 2008). There is strong evidence of the latter, with observations of female octopods (*O. oliveri*) initiating copulation with the first male they are introduced to and become more selective as more mates are presented (Ylitalo et al., 2019).
Previous studies revealed that in captivity, female *O. tetricus* brood their eggs for ~1 month and then survive for 1–3 weeks after their eggs hatch (Joll, 1976). The spent individual caught in April thus indicates a potential spawning event around February or March. The exact length of the paralarval stage for *O. tetricus* has not been determined however it is likely to be similar to *O. vulgaris*, which has a duration of 47-54 days at 21.1 °C and 30-35 days at 23 °C (Iglesias et al., 2007). Furthermore, the number of very small juvenile (<80 mm ML) octopuses observed was notably lower than in previous years (P. Jasinski, pers. comm.). The lower number of small octopuses may be attributed to different water temperature conditions in the previous year. Preliminary investigations of the interaction of SST and successful recruitment were conducted for *O. vulgaris* in the Strait of Sicily, showed a significant correlation of recruitment success and higher SST (Garofalo et al., 2010).

Due to the large proportion of small, immature octopus caught within the waters of North Fremantle, it is likely that this site acts as a nursery area for juvenile octopuses. The significant proportion of small octopus’ within the North Fremantle study site indicates an ontogenetic migration of juveniles occurs to the shallower regions of North Fremantle, potentially due to the differences in prey availability. It is well established that juvenile octopuses frequently prey on crustaceans, and shift to teleosts and other cephalopods as body size increases (Smith, 2003; Villanueva et al., 2017; Vincent et al., 1998). This is consistent with the preliminary analysis of diets conducted in this study which shows that >40% of octopuses had consumed at least one crustacean. These preliminary findings contrast with those on *O. tetricus* diet from an abalone sea ranch in Flinders Bay, approximately 300 km south of Cockburn Sound. Octopus consumed much greater amounts abalone, non-cephalopod molluscs, on the sea ranch (Greenwell et al. 2019) than in North Fremantle. Future studies of *O. tetricus* should examine the differences in the dietary composition of juvenile and adults, including samples from deeper depth regions than those evaluated in this research.

### 4.4 Occurrence of nematodes

This study was the first to examine the presence of anisakid nematodes in *O. tetricus*. Little information and very few publications are available on infestations of nematodes in cephalopods, especially octopuses. Nearly 60% of octopuses sampled from North Fremantle site were infested with nematodes. Modelling on nematode prevalence showed that the probability of infection doubled for medium and larger octopus and that the incidence of
nematodes was much greater in March than June (about double). Temporal variation in nematode prevalence does not appear to be related to octopus’ size, as the smallest mean sizes was recorded in May. Potentially, the difference in prevalence due to size could be attributed to changes in feeding regimes between the warmer and cooler months for *O. tetricus*, as seasonal variation in diets has been identified for *O. bimaculatus* (Villegas et al., 2014) and *E. cirrhosa* (Regueira et al., 2017). Additionally, the nematode reproductive cycle is known to slow at lower temperatures, which may reduce the incidence of infection during the cooler months (Fazio et al., 2012; Nagasawa et al., 1994).

The prevalence of nematodes in early April was almost three times in North Fremantle than those from Cockburn Sound. The probability of infection by at least one nematode for North Fremantle octopus was nearly ten times that of octopus in Cockburn Sound. The regional differences of nematode infection between Cockburn Sound and North Fremantle may demonstrate the existence of two separate populations of *O. tetricus*. *Anisakis* infections have been applied as biological tags to assess sub-populations of pelagic and demersal octopus species from the Mediterranean Sea (Mattiucci et al., 2015; Rello et al., 2009; Roumebedakis et al., 2018a).

There is a high probability that *O. tetricus* ingested the nematodes from predating on crustaceans, which are usually the first intermediate host of anisakid nematodes (Mattiucci et al., 2018; Roumebedakis et al., 2018a). This coincides with the preliminary dietary analysis performed on *O. tetricus* which demonstrated that a major component of the stomach contents of the North Fremantle population was crustaceans. Predation on other cephalopods could also result in the spread of nematodes throughout the population, as the incidence of cannibalism detected in the diet analysis was quite high.

Infested crustaceans are likely to inhabit the same areas as *O. tetricus* due to the limited migration of the species. At present, the extent of *O. tetricus* broad-scale movement is unknown, however demersal octopuses tend to have restricted migrations (tens of kilometres) (Semmens et al., 2007), and mark-recapture trials on similar species such as *O. vulgaris* have demonstrated movement was limited to a home range of <1 km (Arechavala-Lopez et al., 2019; Mereu et al., 2015). Minimal broad-scale movement accounts for the vast difference in infection levels between the two sites, as they are unlikely to be sharing feeding grounds. Other fish and cephalopods, particularly squid, can exhibit large broad-scale movement from feeding.
and mating ground, therefore there is a higher likelihood that they will intermix with other populations (Nadolina & Podolska, 2014; Semmens et al., 2007).

*Octopus* aff. *O. tetricus* is a suitable host due to their trophic position and morphological adaptation. Coleoid cephalopods are susceptible to parasites due to the loss of an external shell, which has allowed high-speed locomotion but increased their vulnerability, and the complex structure of their skin, which is prone to lesioning (Kinne, 1990). Moreover, their function as mesopredators and voracious feeding behaviours mean they easily accumulate multi-host parasites through predation (Boyle & Rodhouse, 2005; Kinne, 1990; Roumbedakis et al., 2018a).

Although it was not possible to identify the species of anisakid nematode, this would be possible with molecular sequencing techniques. Nematodes in the genus *Anisakis* (Anisakidae) are some of the most common and abundant parasites known to cause pathological effects on fish and cephalopods, including ulceration and potential castration (Abollo et al., 1998; Abollo et al., 2001). Examination of anisakid infection on a squid species *Illex coindetii*, *Todaropsis eblanae* and *Totarodes sagittatus* found nematodes within the gonads had caused partial destruction and alteration of the tissue, with partial inhibition of the formation of reproductive cells. Within the North Fremantle site, only one octopus, a maturing female, was found to have had a nematode infestation in the gonads. A small proportion of octopus’ (3%) were found with nematodes encysted in the salivary glands. It not currently known what impact a nematode infestation of the salivary glands has on the health of the octopus; however, other parasitic infections have been known to cause severe damage to salivary glands. Cestode (*Prochristianella* sp.) infection on the salivary glands of *O. maya* caused deterioration of the tissues and the glands were dysfunctional, producing little to no secretions (Guillén-Hernández et al., 2018). Consequently, the damage caused to organs by parasites hinders the hosts capacity to feed and reproduce (Guillén-Hernández et al., 2018).

Multi-host parasites have a complex life history and are transmitted through the food web, with cephalopods and fish acting as secondary intermediate hosts, and marine mammals as definite hosts. A recent review of parasitism in cephalopods outlined several cuttlefish and squid species known to have been infected by nematodes but does not identify any known octopus species (Mattiucci et al., 2018; Roumbedakis et al., 2018a). Currently, there have only been three other published incidents of larval nematode infection within octopuses, *O. vulgaris*.
The presence of anisakid nematodes is also relevant to human health as the third stage anisakids are aetiological agents of painful gastrointestinal diseases in humans if raw or undercooked fish or cephalopods are consumed (Abollo et al., 2001). Anisakidosis, a parasitic infection in humans, occurs when larvae penetrate the gastrointestinal mucosa, which can trigger anaphylactic shock, and may require an endoscopic procedure or surgery (Abollo et al., 2001; Shamsi, 2014). Additionally, anaphylactic reactions can still occur even a dead nematode is ingested (Audicana et al., 2002). The first recorded incident of anisakidosis in Australia was in 2011 and was attributed to the consumption of locally caught mackerel (Shamsi & Butcher, 2011). Due to the popularity of seafood consumption in Australia, in particular sushi and sashimi, more undocumented incidents of anisakidosis in Australia are likely (Shamsi, 2014). The extent of nematode infection on the arms of *O. tetricus* is not known and therefore the risk of infection from consuming octopus from Perth waters cannot be assessed.

Due to time constraints of this thesis, and the challenges of nematode taxonomy, the species of nematodes could not be determined. Future studies should perform molecular identification on the nematodes and collect further samples from Cockburn Sound to identify the true extent of the infestation within the commercial fishery.

**Conclusions and Recommendations**

The examination of the spatial and temporal distribution of *O. tetricus* in North Fremantle, Western Australia, based on data collected between March and June 2020 demonstrates that the nearshore area of North Fremantle acts as a nursery area for juvenile and maturing octopus. The changes in sex ratio between March to June were consistent with the known composition of *O. tetricus* within the commercial fishery. Due to their early maturation, mature males were observed throughout the entirety of the study. The distinct lack of mature females within either depth region is consistent with the current biological hypothesis that *O. tetricus* females have a size-related migration during maturation to find mates and suitable dens for brooding.

The data on the mantle length distribution suggest a slower growth period for *O. tetricus* between autumn and winter as SST is declining, and the presence of small juvenile octopus
from April onwards indicates a spawning event in January or February. This slower growth during the time of lower SSTs is consistent with a previous study of the abundance and growth of *O. tetricus* (Larsen, 2008), and with the findings for other octopus species. However, a more detailed examination of the effect of environmental conditions on the population carried out over an 18 to 24-month period is required more accurately patterns of abundance and growth rates in the population.

Female mantle length was significantly longer in Cockburn Sound than North Fremantle, and the overall proportion of maturing individuals was greater, which suggests that Cockburn Sound may have spawned earlier or had a faster growth rate, potentially due to food availability, differing environmental conditions. Moreover, some of the samples from Cockburn Sound could have come from areas where females were maturing or brooding their eggs. It may be worthwhile for future studies evaluate the potentially distinguishing growth rates between the two sites and identifying where reproduction and egg brooding takes place, with the utilisation of samples throughout the year to enable identification of seasonal variances.

This study provides the first record of anisakid nematodes in the viscera of *O. tetricus*. Nematodes occurred in the viscera of 60% of the octopus’ from North Fremantle, and modelling indicated that the probability of North Fremantle octopus having at least one nematode was almost ten times higher than the Cockburn Sound population. The full extent of infection was not determined as the octopus’ arms were not examined during this study. While the ingestion of seafood infected with anisakids by humans is known to cause anisakidosis, the incidences of this in Australia are very rare, and it is likely to be low risk to the public at this time. Molecular identification of the nematode species may help to determine what, if any, potential risks may be associated with the consumption of *O. tetricus* by people. Examination of any potential pathological effects on the octopus infested with nematodes would be beneficial to monitor the health of the *O. tetricus* population.

The priority for future research is to perform a detailed analysis of the dietary composition of *O. tetricus* and to assess any differences that may occur between different size classes, sexes or depth regions. For a better understanding of the ingestion of nematodes by octopuses, comparative assessment on diets between North Fremantle and Cockburn Sound populations would be very valuable Furthermore, taking invertebrates from identified feeding grounds of octopus may help identify the first intermediate nematode hosts.
References


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