Trophic studies of mesopelagic lanternfishes
(Myctophidae) in the Perth Canyon

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Declaration

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

Daniel L. Cohen
Abstract

The diet of mesopelagic lanternfishes (Myctophidae) was investigated in the Perth Canyon off the Western Australian coast. Previous studies have investigated the abundance and diversity of larval fishes in the waters of the canyon but most components of the pelagic food web and their relationships have not yet been studied. Taking advantage of their diel migration pattern, larval Diaphus sp. (n = 80) and adult Myctophum asperum (n = 41) and Myctophum phengodes (n = 7) were captured at night using a large 1 mm mesh size surface net. Concurrent netting of zooplankton was also undertaken to assess the availability of prey using 150 µm, 500 µm and 1 mm mesh nets. The gut contents of the myctophids were identified and revealed almost no nighttime feeding among larval Diaphus sp. confirming the relationship between ontogenetic stage, eye development and an inability to feed at night. In contrast, the morphometrics of adult M. asperum and M. phengodes and the size and number of prey in the diet revealed a positive relationship between fish size and prey size, but no significant increase in the number of prey as fish size increased. Calanoid copepods were consumed by 83% of M. asperum and 100% of M. phengodes specimens and, respectively, they constituted 39% and 62% of the number of prey items in their guts. Euphausiid adults were consumed by 48% of M. asperum and 100% of M. phengodes specimens and, respectively, constituted 15% and 25% of the number of prey items in their guts. Selectivity was assessed using Ivlev’s and Chesson’s indices and, contrary to their abundance in the diet, calanoids were not typically positively selected with both Myctophum species showing a preference for euphausiids. This investigation represents the first study of the role of myctophids in the pelagic food web in the Perth Canyon and Southeast Indian Ocean and as such is an initial baseline to fill this knowledge gap.
Table of Contents

Abstract .......................................................................................................................... III
Acknowledgements ....................................................................................................... V

1.0 Introduction .............................................................................................................. 6
  1.1 Family Myctophidae ......................................................................................... 7
  1.2 Feeding ecology ............................................................................................. 10
    1.2.1 Diel vertical migration ............................................................................ 11
    1.2.2 Prey selectivity ...................................................................................... 13
  1.3 Rationale for project ......................................................................................... 14

2.0 Methods .................................................................................................................. 15
  2.1 Site Description ............................................................................................... 15
  2.2 Field sampling ............................................................................................... 18
  2.3 Voyage 1 .......................................................................................................... 18
  2.4 Voyage 2 .......................................................................................................... 19
  2.5 Zooplankton prey abundance ......................................................................... 20
  2.6 Fish analysis ..................................................................................................... 21
  2.7 Dietary analysis ............................................................................................... 22
  2.8 Statistical analysis ........................................................................................... 22

3.0 Results .................................................................................................................. 25
  3.1 Voyage 1 .......................................................................................................... 25
    3.1.1 Size frequency .......................................................................................... 26
    3.1.2 Jaw morphology ......................................................................................... 26
  3.2 Voyage 2 .......................................................................................................... 29
    3.2.1 Size frequency .......................................................................................... 29
    3.2.2 Jaw morphology ......................................................................................... 29
  3.3 Zooplankton prey abundance ......................................................................... 32
  3.4 Dietary analysis (Voyage 1) ............................................................................. 38
  3.5 Dietary analysis (Voyage 2) ............................................................................. 38
    3.5.1 Myctophum asperum .................................................................................. 38
    3.5.2 Myctophum phengodes .............................................................................. 39
  3.6 Dietary and morphological relationships ....................................................... 41
  3.7 Selectivity: Myctophum asperum .................................................................... 45
  3.8 Selectivity: Myctophum phengodes .................................................................. 48

4.0 Discussion .............................................................................................................. 50
  4.1 Zooplankton and myctophids in the Perth Canyon ........................................ 51
  4.2 Diet .................................................................................................................... 54
  4.3 Prey selectivity in Myctophum asperum and Myctophum phengodes ............. 60
  4.4 Other methods of dietary analysis .................................................................... 63
  4.5 Importance of myctophids .............................................................................. 66

5.0 Conclusion ............................................................................................................. 66

6.0 References ............................................................................................................. 69

7.0 Appendix ............................................................................................................... 82
  Appendix 1 .............................................................................................................. 82
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1.0 Introduction

The mesopelagic zone occurs at depths between 200 - 1,000 m and is an important, but poorly understood, part of the oceanic ecosystem (Pinet 1992, Robinson et al. 2010, Naito et al. 2013). Understanding of the mesopelagic zone is largely hindered by logistical constraints which have resulted in knowledge of the diversity and function of the biota that lives there to be, for the most part, inadequate (Robinson et al. 2010). This part of the oceanic environment is characterised by increased hydrostatic pressure, diminished light, high inorganic nutrient concentrations and episodic food supply (Robinson et al. 2010). Despite being a harsh environment, the mesopelagic zone supports a diverse and functional community of viruses, bacteria, archaea, protists, zooplankton and mesopelagic fishes (Gjøsaeter & Kawaguchi 1980, Koppelmann & Frost 2008, Aristegui et al. 2009, Robinson et al. 2010).

Mesopelagic fishes are among the most abundant marine organisms and consist of a number of diverse families such as Astronesthidae (Snaggletooths), Chiasmodontidae (Snaketoohs), Gonostomatidae (Bristlemouths), Myctophidae (Lanternfishes), Omosudidae (Hammerjaws), Phosichthyidae (Lightfishes) and Sternoptychidae (Hatchetfishes) (Gjøsaeter & Kawaguchi 1980, Salvanes & Kristofersen 2001). Mesopelagic fishes were estimated to have a global biomass of 948 million tonnes (Gjøsaeter & Kawaguchi 1980), a figure which was subsequently revised to 999 million tonnes by Lam and Pauly (2005). However, it has been suggested that this might be grossly underestimated because of net avoidance (Kaartvedt et al. 2012, Davison et al. 2013, Irigoien et al. 2014). To negate the issue of net avoidance, mesopelagic fishes have been surveyed using acoustics and the global biomass is now estimated to be ~10,000 million tonnes, one magnitude higher than previous estimates (Kaartvedt et al.
This large biomass is of paramount importance as mesopelagic fish make up an integral component of the oceanic food web. The new, greater, estimate of biomass could suggest that the efficiency of energy transfer through mesopelagic fishes might be greater than previously surmised (Kaartvedt et al. 2012, Irigoien et al. 2014).

1.1 Family Myctophidae

Most noteworthy of the mesopelagic fishes are those from the family Myctophidae, commonly referred to as lanternfishes owing to their conspicuous display of bioluminescent photophores. The name myctophid comes from the Greek word “mykter” meaning nose and “ophis” meaning serpent. The myctophids make up about 65% of mesopelagic fishes and reportedly account for at least 20% of ocean ichthyofauna biomass, thereby making them one of the most abundant families in the ocean (Gjøsaeter & Kawaguchi 1980, McGinnis 1982, Hulley 1998, Priede 2017). Myctophid dominance has been reported from various locations in all oceans, except the Arctic, making them the most widely distributed family of mesopelagic fishes (Moser 1970, Priede 2017). This wide distribution and large abundance have led to myctophids being recognised since the 1960s as a viable food source able to support globally significant fisheries (FAO-Fisheries 1997). However, myctophids are currently of little economic importance with an estimated global catch of less than 20,000 tonnes per year (Priede 2017). The majority of this catch is not directly consumed by humans owing to the difficulties in processing due to their high content of lipid and wax esters and their primary commercial use is as animal feed or crop fertiliser (Nair et al. 1983, Shaviklo & Rafipour 2013, Priede 2017). New product development of myctophid surimi (raw fish paste) has shown promising results and popularity owing to their high protein and lipid content (Shaviklo & Rafipour 2013).
The order Myctophiformes has two families, the blackchins (Neoscopelidae) and the lanternfishes (Myctophidae) (Paxton 1979, Moser & Ahlstrom 1996). It is hypothesised that myctophids evolved from the neoscopelids and diverged into a far more diversified family consisting of approximately 250 species from 33 genera (Paxton 1979, Moser & Ahlstrom 1996, Priede 2017). The family Myctophidae is currently recognised as having seven tribes, three from the subfamily Myctophinae (Electronini, Gonichthyini and Myctophini) and four from subfamily Lampanyctinae (Diaphini, Gymnosopelini, Lampanyctini and Notolychini) (Martin et al. 2018). The myctophiform phylogenetic tree has been revised many times over the years based on their morphology, ontogeny and identification through species-specific photophore patterns and, in recent years, genetics and DNA barcoding have been used (Poulsen et al. 2013, Bernal et al. 2014, Martin et al. 2018).

Myctophids are oviparous, non-guarding, pelagic spawners with a lifespan of approximately 1 – 5 years, exhibiting fast growth coupled with low fecundity (100 – 2,000 eggs per spawning) and high mortality rates (Gjøsaeter & Kawaguchi 1980, Hayashi et al. 2001, Karuppasamy et al. 2008). Adult myctophids are small fish < 30 cm, characterised by high myomere counts, a compressed body and head, large eyes and moderate to large jaws with bands of closely set teeth. Myctophid guts are slightly sigmoid, extending to the mid-body with distinct transverse muscular folds (Nafpaktitis 1982, Moser & Ahlstrom 1996). Their body is covered with cycloid or ctenoid scales formed over luminescent photophores (Figure 1). Fish colour varies depending on where in the water column the species lives. Species found in shallow water tend to exhibit iridescent blue, green or silver, whereas deeper-living species tend to be dark brown or black (Moser & Ahlstrom 1996).
Myctophid bioluminescence stems from a biosynthesis coelenterazine system (a light emitting luciferin molecule) (Widder 2010, Shimomura 2012, Poulsen et al. 2013). This system allows myctophids to communicate in a firefly-like manner using duration and intensity of flashes to create species-specific and sexually dimorphic patterns (Poulsen et al. 2013). All myctophid species are bioluminescent (except for Taaningichthys paurolychnus), possessing dermal photophores over the ventral and lateral surfaces of the body. Photophores take the form of complex cup-like structures containing photogenic tissue overlain by a scale modified as a lens (Tsuji & Haneda 1971, Anctil & Case 1977, Richards 2005, de Busserolles et al. 2014).

Myctophids display a wide variety of morphological forms throughout their ontogenetic development (Moser et al. 1984, Moser et al. 2001). This diversity suggests that in their evolutionary history, competition may have resulted in a myriad of morphological adaptions (Conley & Hopkins 2004). Larval gut length can vary from short in Lampanyctus acanthurus to elongate and free trailing from the body in Myctophum aurolaternatum (Moser et al. 1984, Moser & Ahlstrom 1996). Eye characteristics of larvae show a wide range of sizes, shapes, and specialisations, such as stalked or oval
eyes and are composed exclusively of cone receptors, limiting their use to photic conditions with rod receptors developing over time allowing for use in scotopic conditions (Blaxter 1986, Moser & Ahlstrom 1996, Bozzano et al. 2007). These features recede during transformation from larvae to juvenile, resulting in a fairly uniform appearance in adults (Moser & Ahlstrom 1996). In most myctophids, the branchiostegal photophores (Br2) are the first to develop during the larval stage (Figure 1), after which the sequence of development varies between species and, as such, constitute useful characteristics for identification during larval stages (Moser et al. 1984).

1.2 Feeding ecology

Myctophids interact, directly and indirectly, at many trophic levels. They are a profitable food source in terms of energy content and are preyed upon by primary and tertiary consumers. These predators include commercially significant species such as tuna, mackerel, sharks and squids as well as ecologically significant species such as cetaceans, pinnipeds, diving seabirds and penguins (Hopkins et al. 1996, Hulley 1998, Beauplet et al. 2004, Cherel et al. 2010, Saunders et al. 2014). As a result, myctophids are increasingly recognised as significant, but poorly understood, components in open ocean dynamics (Merrett & Roe 1974, Williams et al. 2001, Brodeur & Yamamura 2005, Suntsov & Brodeur 2008). They occupy an important position in the oceanic food web by consuming zooplankton in the upper layer (0 – 200 m), transporting this organic matter through the water column via their vertical migration, providing forage for larger predatory species and their faeces contribute to the rain of particulate organic matter descending towards the sea floor (Gjøsaeter & Kawaguchi 1980, Pakhomov et al. 1996, Suntsov & Brodeur 2008, Bernal et al. 2015, Priede 2017). Information on the feeding
ecology and trophic positions of this family is important to the understanding of pelagic food webs and modelling of ecological processes in the ocean.

Myctophids are carnivorous, preying predominantly on small invertebrates in the zooplankton, which are a valuable source of protein, amino acids and fatty acids (Gjøsæter & Kawaguchi 1980, Watanabe et al. 1983, Watanabe et al. 2002). Their vast abundance makes them huge consumers of these organisms, reportedly removing up to 10% of zooplankton biomass from the water column per night (Watanabe et al. 2002). Dietary studies have revealed that myctophids consume a wide range of microzooplankton (20 µm – 200 µm) such as larvaceans and nauplii, as well as mesozooplankton (0.2 mm – 20 mm) such as ostracods, copepods, amphipods, euphausiids, decapods, chaetognaths, polychaetes, and gastropods (Gjøsæter & Kawaguchi 1980, Dalpadado & Gjøsæter 1988, Hopkins & Gartner 1992, Watanabe et al. 2002, Bernal et al. 2015). However, due to their soft-bodied nature (thus the tendency to be digested faster), some prey items are rarely found intact, with their presence inferred from identifiable features such as the hooks of chaetognaths or the chaeta from polychaetes (Dalpadado & Gjøsæter 1988).

1.2.1 Diel vertical migration

Diel vertical migration is referred to as a trade-off between feeding opportunities and predation risk induced by changes in light intensities (Lampert 1989, Ringelberg 2010). As a way of reducing light dependent mortality, myctophids remain in the dark waters of the mesopelagic zone throughout the day (Nafpaktitis & Nafpaktitis 1969, Kerfoot 1985, Robison 2003, Collins et al. 2008). At night, myctophids undergo diel vertical migration, ascending towards the surface where they feed on zooplankton (Merrett &

Typically this vertical migratory behaviour takes place after the transformation from larvae to juveniles (Watanabe et al. 2002, Sassa et al. 2004). However, there is great variability, with some migratory patterns being ontogenetic or species-specific, and even dependent on the sex, latitude, hydrography, topography and season (Nafpaktitis 1982, Kerfoot 1985, Watanabe et al. 1999, Sassa et al. 2004). Some deeper-living species may not migrate at all or at irregular intervals, while larvae tend to remain in the epipelagic zone until they are developed enough to migrate (Moser & Ahlstrom 1996, Watanabe et al. 2002). In some oceanic regions, myctophids have adapted to live in oligotrophic and oxygen minimum conditions, as this provides safety from predators allowing them to fill a niche in an otherwise inhospitable environment (Smith et al. 1998, Gibson & Atkinson 2003, Gilly et al. 2013). According to Watanabe et al. (1999), myctophids show four migratory variations: migrants, semi-migrants, passive-migrants and non-migrants. The strong diel variations in the myctophids vertical distribution increases the incidence of trophic level interactions, thereby enhancing the complexity of the oceanic food web structure (Bernal et al. 2015).

The act of diel vertical migration means that many different species of myctophids and other fish occupy similar habitats and feeding niches at night (Hopkins & Gartner
As a result, significant dietary overlap can occur (Tyler Jr 1970, Young & Blaber 1986, Pakhomov et al. 1996). Typically myctophids prey on the most abundant species of zooplankton available (Sameoto 1988, Pakhomov et al. 1996). However, this is not always the case and myctophids have been found to coexist in similar feeding niches by selecting different species or sizes of prey, thus avoiding competition for the same resource (Sameoto 1988, Hopkins & Gartner 1992, Rissik & Suthers 2000).

1.2.2 Prey selectivity

Functional similarities of coexisting species influence the level of niche overlap. Species that share similar feeding ecology (diel vertical migration) and morphologies such as size and mouth structure or have the same habitat (mesopelagic zone) tend to have a higher niche overlap and therefore greater competition for resources (Schoener 1974, Schael et al. 1991, Landaeta et al. 2011). An effective method of reducing competition between species is to employ resource partitioning by selecting specific prey from a finite resource in an effort to facilitate their coexistence (Schoener 1974). Diet varies between myctophid species and as such species-specific diets show positive correlations between inter-specific fish morphology and variations in their migratory behaviour (Sabatés & Saiz 2000, Pusch et al. 2004, Shreeve et al. 2009). Stage-specific prey selectivity is consistent with stage-specific variations in functional morphology (Landaeta et al. 2011). Myctophids have been reported to prey on microzooplankton during early life stages and mesozooplankton in their later life stages (Pakhomov et al. 1996, Bernal et al. 2013). It has been proposed that mouth gape, linked to fish size and inter-specific variations, is one of the main morphological constraints with respect to maximum prey size (González-Quirós & Anadóan 2001). Further, as the ability to be selective is closely linked with the ability to search for prey, stronger swimmers have
access to a wider range and size of prey (Sabatés & Saiz 2000). It is important to understand the nature of selective feeding in myctophids as their huge abundance has the potential to instigate changes in zooplankton community structure and influence energy transfer through the ecosystem (Rapport & Turner 1970, Shreeve et al. 2009, Van Noord et al. 2013).

1.3 Rationale for project

Despite their ecological importance, wide distribution and large global abundance, relatively little is known about the diets and prey selectivity of myctophids, especially in the Southeast Indian Ocean off the Western Australian coast where no prior dietary studies on myctophids have been conducted. Dietary research is necessary for understanding the role of myctophids in the oceanic food web and the influences they have on community structure. The Perth Canyon has recently been declared a commonwealth Marine Park and presents a unique opportunity to study the feeding of myctophids off Western Australia as it provides easily accessible deep water (>500 m) close to the port of Fremantle. Several studies have previously sampled myctophids in, and around, the Perth Canyon highlighting their abundance (Muhling et al. 2008, Holliday et al. 2011, Holliday et al. 2012, McCauley & Cato 2016).

The primary objective of this study was to determine the diets of myctophids occurring at night in the surface waters of the Perth Canyon based on the identification and quantification of the different prey items found in their guts. The main aims of this study were to: 1) Determine the prey items in the diets of adult and larval myctophids; 2) Identify the abundance and composition of zooplankton occurring in the Perth Canyon surface waters at night; and 3) Compare the gut contents of the myctophids to
zooplankton availability to establish if there is any prey selectivity occurring. The hypothesis was that myctophids in the Perth Canyon prey on zooplankton taxa based on the zooplankters proportional abundance in the surface waters. It is also hypothesised that diet and prey selectivity will be species-specific and linked to fish ontogeny, prey species and size.

2.0 Methods

2.1 Site Description

The study was conducted off the coast of south-western Australia in the Perth Canyon located 25 km west of Rottnest Island at around 32˚S, 115˚E (Figure 2).

Figure 2: Bathymetry (in metres) and location of sampling sites for voyages 1 and 2 (V1 and V2, respectively) in the Perth Canyon. Sourced from Rennie et al. (2009a).

The Perth Canyon is a significant bathymetric feature of the Western Australian coast and is suggested to be a geological relic of the Swan River drainage system (Rennie et
al. 2009a, Huang et al. 2014). However, a recent study involving the first exploration of the Perth Canyon using a remote underwater vehicle suggests there is no geomorphological evidence to support this (Trotter et al. 2019). From the 200 m isobath the canyon descends rapidly to 1,000 m depth and opens onto the abyssal plain at > 4,000 m depth (Rennie et al. 2006, Trotter et al. 2019) (Figure 2). The sides of the canyon are steep, particularly near the seafloor where slopes of 30 – 50% are common (Rennie et al. 2007). The canyon has a bend at its midpoint with two shorter tributary canyons on the southern rim (Rennie et al. 2007, 2009b, Trotter et al. 2019).

Oceanographically, the region is dominated by the Leeuwin Current, an anomalous surface current flowing southwards along the shelf break towards Cape Leeuwin, where it turns east following the shelf-edge into the Great Australian Bight (Cresswell & Golding 1980, Cresswell & Peterson 1993, Meuleners et al. 2007). The Leeuwin current is narrow (~100 km wide), relatively shallow (< 300 m deep) and transports warm, nutrient-poor, tropical and subtropical water southwards. While the current typically flows in a linear fashion it often consists of complex meanders, eddies and jet-like streams (Cresswell & Golding 1980, Cresswell & Peterson 1993, Waite et al. 2007). The current reaches speeds of over 1 ms$^{-1}$, averaging 0.4 ms$^{-1}$ and is characterised by high mesoscale, seasonal and inter-annual variability in strength (Godfrey & Ridgway 1985, Meuleners et al. 2007, Rennie et al. 2009b). The Leeuwin Current is generally strongest during winter and weakest during summer when the southerly sea breezes counter its southward flow and drive the northward-flowing coastal Capes Current (Smith et al. 1991, Gersbach et al. 1999, Pattiaratchi & Woo 2009). The Leeuwin Undercurrent, a deep (> 300 m), cool, northward flowing current follows the continental shelf-edge and attains speeds of 0.1 – 0.4 ms$^{-1}$ (Woo & Pattiaratchi 2008). This current
is responsible for small subsurface eddies along the length of the Perth Canyon (Rennie et al. 2006, Rennie et al. 2007).

The Perth Canyon has strong vertical transport brought on by upwelling associated with the Leeuwin Undercurrent, although the Leeuwin Current acts as a barrier reducing cold-water expression on the surface. Upwelling of nutrients to the epipelagic zone is dependent on the location and thickness of the Leeuwin current (Rennie et al. 2009b). Canyons are important to continental shelf ecosystems as they can strongly influence shelf circulation and the distribution of biota (Brodeur 2001, Rennie et al. 2009a). The Perth Canyon supports an abundance of marine life (Koslow et al. 2008, Rennie et al. 2009a). This is hypothesised to be as a result of the complex upwelling of cold nutrient-rich water from the canyon depths resulting in high prey densities (Rennie et al. 2009a, Double et al. 2014).

The canyon is frequented by megafauna such as endangered blue whales (*Balaenoptera musculus*) and pygmy blue whales (*Balaenoptera musculus brevicauda*) where they feed on large aggregations of krill (Rennie et al. 2006, Jenner et al. 2008, Rennie et al. 2009a, Double et al. 2014, Erbe et al. 2015, Sutton et al. 2015). The presence of feeding whales indicates the potential of the oceanic processes in the canyon to support a high biomass of marine prey (Rennie et al. 2006). Acoustic evidence has emerged suggesting that fish choruses recorded in the Perth Canyon could be those of feeding myctophids (Hawkins et al. 2016, McCauley & Cato 2016). This indication of potentially high myctophid biomass combined with the easily accessible deep-water makes the Perth Canyon a highly suitable and convenient site for studying myctophids.
2.2 Field sampling

The known diel vertical migration of myctophids enables sampling of these fishes in the surface waters at night from a relatively small research vessel. For this project, the 28 m research vessel *Whale Song* was used and several different nets were deployed to capture both myctophids and their zooplankton prey (Table 1).

Table 1: Summary of sampling effort in the collection of myctophids and zooplankton from the Perth Canyon in 2018.

<table>
<thead>
<tr>
<th>Voyage</th>
<th>Target</th>
<th>Net mesh</th>
<th>Tows</th>
<th>Time (24 hr)</th>
<th>Position Initial</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>In</td>
<td>Out</td>
<td></td>
</tr>
<tr>
<td>Voyage 1</td>
<td>Myctophids</td>
<td>1 mm</td>
<td>NA</td>
<td>03:35</td>
<td>06:10</td>
<td>31°59′09″S 115°07′12″E</td>
</tr>
<tr>
<td>3/4/18</td>
<td>Zooplankton</td>
<td>150 µm</td>
<td>5</td>
<td>03:40</td>
<td>05:21</td>
<td>Same area as above</td>
</tr>
<tr>
<td></td>
<td>Myctophids &amp; Zooplankton</td>
<td>1 mm</td>
<td>NA</td>
<td>17:00</td>
<td>17:57</td>
<td>31°56′51″S 115°09′08″E</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18:56</td>
<td>20:50</td>
<td>31°56′18″S 115°06′10″E</td>
</tr>
<tr>
<td></td>
<td>Zooplankton</td>
<td>150 µm</td>
<td>5</td>
<td>19:00</td>
<td>19:51</td>
<td>Same area as above</td>
</tr>
<tr>
<td></td>
<td>Myctophids &amp; Zooplankton</td>
<td>500 µm</td>
<td>8</td>
<td>21:13</td>
<td>23:57</td>
<td>31°56′28″S 115°04′10″E</td>
</tr>
</tbody>
</table>

2.3 Voyage 1

Sampling took place in the Perth Canyon during the hours of darkness from 03:00 – 06:00 on April 3rd 2018 (Table 1). To capture myctophids, the vessel’s crane was used to deploy a 1 mm mesh size net (1 m² mouth area, 4 m long), within 1 m of the surface alongside the vessel. It was towed at an average of 2 – 3 knots almost continuously for three hours with the net only raised briefly from the water about every 20 – 30 minutes in order to empty the cod end. The volume of water sampled was not recorded, as fish abundance was not required for this dietary analysis. The contents of the cod end were preserved in plastic sample jars in a solution of 5% formalin and seawater.
To determine prey availability, zooplankton were sampled in the surface waters using a 150 µm mesh size plankton net (34 cm diameter, 1.5 m long). Five replicate tows were conducted behind the vessel concurrently with the deployment of the 1 mm mesh net for the sampling of fishes to ensure a good representation of the available zooplankton prey. To determine the volume of water sampled during each tow, a General Oceanics flowmeter was placed in the mouth of the net and readings were recorded before and after each tow. The zooplankton samples were preserved in 5% formalin and seawater.

2.4 Voyage 2

The second round of sampling took place in the Perth Canyon on the 1st of September 2018 with the research vessel Whale Song in a similar but slightly wider sampling area than the previous voyage (Table 1).

From 17:00 – 18:00, while it was still light, two surface tows of the 1 mm mesh net (1 m² mouth area, 4 m long) net were conducted to determine whether larval myctophids feed in the surface waters during daylight, however, no myctophids where captured in these tows. Sampling resumed at 19:00 after dark and the net was towed almost continuously until 21:00 with the cod end emptied every 20 – 30 minutes. Concurrently with the large 1 mm mesh net tows, five zooplankton surface tows were also conducted from behind the vessel with the 150 µm mesh net (34 cm diameter, 1.5 m long) equipped with a General Oceanics flowmeter. In an effort to obtain additional samples while underway, further surface tows (15 – 20 minutes duration) were undertaken at a speed of 2 knots between 21:00 – 23:55 from the stern of the vessel with a 500 µm mesh size net (50 cm diameter, 4.5 m long). Samples from voyage 2 were preserved with 5% formalin and seawater.
2.5 Zooplankton prey abundance

Each 150 µm mesh plankton sample from both voyages was reduced to a volume of 200 ml by pouring off any excess formalin through a 106 µm sieve. The 200 ml samples were aerated with a small pump and 10 x 2 ml homogenous subsamples taken with a wide mouth pipette so that in total 20 ml (10%) of the zooplankton sample was counted from each tow. The subsamples were enumerated and zooplankton identified under a dissecting microscope to the lowest possible taxonomic level or ontogenetic stage for euphausiids and decapods with the use of relevant plankton identification literature (e.g. Todd et al. 1996, Gibbons 1997, Richards 2005, Suthers & Rissik 2009, Johnson & Allen 2012). Any myctophids found in zooplankton samples were extracted and added to the fish samples.

The volume of water sampled in each zooplankton tow was calculated using:

\[ V = \pi R^2 \times L \]

Where V is the sampled volume of water, R is the net radius and L is the length of the tow derived from the difference in flowmeter readings divided by the rotor constant. Zooplankton counts from the 10% of each sample counted were multiplied up to represent the whole sample and divided by the volume of water (per m³) sampled in each tow to obtain the number per m³. The mean abundance and standard error for each zooplankton taxon was calculated from the five replicate plankton samples. In an effort to assess the availability of larger zooplankton, which might have avoided the small net, the abundances of zooplankton in the 500 µm and 1 mm mesh net tows from voyage 2 were also determined. The samples were split to 1/8th with a Folsom Splitter and the zooplankton identified and counted as per the above methods. The prey counts were multiplied by eight to represent the full sample. Fish and euphausiids were separated by
eye and counted for the whole sample. As the 500 µm and 1 mm mesh nets were not equipped with flowmeters only the proportion of each prey group was calculated.

2.6 Fish analysis

All fish were extracted from the 500 µm and 1 mm mesh net samples by hand sorting in plastic trays; the mesh size used to capture the myctophids is inconsequential to the dietary study. Adult myctophids were identified to species level based on species-specific photophore patterns and meristics, but myctophid larvae could only be identified to genus level. Identification was facilitated with the use of appropriate literature (Olivar & Fortuño 1991, Moser & Ahlstrom 1996, Moser et al. 2001, Richards 2005, Paxton & Williams in press). Prior to each dissection the standard length (tip of snout to base of caudal fin), total length (tip of the snout to end of caudal fin), body depth (dorsal to ventral at the deepest point of the body typically behind the gills) and upper jaw and lower jaw lengths (tip of the premaxilla to the posterior end of the maxilla and tip of the lower jaw to the posterior end of the maxilla) were measured using an eyepiece micrometer (Figure 3). As an indicator of the potential size of prey that could be consumed, the mouth gape (45°) of myctophids was calculated using the Pythagoras theorem.

Figure 3: Larval Diaphus sp. and morphometric measurements (top left). Dissection and gut (top right). M. asperum (bottom left) and M. phengodes (bottom right).
2.7 Dietary analysis

The guts of larva and adult myctophids were removed and examined by making an incision on the ventral side of the fish and detaching the gut from the posterior end of the esophagus and the pyloric valve (Sameoto 1988, 1989, Bernal et al. 2013) (Figure 3). The guts were opened with a fine needle and the gut contents placed on a glass slide and viewed under a dissecting microscope or a compound microscope if necessary. Prey items were identified to the lowest possible taxonomic level or ontogenetic stage for euphausiids and decapods with the use of the appropriate zooplankton literature (listed above). In several instances, much of the gut contents were unidentifiable, although it was possible to identify the presence of euphausiids based on the remnants with identifiable characteristics, such as appendages, eyes, carapace or photophores. In such cases, this was recorded as one euphausiid adult in the gut. Using an eyepiece micrometer, prey size was recorded as length and depth/width (the largest between depth and width) of intact prey items occurring in fish.

2.8 Statistical analysis

Data were compiled in a Microsoft Excel spreadsheet and imported into R-studio. Statistical analysis and preparation of figures were carried out using R-studio and Microsoft Excel. Linear regression was used to study the relationship between myctophid morphometrics as well as to quantify any relationships present between fish and prey. Differences in data were tested for significance ($\alpha = 0.05$) with a Student’s $t$ test or non-parametric equivalents (Wilcoxon sign rank test). Frequency of occurrence (FO%) (percentage of how many fish each prey group occurred in) and mean percentage by number (MNi,%) (percent composition of each prey group in the diet of each myctophid species) were used as indicators of the diet of myctophids (Table 2).
Table 2: Description of metrics and indices used in the dietary analysis of myctophids sampled in the Perth Canyon.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Frequency of occurrence:</strong></td>
<td>Where $J_i$ is the number of fish containing prey group $i$ and $P$ is the number of fish with food in their guts (Chipps &amp; Garvey 2007).</td>
</tr>
<tr>
<td>$F_{O%} = \frac{J_i}{P} \times 100$</td>
<td></td>
</tr>
<tr>
<td><strong>Mean percentage by number:</strong></td>
<td>Where $N_{ij}$ is the count of prey group $i$ in fish $j$, $Q$ is the number of prey groups in the guts of fish $j$ and $P$ is the number of fish with food in their guts (Chipps &amp; Garvey 2007).</td>
</tr>
<tr>
<td>$M_{N_{ij}}% = \frac{1}{P} \sum_{j=1}^{P} \left( \frac{N_{ij}}{\sum_{i=1}^{Q} N_{ij}} \right) \times 100$</td>
<td></td>
</tr>
<tr>
<td><strong>Ivlev’s electivity index:</strong></td>
<td>Where $r_i$ is the proportional abundance of prey in a predator’s diet and $n_i$ is the prey’s proportional abundance in the environment. Prey selectivity occurs when $E_i = 1$, negative selectivity occurs when $E_i = -1$ and non-selectivity occurs when $E_i = 0$ (Ivlev 1961).</td>
</tr>
<tr>
<td>$E_i = \frac{(r_i - n_i)}{(r_i + n_i)}$</td>
<td></td>
</tr>
<tr>
<td><strong>Chesson’s selectivity index:</strong></td>
<td>Where $m$ is the number of prey groups eaten by each species, $r_i$ is the proportion of prey group $i$ in the diet of each species, $n_i$ is the proportion of that prey group found in the environment and $j$ refers to all prey groups. $\alpha_i$ is the numerical proportion of the diet that might consist of prey group $i$ if all prey groups were present in equal numbers in the environment. To determine whether selectivity is taking place we refer to $\alpha_{\text{neutral}}$, defined as $m^{-1}$ or $1/m$ and is the proportion of a prey group out of all prey groups eaten by a species. Positive selectivity occurs when $\alpha_i &gt; \alpha_{\text{neutral}}$, negative selectivity (prey avoidance) occurs when $\alpha_i &lt; \alpha_{\text{neutral}}$ and non-selectivity occurs when $\alpha_i = \alpha_{\text{neutral}}$. $\alpha_i$ is equal to zero when the prey is not found in the gut but is present in the environment (Chesson 1983).</td>
</tr>
<tr>
<td>$\alpha_i = \frac{\left( \frac{r_i}{n_i} \right)}{\sum_{j=1}^{m} \left( \frac{r_j}{n_j} \right)}$</td>
<td></td>
</tr>
</tbody>
</table>
An effort was made to establish the difference in prey size captured in the three net sizes and which net best represented the prey size found in the diet of the *Myctophum* species (*Myctophum asperum* and *Myctophum phengodes*). Prey body depth or width (whichever was largest) was used, as it is the best indicator of their potential to pass through the net’s mesh (Barkley 1972) or be consumed by a fish. The body depth/width (mm) of 30 (where possible) random individuals from seven prey groups important in the diet were measured from the 150 µm, 500 µm and 1 mm mesh net samples, namely calanoids, poecilostomatoids, amphipods, furcilia larvae, euphausiid adults, zoea larvae and teleost larvae. The two most common teleost larvae (*Sardinops* and *Scomberesox*) measurements were used (elongate morphology). The difference in prey depth/width between each net was assessed using linear regression and the difference between the prey depth/width in the net and those in the gut was assessed with multiple t tests. The net size that did not show a significant difference ($p > 0.05$) between the prey depth/width would theoretically best represent the prey size consumed by the *Myctophum* species.

For comparison, prey selectivity was assessed with two different indices, namely Ivlev’s electivity index (dependent on prey abundance) (Ivlev 1961) and Chesson’s selectivity index (independent of prey abundance) (Chesson 1983) (Table 2). Although both indices determine the prey selectivity, Chesson’s selectivity values are interpreted as the proportion of the diet that would consist of the prey if all prey found in the environment were present in equal numbers. This creates an unbiased environment where selectivity is based on fish preference rather than the abundance of prey items in the environment (Chesson 1983, Kremers 1984). In contrast, the Ivlev’s electivity index is not as versatile at determining food selection of a species when comparing prey
groups with different relative abundances as it is dependent on these values (Jacobs 1974). The relative prey abundance from voyage 2 was determined for the 150 µm, 500 µm and 1 mm mesh nets from which the Ivlev’s and Chesson’s indices were calculated for both Myctophum species. Some prey items were found in low numbers in the diet but were absent in the zooplankton samples; in such cases, they were removed from the selectivity analysis, as values cannot be calculated without a proportional abundance. In cases where a prey group was absent from the diet but present in the environment a Chesson’s value of 0 will be calculated and cannot, therefore, be assessed statistically; these cases represent absolute negative selectivity (Bernal et al. 2013).

3.0 Results

3.1 Voyage 1

Sampling from voyage 1 yielded 80 Diaphus sp. larvae and a single adult Myctophum asperum identified with the use of meristics (Table 3), photophore patterns and in concurrence with the values given in Paxton and Williams (in press).

Table 3: Meristics of Diaphus sp., Myctophum asperum and Myctophum phengodes sampled in the Perth Canyon during voyages 1 and 2.

<table>
<thead>
<tr>
<th>Meristics</th>
<th>Diaphus sp.</th>
<th>Myctophum asperum</th>
<th>Myctophum phengodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertebrae:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total myomeres</td>
<td>35 – 36</td>
<td>36 – 38</td>
<td>38 - 41</td>
</tr>
<tr>
<td>Number of fin rays:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorsal</td>
<td>13</td>
<td>12 – 14</td>
<td>12 – 13</td>
</tr>
<tr>
<td>Anal</td>
<td>14 – 15</td>
<td>17 – 19</td>
<td>21 – 22</td>
</tr>
<tr>
<td>Pectoral</td>
<td>11 – 12</td>
<td>12 – 13</td>
<td>15 – 16</td>
</tr>
<tr>
<td>Pelvic</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Caudal:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorsal Secondary</td>
<td>5 – 6</td>
<td>8 – 9</td>
<td>7</td>
</tr>
<tr>
<td>Principal</td>
<td>10 + 9</td>
<td>10 + 9</td>
<td>10 + 9</td>
</tr>
<tr>
<td>Ventral Secondary</td>
<td>6</td>
<td>8 – 9</td>
<td>7</td>
</tr>
<tr>
<td>Gill rakers on first arch:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>Fish too small</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Lower</td>
<td>Fish too small</td>
<td>11 – 12</td>
<td>18 – 19</td>
</tr>
<tr>
<td>Total</td>
<td>Fish too small</td>
<td>15 – 16</td>
<td>26 – 27</td>
</tr>
</tbody>
</table>
3.1.1 Size frequency

Standard lengths of *Diaphus* sp. ranged from 5.3 – 9.9 mm (mean ± SE: 6.7 ± 0.11 mm) with 86% of fish occurring within the 5 – 7.5 mm range (Figure 4). Body depth ranged from 1.4 to 3.2 mm (1.99 ± 0.04 mm) and as a percentage of standard length was, on average, (mean ± SE) 29.7 ± 0.2% indicating a deep morphology (Figure 4). A significant positive relationship (*p* < 0.001, linear regression) was found between standard length and body depth (Figure 4). The single *M. asperum* had a standard length of 18.5 mm with a body depth of 4.2 mm, 22.7% of its standard length.

3.1.2 Jaw morphology

Upper and lower jaw lengths of *Diaphus* sp. ranged from 0.85 – 2.03 mm (1.28 ± 0.25 mm) and 0.75 – 1.88 mm (1.19 ± 0.24 mm), respectively. There was a strong positive correlation between standard lengths and the upper and lower jaw lengths (upper jaw $R^2 = 0.86$, *p* < 0.001 and lower jaw $R^2 = 0.85$, *p* < 0.001, linear regression) (Figure 5). *Diaphus* sp. showed a significant difference between upper and lower jaw lengths (*p* = 0.009, one-sided *t* test), indicating that the upper jaw is typically longer than the lower jaw.

Mouth gape (45°) in the sample of *Diaphus* sp. ranged from 0.62 – 1.49 mm (0.95 ± 0.19 mm) equivalent to an average of 14.1 ± 0.1% of its standard length. Mouth gape (45°) showed strong positive correlation to standard length ($R^2 = 0.86$, *p* < 0.001, linear regression) (Figure 5). The single *M. asperum* had an upper jaw and lower jaw lengths of 3.05 and 2.95 mm, respectively, with a mouth gape (45°) of 2.3 mm or 12.4% of its standard length.
Figure 4: Length and body depth frequencies and their relationship in *Diaphus* sp. sampled in the Perth Canyon. No standard length from two fish with damaged tails.
Figure 5: Correlation between standard length, upper and lower jaw lengths and mouth gape (45°) in *Diaphus* sp. sampled in the Perth Canyon. Two tails, one upper jaw and three lower jaws were damaged and not included.
3.2 Voyage 2

Sampling during voyage 2 yielded adults of 41 *Myctophum asperum* and seven *Myctophum phengodes*, identified with the use of meristics (Table 3), photophore patterns and in concurrence with the values given in Paxton and Williams (in press).

3.2.1 Size frequency

Standard lengths of *M. asperum* ranged from 15.7 – 40 mm (mean ± SE: 31.37 ± 0.81 mm) with 90% of specimens from 25 – 40 mm (Figure 6). Body depth ranged from 3.7 – 9 mm (7 ± 0.18 mm) (Figure 6) and was, on average, (mean ± SE) 22.3 ± 0.1% of its standard length. A significant positive relationship (*p* < 0.001, linear regression) was found between standard length and body depth (Figure 6).

*Myctophum phengodes* was the largest species caught, with standard length ranging from 45.6 – 71.6 mm (59.03 ± 3.14 mm) (Figure 7). The body depths ranged from 10.5 – 16 mm (13.07 ± 0.67 mm). A significant positive relationship (*p* < 0.001, linear regression) existed between standard length and body depth (Figure 7). The body depth for *M. phengodes* was, on average, 22.2 ± 0.2% of its standard length, nearly the same percentage as *M. asperum* indicating similarity in the morphologies of the two species (*p* = 0.56, two-sided *t* test). Compared to *Diaphus* sp., both *M. asperum* and *M. phengodes* have significantly smaller body depth to standard length ratios (*p* < 0.001 and *p* < 0.001, respectively, one-sided *t* test), indicating a more elongate morphology.

3.2.2 Jaw morphology

Upper and lower jaw lengths of *M. asperum* ranged from 2.8 – 6.2 mm (4.92 ± 0.12 mm) and 2.7 – 5.9 mm (4.67 ± 0.12 mm), respectively. There was a strong positive
Figure 6: Length and body depth frequencies and their relationship in *M. asperum* sampled in the Perth Canyon.
Figure 7: Length and body depth frequencies and their relationship in *M. phengodes* sampled in the Perth Canyon.
correlation between the standard lengths of *M. asperum* and the upper and lower jaw lengths (upper jaw $R^2 = 0.95$, $p < 0.001$ and lower jaw $R^2 = 0.91$, $p < 0.001$, linear regression) (Figure 8). There was no significant difference between upper and lower jaw lengths ($p = 0.13$, two-sided $t$ test). Mouth gape ($45^\circ$) ranged from 2.11 – 4.64 mm (3.68 ± 0.09 mm) an average of 11.8 ± 0.1% of its standard length and showed strong positive correlation to standard length ($R^2 = 0.95$, $p < 0.001$, linear regression).

The upper and lower jaw lengths of *M. phengodes* ranged from 7.7 – 11.4 mm (9.67 ± 0.47 mm) and 7.6 – 11.2 mm (9.5 ± 0.44 mm), respectively. Both jaw lengths exhibited a strong positive relationship with standard length (upper jaw $R^2 = 0.95$, $p < 0.001$ & lower jaw $R^2 = 0.91$, $p < 0.001$, linear regression) (Figure 9). There was no significant difference between the upper and lower jaw lengths ($p = 0.41$, two-sided $t$ test). Mouth gape ($45^\circ$) ranged from 5.86 – 8.65 mm (7.35 ± 0.35 mm) correlating strongly with standard length ($R^2 = 0.94$, $p < 0.001$, linear regression). Mouth gape ($45^\circ$) of *M. phengodes* was, on average, 12.5 ± 0.2% of its standard length and had a significantly larger mouth gape ($45^\circ$) proportional to its standard length than *M. asperum* ($p = 0.002$, one-sided $t$ test), but proportionally smaller than that of *Diaphus* sp. ($p < 0.001$, one-sided $t$ test).

### 3.3 Zooplankton prey abundance

Eighteen zooplankton groups were identified and enumerated from the five 150 µm mesh net samples collected during voyage 1. Calanoid copepods were dominant accounting for around 80% of all zooplankton captured (mean abundance: 1459.39 per m$^3$; mean percent composition: 80.55%). Cyclopoid copepods were the second most abundant group (169.28 per m$^3$; 9.2%), followed by poecilostomatoid copepods (146.17
Figure 8: Relationship between standard length, upper and lower jaw lengths and mouth gape (45°) for *M. asperum* sampled in the Perth Canyon.
Figure 9: Relationship between standard length, upper and lower jaw lengths and mouth gape (45°) for *M. phengodes* sampled in the Perth Canyon.
per m$^3$; 8.01%), chaetognaths (13.35 per m$^3$; 0.75%) and harpacticoid copepods (7.88 per m$^3$; 0.44%) (Table 4 & Figure 10).

Twenty-one zooplankton groups were identified and enumerated from four 150 µm mesh net samples from voyage 2 (flowmeter jammed on the fifth tow so the sample was not used). Similar to the first voyage, calanoid copepods were most abundant in the net samples contributing about 70% of all zooplankton captured (996.61 per m$^3$; 73.4%). Poecilostomatoid copepods were the next most abundant (153.6 per m$^3$; 11.24%), followed by cyclopoid copepods (127.21 per m$^3$; 9.38%), nauplii larvae (38.27 per m$^3$; 2.89%), and chaetognaths (15.48 per m$^3$; 1.1%) (Table 4 & Figure 10).

From the five 500 µm mesh net tows taken during voyage 2, twenty-two zooplankton groups were identified and enumerated. Although proportionally less abundant than in the 150 µm mesh net samples, calanoids were still the most abundant, constituting 58.4% of zooplankton, followed by cnidaria (9.6%), crab zoea larvae (8.3%), chaetognaths (6.3%) and euphausiid furcilia larvae (3.9%) (Table 4 & Figure 10).

Nineteen zooplankton groups were identified and enumerated from the 1 mm mesh net samples taken during voyage 2. A significant drop in calanoid copepod abundance was observed and, although still the most abundant group, they constituted only 23.25%. Euphausiid adults were the next most abundant at 21.85%, an increase of over 20% from their abundances in the 500 µm mesh net. Cnidaria were the next most abundant (17.97%), followed by zoea larvae (10.37%) and decapod adults (8.36%) (Table 4 & Figure 10). The majority of euphausiid adults from the zooplankton samples were identified as *Euphausia recurva*. 

35
Table 4: Mean abundances of zooplankton per m$^3$ of seawater (No. m$^3$) and mean percent composition (%) from voyage 1 and 2 using the 150 µm mesh size net in the Perth Canyon. Note that the 500 µm and 1 mm mesh size nets used in voyage 2 were not fitted with flowmeters so only the mean percent composition (%) values are given.

<table>
<thead>
<tr>
<th>Zooplankton</th>
<th>Voyage 1 150 µm mesh</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. m$^{-3}$ (± SE)</td>
<td>% (± SE)</td>
<td>No. m$^{-3}$ (± SE)</td>
<td>% (± SE)</td>
<td>No. m$^{-3}$ (± SE)</td>
<td>% (± SE)</td>
<td>No. m$^{-3}$ (± SE)</td>
<td>% (± SE)</td>
</tr>
<tr>
<td>Cnidaria</td>
<td>0</td>
<td>0</td>
<td>4.75 (± 2.01)</td>
<td>0.31 (± 0.12)</td>
<td>9.63 (± 1.36)</td>
<td>0.04 (± 0.03)</td>
<td>17.97 (± 4.55)</td>
<td></td>
</tr>
<tr>
<td>Polychaeta</td>
<td>0.35 (± 0.21)</td>
<td>0.02 (± 0.01)</td>
<td>1.46 (± 0.51)</td>
<td>0.11 (± 0.04)</td>
<td>0.04 (± 0.03)</td>
<td>0.94 (± 0.22)</td>
<td>0.34 (± 0.05)</td>
<td></td>
</tr>
<tr>
<td>Ostracoda</td>
<td>0.83 (± 0.52)</td>
<td>0.04 (± 0.03)</td>
<td>0</td>
<td>0</td>
<td>0.94 (± 0.22)</td>
<td>0.34 (± 0.05)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Copepoda</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calanoida</td>
<td>1459.39 (± 71.22)</td>
<td>80.55 (± 0.89)</td>
<td>996.61 (± 124.02)</td>
<td>73.4 (± 0.77)</td>
<td>58.36 (± 2.61)</td>
<td>23.25 (± 6.32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclopoida</td>
<td>169.28 (± 21.34)</td>
<td>9.2 (± 0.58)</td>
<td>127.21 (± 20.29)</td>
<td>9.38 (± 1.54)</td>
<td>0.12 (± 0.06)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harpacticoida</td>
<td>7.88 (± 1.93)</td>
<td>0.44 (± 0.12)</td>
<td>0.88 (± 0.26)</td>
<td>0.06 (± 0.02)</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pocilostomatoida</td>
<td>146.17 (± 13.53)</td>
<td>8.01 (± 0.37)</td>
<td>153.6 (± 23.41)</td>
<td>11.24 (± 1.13)</td>
<td>0.95 (± 0.11)</td>
<td>0.38 (± 0.26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nauplii larvae</td>
<td>7.04 (± 1.88)</td>
<td>0.4 (± 0.12)</td>
<td>38.27 (± 6.62)</td>
<td>2.89 (± 0.62)</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isopoda</td>
<td>0.15 (± 0.15)</td>
<td>0.01 (± 0.01)</td>
<td>0.39 (± 0.22)</td>
<td>0.03 (± 0.02)</td>
<td>0.41 (± 0.11)</td>
<td>0.36 (± 0.36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphipoda</td>
<td>0.82 (± 0.45)</td>
<td>0.05 (± 0.03)</td>
<td>0.54 (± 0.28)</td>
<td>0.05 (± 0.03)</td>
<td>1.93 (± 0.13)</td>
<td>4.29 (± 1.26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mysidacea</td>
<td>0</td>
<td>0</td>
<td>0.03 (± 0.03)</td>
<td>0.002 (± 0.002)</td>
<td>0.57 (± 0.17)</td>
<td>0.45 (± 0.21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euphausiidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calyptopis larvae</td>
<td>0.49 (± 0.49)</td>
<td>0.03 (± 0.03)</td>
<td>0.91 (± 0.54)</td>
<td>0.06 (± 0.04)</td>
<td>0.03 (± 0.02)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furcilia larvae</td>
<td>1.47 (± 0.92)</td>
<td>0.07 (± 0.04)</td>
<td>3.29 (± 1.19)</td>
<td>0.27 (± 0.1)</td>
<td>3.85 (± 1.6)</td>
<td>2.63 (± 0.33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euphausiid adults</td>
<td>0.05 (± 0.03)</td>
<td>0.001 (± 0.001)</td>
<td>0.39 (± 0.19)</td>
<td>0.03 (± 0.02)</td>
<td>0.99 (± 0.36)</td>
<td>21.85 (± 7.46)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decapoda</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protozoa larvae</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.03 (± 0.03)</td>
<td>0.09 (± 0.09)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zoa larvae</td>
<td>2.9 (± 1)</td>
<td>0.15 (± 0.04)</td>
<td>3.79 (± 1.18)</td>
<td>0.29 (± 0.11)</td>
<td>8.29 (± 2.71)</td>
<td>10.37 (± 1.29)</td>
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<tr>
<td>Postlarvae</td>
<td>0.69 (± 0.48)</td>
<td>0.04 (± 0.03)</td>
<td>0</td>
<td>0</td>
<td>0.05 (± 0.04)</td>
<td>2.43 (± 0.91)</td>
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<tr>
<td>Decapod adults</td>
<td>0</td>
<td>0</td>
<td>0.27 (± 0.24)</td>
<td>0.05 (± 0.03)</td>
<td>0.77 (± 0.09)</td>
<td>8.36 (± 0.92)</td>
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</tr>
<tr>
<td>Chaetognatha</td>
<td>13.35 (± 1.25)</td>
<td>0.75 (± 0.09)</td>
<td>15.48 (± 3.12)</td>
<td>1.1 (± 0.1)</td>
<td>6.33 (± 0.59)</td>
<td>2.62 (± 0.82)</td>
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<tr>
<td>Gastropoda</td>
<td>1.57 (± 0.65)</td>
<td>0.08 (± 0.03)</td>
<td>2.75 (± 1.48)</td>
<td>0.17 (± 0.08)</td>
<td>1.97 (± 0.64)</td>
<td>0.98 (± 0.12)</td>
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<tr>
<td>Dolichidea</td>
<td>0.52 (± 0.34)</td>
<td>0.03 (± 0.02)</td>
<td>0.28 (± 0.25)</td>
<td>0.02 (± 0.02)</td>
<td>1.41 (± 0.24)</td>
<td>0.85 (± 0.46)</td>
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<td>Larvacea</td>
<td>2.55 (± 1.07)</td>
<td>0.13 (± 0.05)</td>
<td>7.33 (± 2.39)</td>
<td>0.51 (± 0.14)</td>
<td>0.99 (± 0.2)</td>
<td>0.66 (± 0.39)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Teleostei larvae</td>
<td>0</td>
<td>0</td>
<td>0.23 (± 0.11)</td>
<td>0.02 (± 0.01)</td>
<td>0.96 (± 0.17)</td>
<td>1.7 (± 0.74)</td>
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<td>0</td>
<td>0</td>
<td>0.59 (± 0.31)</td>
<td>0.04 (± 0.03)</td>
<td>1.38 (± 0.29)</td>
<td>0.41 (± 0.34)</td>
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</table>
Figure 10: Summary of the five most abundant (% composition) zooplankton groups in each net size sampled in the Perth Canyon for voyage 1 (150 µm mesh net) and voyage 2 (150 µm, 500 µm and 1 mm mesh nets).
3.4 Dietary analysis (Voyage 1)

Of all 80 larval *Diaphus* sp. sampled at night in the surface waters of the Perth Canyon, the gut of only one fish contained prey and the rest were empty. The second largest specimen of *Diaphus* sp. (standard length = 9.5 mm) had consumed two copepod nauplii larvae. The single *M. asperum* captured during voyage 1 contained 55 individual prey items from four out of the 18 prey groups sampled during voyage 1. The single *M. asperum* consumed predominately calanoid copepods (percentage of diet: 89.09%), followed by poecilostomatoid copepods (7.27%), euphausiid adults (1.82%) and amphipods (1.82%).

3.5 Dietary analysis (Voyage 2)

3.5.1 *Myctophum asperum*

Of the 41 *M. asperum* examined, 40 of them had consumed prey with the gut contents including 15 out of the over all 24 prey groups found in the zooplankton samples from the Perth Canyon during voyage 2. The 41 *M. asperum* consumed an average of (mean ± SE) 6.5 ± 0.15 individual prey items per fish (range of 0 – 25 prey items per fish). Calanoid copepods were the most common prey, consumed by 82.5% of *M. asperum* (by FO%), with 47.5% consuming euphausiid adults, 32.5% consuming furcilia larvae, 30% consuming poecilostomatoid copepods and 27.5% consuming zoea larvae (Table 5). The diet of *M. asperum* was dominated by calanoid copepods (MNi% ± SE: 38.57 ± 4.38%), and euphausiid adults (15.23 ± 4.36%), followed by a small decrease in proportion with zoea larvae (12.11 ± 3.87%), poecilostomatoid copepods (9.27 ± 3.23%) and furcilia larvae (9.24 ± 2.97%) and the remaining prey groups making up 15.6 ± 3.5% of the diet (Table 5 & Figure 11).
All seven *M. phengodes* specimens contained prey and consumed ten out of the twenty-four prey groups found in the Perth Canyon zooplankton during voyage 2. The seven *M. phengodes* consumed an average of 21.4 ± 0.97 individual prey items per fish (range of 10 – 41 prey items per fish), significantly more than *M. asperum* (*p* = 0.005, one-sided *t* test). *Myctophum phengodes* diet contained five prey groups less than that of *M.
*asperum*. Calanoid copepods and euphausiid adults were consumed by 100% of *M. phengodes* (by FO%), 42.9% consumed furcilia larvae and 28.6% consumed ostracods and zoea larvae (Table 6). The diet of *M. phengodes* was dominated by calanoids (MN, % ± SE: 62.13 ± 8.65%), and euphausiid adults (25.15 ± 11.11%), followed by furcilia larvae (2.9 ± 1.98%), ostracods (2.38 ± 1.56%), and zoea larvae (2.25 ± 1.73%) with the remaining prey groups making up 5.2 ± 1.6% of the diet (Table 6 & Figure 11).

Table 6: Percent frequency of occurrence (FO%), mean number per fish (Mean n) and mean percentage by number (MN, %) in the diet of *M. phengodes*.

<table>
<thead>
<tr>
<th>Prey groups</th>
<th>FO%</th>
<th>Mean n (± SE)</th>
<th>MN, % (± SE)</th>
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</thead>
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<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Polychaeta</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ostracoda</td>
<td>28.57</td>
<td>0.43 (± 0.3)</td>
<td>2.38 (± 1.56)</td>
</tr>
<tr>
<td>Copepoda</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Calanoida</td>
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<td>14.57 (± 3.9)</td>
<td>62.13 (± 8.65)</td>
</tr>
<tr>
<td>Cyclopoida</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Harpacticoida</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Poecilostomatoida</td>
<td>57.14</td>
<td>0.57 (± 0.2)</td>
<td>2.11 (± 0.79)</td>
</tr>
<tr>
<td>Nauplii larvae</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Isopoda</td>
<td>14.29</td>
<td>0.14 (± 0.14)</td>
<td>0.35 (± 0.35)</td>
</tr>
<tr>
<td>Amphipoda</td>
<td>14.29</td>
<td>0.14 (± 0.14)</td>
<td>0.51 (± 0.51)</td>
</tr>
<tr>
<td>Mysidacea</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Euphausiidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calyptopis larvae</td>
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<td>0</td>
</tr>
<tr>
<td>Furcilia larvae</td>
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<td>0.71 (± 0.42)</td>
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<tr>
<td>Euphausiid adults</td>
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<td>3.57 (± 1.23)</td>
<td>25.15 (± 11.11)</td>
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<td>Decapoda</td>
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<tr>
<td>Protozoa larvae</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Zoea larvae</td>
<td>28.57</td>
<td>0.86 (± 0.7)</td>
<td>2.25 (± 1.73)</td>
</tr>
<tr>
<td>Postlarvae</td>
<td>14.29</td>
<td>0.14 (± 0.14)</td>
<td>0.51 (± 0.51)</td>
</tr>
<tr>
<td>Decapod adults</td>
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</tr>
<tr>
<td>Chaetognatha</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gastropoda</td>
<td>28.57</td>
<td>0.29 (± 0.18)</td>
<td>1.7 (± 1.13)</td>
</tr>
<tr>
<td>Doliolida</td>
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<td>0</td>
</tr>
<tr>
<td>Larvaea</td>
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<td>0</td>
</tr>
<tr>
<td>Teleostei larvae</td>
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<td>0</td>
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</tr>
<tr>
<td>Total</td>
<td>NA</td>
<td>21.4 (± 0.97)</td>
<td>100</td>
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</table>
3.6 Dietary and morphological relationships

Although there was a visual trend of decreasing number of prey items per fish with increasing standard length for both *M. asperum* and *M. phengodes* (Figure 12), this was not significant (*p* = 0.49 and *p* = 0.22, respectively, linear regression). This indicated that for both species the number of prey items consumed did not change over the size range. Likewise, because of the correlation between morphometrics the same was true for all morphological measurements recorded from both species, with a non-significant *p* value > 0.05 reported for each morphological measurement.

As the standard length and depth of *M. asperum* increased a significant positive increase in body depth/width of prey was found (*p* = 0.004 and *p* = 0.005, respectively, log-linear regression), but the R² value was low (Figure 13). The same was found when body depth/width of prey was compared to mouth gape (45°). The significant increase in prey body depth/width as mouth gape (45°) increased (*p* = 0.009, log-linear...
Figure 12: The number of prey items consumed by *M. asperum* and *M. phengodes* compared to their standard lengths sampled in the Perth Canyon.
regression) suggested that the potential to consume larger prey increases with fish size. Similarly, a positive relationship was observed for the standard length, body depth and mouth gape (45°) of *M. phengodes* when compared to the body depth/width of prey (*p* < 0.001, *p* < 0.001 and *p* < 0.001, respectively, log-linear regression). However, the sample size for *M. phengodes* was small and the R² value low (Figure 13). In this study, *M. phengodes* specimens were significantly larger (mean standard length ± SE: 59.03 ± 3.14 mm) than those of *M. asperum* (31.37 ± 0.81 mm) (*p* < 0.001, one-sided *t* test), and were found to have consumed significantly larger prey (body depth/width) (*p* < 0.001, Wilcoxon rank sum test).

Analysis of the prey body depth/width of the seven major prey groups measured from each net indicated that a significant positive relationship (*p* value < 0.001, linear regression) existed between the net mesh size and depth/width of prey captured in each net (Figure 14). This indicates that the average depth/width of prey inside each net increased with each subsequent net mesh size (Figure 14). The 150 µm, 500 µm and 1 mm mesh net sizes had a combined average prey depth/width of (mean ± SE) 0.58 ± 0.04 mm, 1.06 ± 0.04 mm and 1.46 ± 0.05 mm, respectively. Seven prey groups were used to compare the prey depth/width in the nets to the prey depth/width from the guts. This revealed that those from the 150 µm mesh net were the most similar to those in the guts of *M. asperum*, having no significant difference for four prey groups, namely amphipods, furcilia larvae, zoea larvae and teleost larvae (*p* value > 0.05, two-sided *t* test) (Table 7 & Figure 14). For *M. phengodes*, the prey depth/width from the 500 µm and 1 mm mesh net sizes were most similar to the prey depth/width in the guts, both net sizes having three non-significant difference, namely furcilia larvae, euphausiid adults and zoea larvae (*p* value > 0.05, two-sided *t* test) (Table 7 & Figure 14).
Figure 13: Prey depth/width (log) compared to standard lengths of *M. asperum* and *M. phengodes* sampled in the Perth Canyon.
3.7 Selectivity: *Myctophum asperum*

The Ivlev’s electively index indicated that prey positively selected by *M. asperum* were typically those found in relatively high proportions in the diet, namely poecilostomatoid
copepods, furcilia larvae, euphausiid adults and zoea larvae, but not calanoid copepods (Tables 5 & 8). *Myctophum asperum* showed consistent positive selection towards isopods, furcilia larvae, zoea larvae, larvaceans and teleost larvae based on the proportional abundances for all three net sizes (Table 8). Based on the proportional abundances in the 150 µm mesh net, the highest positive selectivity was shown towards amphipods and euphausiid adults, but both were negatively selected when using the proportional abundances from the 1 mm mesh net (Table 8). Despite their dominance in the diet, calanoids were negatively selected when the proportional abundances from the 150 and 500 µm mesh nets were used but positively selected using the 1 mm mesh net, where their proportion in the net catch was significantly less (Tables 4 & 8).

The Chesson’s selectivity index for *M. asperum* using the proportional abundances from the 150 µm, 500 µm and 1 mm mesh nets indicated a more neutrally selective feeding strategy, with the majority of the diet of *M. asperum* consisting of neutrally selected prey, (by MN,%) 40%, 83% and 85%, respectively (Table 9). Amphipods, furcilia larvae, zoea larvae, larvaceans and teleost larvae were identified as being neutrally selected based on the proportional abundances in all three nets (Table 8). Typically, those prey that were positively selected using the Ivlev’s analysis were neutrally selected using the Chesson’s. Calanoids were neutrally selected using the proportional abundances in the 500 µm and 1 mm mesh nets, but negatively selected based on their largest proportional abundance in the 150 µm mesh net (Table 8). *Myctophum asperum* showed significant positive selectivity towards euphausiid adults based on the proportional abundances in the 150 and 500 µm mesh nets. However, using the proportional abundances in the 1 mm mesh net indicated euphausiid adults as being neutrally selected and poecilostomatoid copepods as positively selected (Table 8).
Table 8: Ivlev’s electivity index, mean Chesson’s alpha ($\alpha_i$) from the zooplankton proportional abundances (150 µm, 500 µm and 1 mm mesh nets), and $p$ values from two-sided $t$ tests (* indicates significance) for the prey items consumed by *M. asperum*. NP indicates the prey group was not present in the net tows and was removed from the analyses; NA indicates that the prey group was not present in the guts and, therefore, Chesson’s $\alpha_i$ could not be compared to $\alpha_{neutral}$ with a $t$ test and prey avoidance was inferred. Green = Positive, Blue = Neutral and Red = Negative.

<table>
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<tr>
<th>Prey groups</th>
<th>150 µm net</th>
<th>500 µm net</th>
<th>1 mm net</th>
<th>Ivlev’s electivity index</th>
<th>150 µm net</th>
<th>500 µm net</th>
<th>1 mm net</th>
<th>Chesson’s selectivity index</th>
<th>150 µm net</th>
<th>500 µm net</th>
<th>1 mm net</th>
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<td></td>
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<td>$\alpha_{neutral} = 0.071$</td>
<td>$\alpha_{neutral} = 0.077$</td>
<td>$p$ value</td>
<td>$\alpha_{neutral} = 0.077$</td>
<td>$\alpha_{neutral} = 0.071$</td>
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<td>$p$ value</td>
<td>$\alpha_{neutral} = 0.077$</td>
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<td>NP</td>
<td>NP</td>
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<td>Postlarvae</td>
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<td>0.93</td>
<td>-0.18</td>
<td>0.09</td>
<td>0.09</td>
<td>0.7</td>
<td>0.02</td>
<td>&lt;0.01*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decapod adults</td>
<td>0.95</td>
<td>-0.01</td>
<td>-0.84</td>
<td>0.03</td>
<td>0.01*</td>
<td>0.01</td>
<td>&lt;0.01*</td>
<td>0.001</td>
<td>&lt;0.01*</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
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<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastropoda</td>
<td>0.77</td>
<td>-0.13</td>
<td>0.23</td>
<td>0.03</td>
<td>0.04*</td>
<td>0.02</td>
<td>0.06</td>
<td>0.02</td>
<td>0.03*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dolichopsis</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larvae</td>
<td>0.66</td>
<td>0.46</td>
<td>0.61</td>
<td>0.03</td>
<td>0.08</td>
<td>0.05</td>
<td>0.55</td>
<td>0.06</td>
<td>0.54</td>
<td></td>
<td></td>
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<tr>
<td>Teleostei larvae</td>
<td>0.99</td>
<td>0.61</td>
<td>0.43</td>
<td>0.06</td>
<td>0.67</td>
<td>0.05</td>
<td>0.43</td>
<td>0.05</td>
<td>0.3</td>
<td></td>
<td></td>
<td></td>
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<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 9: The total percentage of the diet made up by prey groups specific to each selectivity \((n = \text{number of prey groups with that selection})\) from the Ivlev’s and Chesson’s analyses using the proportional abundances from the 150 \(\mu\)m, 500 \(\mu\)m and 1 mm mesh nets for *M. asperum* and *M. phengodes* sampled in the Perth Canyon.

<table>
<thead>
<tr>
<th>Index</th>
<th>Selection</th>
<th>Myctophum asperum</th>
<th>Myctophum phengodes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>150 (\mu)m net</td>
<td>500 (\mu)m net</td>
</tr>
<tr>
<td>Ivlev’s electivity index</td>
<td>Positive</td>
<td>49.1% (n = 10)</td>
<td>58.7% (n = 10)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>48.2% (n = 11)</td>
<td>41% (n = 12)</td>
</tr>
<tr>
<td>Chesson’s selectivity index</td>
<td>Positive</td>
<td>15.2% (n = 1)</td>
<td>15.2% (n = 1)</td>
</tr>
<tr>
<td></td>
<td>Neutral</td>
<td>40% (n = 6)</td>
<td>82.8% (n = 10)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>42% (n = 14)</td>
<td>1.6% (n = 11)</td>
</tr>
</tbody>
</table>

3.8 Selectivity: *Myctophum phengodes*

As the majority of the diet of *M. phengodes* consisted of calanoid copepods and euphausiid adults, there did not appear to be a connection between the prey group’s proportion in the diet and their selectivity based on the Ivlev’s electivity index (Tables 6 & 10). Euphausiid adults had the most significant positive selectivity based on the proportional abundances in the 150 and 500 \(\mu\)m mesh nets (Table 10). Isopods were the only prey group to be positively selected using the proportional abundances from all three nets. *Myctophum phengodes* positively selected similar prey groups to *M. asperum*, namely isopods, furticia larvae, euphausiids adults and gastropods, although there were fewer positive selections than *M. asperum* (Tables 9 & 10).
Table 10: Ivlev’s electivity index, mean Chesson’s alpha ($\alpha_i$) from the zooplankton proportional abundances (150 µm, 500 µm and 1 mm mesh nets), and $p$ values from two-sided $t$ tests (* indicates significance) for the prey items consumed by *M. phengodes*. NP indicates the prey group was not present in the net tows and was removed from the analyses; NA indicates that the prey group was not present in the guts and, therefore, Chesson’s $\alpha_i$ could not be compared to $\alpha_{neutral}$ with a $t$ test and prey avoidance was inferred. Green = Positive, Blue = Neutral and Red = Negative.

<table>
<thead>
<tr>
<th>Prey groups</th>
<th>Ivlev’s electivity index</th>
<th>150 µm net</th>
<th>500 µm net</th>
<th>1 mm net</th>
<th>Chesson’s selectivity index</th>
<th>150 µm net</th>
<th>500 µm net</th>
<th>1 mm net</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$a_{neutral} = 0.125$</td>
<td>$a_{neutral} = 0.1$</td>
<td>$a_{neutral} = 0.1$</td>
<td></td>
<td>$a_{neutral} = 0.125$</td>
<td>$a_{neutral} = 0.1$</td>
<td>$a_{neutral} = 0.1$</td>
</tr>
<tr>
<td>Cnidaria</td>
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<td>-1</td>
<td>-1</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>Polychaete</td>
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<td>-1</td>
<td>NP</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>NP</td>
</tr>
<tr>
<td>Ostracoda</td>
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<td>0.36</td>
<td>0.73</td>
<td>NP</td>
<td>NA</td>
<td>0.11</td>
<td>0.91</td>
<td>0.17</td>
</tr>
<tr>
<td>Copepoda</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calanoidia</td>
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<td>0.08</td>
<td>0.46</td>
<td>0.003</td>
<td>&lt;0.01*</td>
<td>0.06</td>
<td>0.08</td>
<td>0.17</td>
</tr>
<tr>
<td>Harpacticoida</td>
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<td>NP</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>NP</td>
</tr>
<tr>
<td>Poecilostomatoida</td>
<td>-0.61</td>
<td>0.48</td>
<td>0.79</td>
<td>0.001</td>
<td>&lt;0.01*</td>
<td>0.12</td>
<td>0.77</td>
<td>0.28</td>
</tr>
<tr>
<td>Nauplii larvae</td>
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<td>NP</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>NP</td>
</tr>
<tr>
<td>Isopoda</td>
<td>0.92</td>
<td>0.24</td>
<td>0.36</td>
<td>0.05</td>
<td>0.24</td>
<td>0.06</td>
<td>0.52</td>
<td>0.05</td>
</tr>
<tr>
<td>Amphipoda</td>
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<td>-0.49</td>
<td>-0.74</td>
<td>0.01</td>
<td>&lt;0.01*</td>
<td>0.003</td>
<td>&lt;0.01*</td>
<td>0.01</td>
</tr>
<tr>
<td>Mysidae</td>
<td>-1</td>
<td>-1</td>
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<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>Calanoidia</td>
<td>-1</td>
<td>-1</td>
<td>NP</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>NP</td>
</tr>
<tr>
<td>Furcilia larvae</td>
<td>0.87</td>
<td>-0.07</td>
<td>-0.12</td>
<td>0.04</td>
<td>0.05*</td>
<td>0.03</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Euphausiid adults</td>
<td>0.99</td>
<td>0.89</td>
<td>-0.09</td>
<td>0.83</td>
<td>&lt;0.01*</td>
<td>0.48</td>
<td>0.03*</td>
<td>0.22</td>
</tr>
<tr>
<td>Decapoda</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protozoa larvae</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>NP</td>
</tr>
<tr>
<td>Zoa larvae</td>
<td>0.87</td>
<td>-0.35</td>
<td>-0.44</td>
<td>0.03</td>
<td>0.01*</td>
<td>0.02</td>
<td>&lt;0.01*</td>
<td>0.01</td>
</tr>
<tr>
<td>Postlarvae</td>
<td>NP</td>
<td>0.85</td>
<td>-0.53</td>
<td>0.03</td>
<td>0.01*</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Decapod adults</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>Chaetognatha</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>Gastropoda</td>
<td>0.74</td>
<td>-0.19</td>
<td>-0.17</td>
<td>0.03</td>
<td>&lt;0.01*</td>
<td>0.04</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>Doliolida</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
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<td>Larvae</td>
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<tr>
<td>Teleostei larvae</td>
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<td>NA</td>
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</tr>
<tr>
<td>Unknown</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>0</td>
</tr>
</tbody>
</table>
The selectivity of *M. phengodes* determined by the Chesson’s selectivity index using the proportional abundances in the 150 μm mesh net indicated a less neutral feeding strategy compared to the proportional abundances from the other nets, only neutrally selecting isopods which made up (by MNi%) only 0.35% of its diet (Tables 9 & 10). The analyses based on the proportional abundances in the larger net sizes (500 μm and 1 mm mesh nets) showed a far more neutrally selective diet with both nets having six neutral selections, accounting for (by MNi%) 69% and 94% of the diet of *M. phengodes*, respectively (Tables 9 & 10). The prey groups positively selected from the Ivlev’s analyses using the proportional abundances in these larger mesh nets were typically neutrally selected using the Chesson’s index. Positive selection was again only apparent for euphausiid adults based on the proportional abundances from the 150 and 500 μm mesh nets, however, no prey was positively selected when the 1 mm mesh net proportional abundances were used.

4.0 Discussion

This study, which aimed to examine the diet of myctophids occurring in the surface waters of the Perth Canyon at night, represents the first study of its kind in the Perth Canyon and the Southeast Indian Ocean. As such this study represents a baseline of myctophid diet and prey selectivity in the Southeast Indian Ocean. The dietary compositions of different prey groups in the guts of myctophids have been quantified and the morphological relationships of both fish and prey have been investigated. In addition, to investigate prey selectivity, an assessment was made of the availability of zooplankton in the Perth Canyon. Dietary studies are fundamental to understanding the formation of trophic interactions and interdependence of species in the food web. Myctophids play a key role in the ocean food web creating trophic links between
zooplankton and piscivorous predators and significantly contribute to the biological pump of nutrients from the upper productive layers to the deeper layers (Gjøsaeter & Kawaguchi 1980, Shreeve et al. 2009).

4.1 Zooplankton and myctophids in the Perth Canyon

Zooplankton in the Perth Canyon was overwhelmingly dominated by calanoid copepods, which contributed 20 – 80% of the zooplankton abundance. Calanoid copepods are well known as the most dominant marine taxon in the world (Longhurst 1985, Banse 1995, Blaxter et al. 1998, Sassa & Kawaguchi 2004, Strzelecki et al. 2007). Zooplankton assemblages from the 150 µm mesh nets towed on both voyages in the Perth Canyon were similar despite sampling taking place in different seasons. Both sets of 150 µm mesh net samples had calanoid, cyclopoid and poecilostomatoid copepods and chaetognaths in the top five most abundant zooplankton taxa, with little variation in the zooplankton compositions. There was a lack of larger zooplankton as the small mesh size can become clogged and create a bow wave in front of the net triggering an avoidance response, while the small mouth size (area = 908 cm²) of the net makes it easier to avoid capture (Fleminger & Clutter 1965, Sameoto et al. 2000).

The zooplankton in the 500 µm mesh net tows from voyage 2 had different proportional abundances to that of the 150 µm mesh net. Larger zooplankton such as furcilia larvae, euphausiid adults, zoea larvae, decapod adults and teleost larvae were in greater abundance compared to the small net. This is most likely a result of the larger mesh reducing the presence of a bow wave in front of the net and increasing the rate that smaller zooplankton pass through the mesh, while the larger mouth (area = 1963 cm²) decreased the chance of escape (Sameoto et al. 2000). This, in turn, meant that the
proportional abundance of smaller zooplankton such as calanoid, cyclopoid and poecilostomatoid copepods decreased (Sameoto et al. 2000).

As expected, the zooplankton proportional abundances in the 1 mm mesh net tows were biased towards larger zooplankton due to the larger net mouth (area = 1 m²) and mesh size. Euphausiid adults were 20% more abundant compared to their proportional abundance in the 500 µm mesh net, amphipods were more than double and decapod adults more than eight times their proportional abundance in the 500 µm mesh net. As a result, the proportional abundance of calanoids, which in the smaller nets far outnumbered all other zooplankton, were less than half their proportion found in the 500 µm mesh net. It is clear from the smaller mesh sizes and literature that calanoid copepods occur in far greater proportional abundance than recorded by the 1 mm mesh net (Longhurst 1985, Banse 1995, Blaxter et al. 1998, Sassa & Kawaguchi 2004, Strzelecki et al. 2007). It is hypothesised that the abundances in the 1 mm mesh net of the larger zooplankton such as amphipods, furchilia larvae, euphausiid adults, zoea larvae, postlarvae and decapod adults were, in fact, representative of their true abundance in the Perth Canyon. However, due to the loss of smaller organisms, the proportional abundance of small zooplankton in the large net may be underrepresented (Wiebe et al. 1982, Sameoto et al. 2000).

Although zooplankton studies in the Perth Canyon are limited, the euphausiid assemblage have been assessed and indicated that Euphausia recurva was the dominant species (Sutton et al. 2015). The majority of krill from the zooplankton samples from this current study and the few intact specimens found in the diet of both Myctophum species were also identified as Euphausia recurva. Zooplankton studies from a pair of
warm and cold core oceanic eddies off the south-western Australian coast indicated a dominance of calanoid copepods, cyclopoid copepods, chaetognaths and furcilia larvae from 100 and 335 µm mesh size bongo nets (Strzelecki et al. 2007), similar to what was found in this study (150 and 500 µm mesh size nets). Zooplankton assemblages at the Integrated Marine Observing System Rottnest Island reference station (50 m depth), indicated a clear dominance of crustaceans, particularly calanoid and cyclopoid copepods from the 100 µm mesh net samples, similar to the abundances reported in the 150 µm mesh net of this study (McCosker 2016, Eriksen et al. 2019).


In this study, three myctophid species from two genera were captured in the Perth Canyon, namely Diaphus sp., M. asperum and M. phengodes, and all have been previously sampled off the coast of Western Australia (Muhling et al. 2007, Muhling et al. 2008, Holliday et al. 2011, Holliday et al. 2012, Paxton & Williams in press). Diaphus sp. was only found in the larval stage with its morphology indicative of the deep morphotype (Moser et al. 1984). Previous studies conducted near the Perth Canyon have sampled larvae of this morphotype in significant numbers around the same
time of year as voyage 1 from this study (Holliday et al. 2011, Holliday et al. 2012). Most Diaphus species spawn during summer and autumn, with timing and duration varying depending on species and latitude (Olivar 1987, Moku et al. 2003, Sassa & Kawaguchi 2004, Muhling et al. 2008). The presence of larval Diaphus sp. in the Perth Canyon could indicate that local spawning was taking place before voyage 1 in April (Holliday et al. 2011, Holliday et al. 2012). The absence of adult or larval Diaphus sp. in samples from voyage 2 could be due to the lack of spawning in September and that the community of larval fishes sampled during voyage 1 had undergone transformation into adults and may no longer have resided in the area.

4.2 Diet

None of the larval Diaphus sp. sampled at night during voyage 1 were found to contain any prey except for the second largest individual (standard length = 9.5 mm), which consumed two copepod nauplii larvae. The lack of prey in the guts suggests that no feeding had occurred amongst the Diaphus sp. larvae during the night or, as sampling took place in the early hours of the morning, any prey that had been eaten earlier may have already been digested (Dalpadado & Gjøsæter 1988). Diaphus garmani, Diogenichthys laternatus, Myctophum asperum and Triphoturus mexicanus are examples of myctophids in which the larvae have been found to specifically feed during the day (Sassa & Kawaguchi 2004, Rodríguez-Graña et al. 2005). Nighttime feeding typically occurs after transformation from larvae into juveniles, indicating an ontogenetic shift in their feeding ecology and behaviour (Ahlstrom 1971, Watanabe et al. 2002, Sabatés et al. 2003, Yatsu et al. 2005, Bozzano et al. 2007). This might explain why one of the largest Diaphus sp. larvae, which was close to the transformation size of ~ 11 mm, was found to have eaten at night while the others had not (Moser et al. 2001).
Literature on the use of sensory systems by larval myctophids, particularly their visual systems and adaptations to feeding in the dark, are sparse. However, it appears that the majority of small fish larvae from most teleost families are strong visual predators at the onset of feeding (Pankhurst & Hilder 1998, Rodriguez & Gisbert 2002). In the early life stages, the retinas of larvae are characterised by having only cone photoreceptors, which limit their visual functions to photopic conditions of surface waters (Bagarinao & Hunter 1983, Blaxter 1986, Bozzano et al. 2007). The ability for larval fishes to function in scotopic conditions, or increasing depths, improves as the rod photoreceptors develop as these are responsible for seeing in the dark (Bozzano et al. 2007). Therefore, the lack of rod photoreceptors in their eyes only allows for daylight feeding in myctophid larvae (Conley & Hopkins 2004, Morote et al. 2011, de Busserolles et al. 2014). Future sampling of Diaphus sp. on the surface and at depth could be carried out over a 24 hour period as this would confirm whether feeding during the day is occurring and reveal the diet of this species in all ontogenetic stages.

Of the *M. asperum* and *M. phengodes* captured in voyage 2, all fish except one *M. asperum* specimen had consumed prey. Frequency of occurrence revealed that calanoid copepods were found in 82% and 100% of *M. asperum* and *M. phengodes*, respectively, a good indication of their importance in the diet. Mean percentage by number revealed that calanoid copepods constituted 39% and 62% of prey in their diets, respectively. This dominance in the diet is likely related to the large abundance of calanoids in the environment and is consistent with the original hypothesis that myctophids would consume prey based on their proportional abundance in the environment (Sameoto 1988, Pakhomov et al. 1996).
Equally as important in the diet were euphausiid adults, found in 48% of *M. asperum* and 100% of *M. phengodes*. *Myctophum asperum* specimens were the smaller of the two *Myctophum* species (max SL = 76 mm) (Paxton & Williams in press) and typically each only had a single, highly digested, euphausiid adult in its gut, and overall accounted for 15% of the diet of *M. asperum*. Individuals of *M. phengodes*, being larger (max SL = 94 mm) (Paxton & Williams in press), consumed up to eight euphausiid adults in this study accounting for 25% of their overall diet and were often tightly packed and squashed into the gut, demonstrating the voracious nature of feeding in these fish (Moku et al. 2000, Clarke et al. 2018). *Myctophum asperum* typically preyed on medium-sized zooplankton such as furcilia and zoea larvae, which together contributed nearly 20% of their diet. These findings are consistent with the hypothesis that the diet would be influenced by prey size, species of fish or ontogeny.

Calanoid copepods and euphausiid adults combined constitute 54% and 87% of the diets of *M. asperum* and *M. phengodes*, respectively. Such dietary compositions are a common occurrence in myctophids with calanoid copepods and euphausiids typically the most prevalent in the diet, in some instances comprising 20 – 80% calanoid copepods and 10 – 35% euphausiids (Bernal et al. 2013, Bernal et al. 2015). This kind of diet is not only restricted to myctophids but many species of mesopelagic fish such as bristlemouths, hatchetfishes and lightfishes (McClain - Counts et al. 2017). The dietary composition of *M. asperum* in Japanese waters assessed by Watanabe et al. (2002) indicated that the majority of the years sampled showed larvaceans as being the main food source comprising nearly 90% of the diet. However, two of the sampling years, showed similar dietary compositions to this study, with calanoid copepods making up around 50% of the fishes diet. While predation of larvaceans by *M. asperum* in the Perth
Canyon did occur, they only made up 3% of the diet. However, due to the soft morphology of larvaceans, they can often be missed in microscope work and are easily digested so it is possible that they were slightly more significant to the diet. A study in the Western North Pacific found that *M. asperum* fed predominantly on ostracods and polychaetes, with copepods and euphausiids only making up a small portion of their diets (Sassa & Kawaguchi 2004). Based on the findings from this study and compared to others, it would appear that differences in the diet of the same species do occur depending on the region, season and zooplankton abundances, highlighting the importance of dietary studies around the world to build on our understanding of the oceanic food web.

Both *Myctophum* species shared four out of the top five most proportionally abundant prey groups in their diet, namely calanoid copepods, euphausiid adults (proportionally most abundant in *M. phengodes*), furcilia larvae and zoea larvae (proportionally most abundant in *M. asperum*). There was a clear difference in the proportional make up of diets; *M. phengodes* had only two prey groups that constituted more than 5% of its diet, while *M. asperum* had six. This could indicate that *M. asperum* expanded its diet by consuming larger proportions of other prey, thus reducing niche overlap, though the differences in diet could be due to the low sample size of *M. phengodes* examined (n = 7). As the diet was assessed using the number of prey items consumed and not the weight of prey items, it should be noted that while calanoids were the most proportionally abundant in the diet they are significantly smaller than other prey and would, therefore, contribute less nutrients per individual than larger prey. However, it is important in that the high number of calanoids in the diet would mean many feeding
attempts using energy each time indicating that the benefits may outweigh the costs when predating on calanoid copepods.

It has been proposed that mouth gape is one of main morphological constraints in maximum prey size (González-Quiróas & Anadóan 2001) and has frequently been demonstrated to influence the diet of myctophids (Sabatés & Saiz 2000, Sabatés et al. 2003, Salas-Berrios et al. 2013, Contreras et al. 2018). The mouth gape of Diaphus sp., M. asperum and M. phengodes all showed significant positive relationships to standard length, indicating that the potential to consume larger prey increases as these fish grow.

Linear regression on the morphology of both Myctophum species against the diet revealed no significant change in the number of prey consumed as the standard length and mouth gape increased. The findings specific to M. asperum are similar to those of Sassa and Kawaguchi (2004) who also found no significant relationship between fish size and the number of prey for this species. As pointed out by Hunter (1981), prey size dominates prey selectivity of fishes. In this study, a positive relationship was established between prey size (body depth/width) and the standard length and mouth gape of both Myctophum species. This specific relationship has also been recognised by Sassa and Kawaguchi (2004) suggesting that the feeding strategy of M. asperum involved taking larger prey rather than a greater number of prey. This is a feeding strategy observed in many myctophid species, and as such, size-related changes in diet may indirectly aid in the distribution of food resources (Schafer et al. 2002, Shreeve et al. 2009, Bernal et al. 2015, Contreras et al. 2018). While there were too few specimens in this study to perform an accurate analysis based on size class, higher frequencies of larger prey were observed in larger specimens (Clarke et al. 2018). This suggests a
transition in prey size based on ontogeny, often referred to as feeding up the food chain and is consistent with the original hypothesis of this study (Wickstead 1962, Sameoto 1988, Cherel et al. 2010, Saunders et al. 2018).

Consuming quality rather than quantity would seem to be the most energy efficient method of feeding. However, smaller prey items, such as calanoid and poecilostomatoid copepods, were consistently consumed by both Myctophum species despite their size and may suggest a level of opportunistic feeding (Sameoto 1988, Bernal et al. 2013). Piscivory was also demonstrated by the smaller species M. asperum but not the larger species M. phengodes and this typically opportunistic behaviour has been occasionally observed in other myctophids (Hopkins et al. 1996, Gaskett et al. 2001, Bernal et al. 2013). This is an interesting comparison as typically it is the larger species in the environment that prey on other fish (Bernal et al. 2015). In some instances in this study, the consumed fish were so large that they were found folded in half taking up the majority of space inside the gut, further demonstrating the voracious feeding nature of myctophids and another example of consuming quality over quantity.

Myctophum asperum had a higher prey diversity, consuming five prey groups more than M. phengodes. These findings are similar to those of a prey diversity analysis by Bernal et al. (2013), where the lowest prey diversity was observed in the largest myctophids. This suggests that the diet may become more specific and less diverse with size. The difference in prey diversity between the two species may be related to the small sample size of M. phengodes. However, disparities in diet are known to occur as a result of inter-specific variances in morphology, migratory behaviour and ability to capture prey (Hopkins & Baird 1985, Pusch et al. 2004, Sassa & Kawaguchi 2004, Bernal et al.
2013, Bernal et al. 2015). *Myctophum asperum* may have expanded its diet to include other prey groups in an effort to potentially minimise competition, thereby reducing the exhaustion of food resources (Schoener 1974, Hopkins & Gartner 1992, Shreeve et al. 2009).

### 4.3 Prey selectivity in *Myctophum asperum* and *Myctophum phengodes*

This study used three different net sizes to determine zooplankton abundances, this has sometimes indicated varying levels of selectivity for the same prey group in both the Ivlev’s and Chesson’s analyses (Van Noord 2013). The comparison between the prey depth/width in the three net mesh sizes and those in the guts of *M. asperum* and *M. phengodes* potentially indicates which net size is best suited for studying the diet of these fish (Barkley 1972, Sameoto et al. 2000). The prey depth/width from the 150 μm mesh net were most similar to those in guts of *M. asperum*, whereas the prey depth/width in the guts of the larger species *M. phengodes* was most similar to those in the 500 μm and 1 mm mesh nets. Based on literature and the results presented in the study, a medium mesh size, such as the 500 μm mesh net may be the most suitable net size for assessing selectivity. This net reduces the number of smaller zooplankton that pass through the mesh and the somewhat large mouth (area = 1963 cm²) may reduce capture avoidance of larger zooplankton (Wiebe et al. 1982, Evans & Sell 1985, Sameoto et al. 2000).

Based on the Ivlev’s electivity analysis using the zooplankton proportional abundances from the three mesh sizes, both *Myctophum* species positively selected many of the same prey groups. However, as in other studies (Sassa & Kawaguchi 2004), the diet of *M. asperum* displayed euryphagous tendencies, positively selecting a wider variety of prey than *M. phengodes*. This difference could be attributed to the small sample size of
M. phengodes available in this study. Myctophum asperum showed strong positive selection towards isopods, furcilia larvae, zoea larvae, larvaceans and teleost larvae, whereas M. phengodes only showed this level of positive selection towards isopods. Positively selected prey from both Myctophum species typically consisted of larger zooplankton, although species in high abundances in the environment, such as calanoid and poecilostomatoid copepods, were also targeted. This is common in adult myctophids as they typically select larger items to supply their higher energy requirements (Bernal et al. 2013), but have also been reported to prey on, but not always positively select, the most abundant zooplankton due to their ease of capture (Sameoto 1988, Pakhomov et al. 1996). The 1 mm mesh net was biased towards the increased capture of larger zooplankton and an increased escape rate of smaller zooplankton. This shift in proportional abundances skewed the electivity analysis, most notably for euphausiid adults, which unlike the 150 and 500 µm mesh nets, were negatively selected based on the proportional abundances in this 1 mm mesh net. Conversely, the decrease in the proportion of calanoid copepods in the 1 mm mesh net resulted in their positive selectivity by M. asperum.

The results from the Chesson’s selectivity analysis using the zooplankton samples from the 150 and 500 µm mesh nets indicated that euphausiid adults were the only prey group to be positively selected by both M. asperum and M. phengodes. The high lipid, protein, fatty acid and omega-3 content of euphausiids are of great nutritional value to consumers, used for growth and reproduction (Phleger et al. 1998, Nicol et al. 2004, Ju et al. 2009, Sutton et al. 2015). All these nutrients packaged into one relatively large prey item makes euphausiids highly desirable and is one possible reason for their positive selection. This selectivity of euphausiid adults differs somewhat from the
original hypothesis, as they were, for the most part, not particularly abundant in the net samples. However, as euphausiid adults are large relative to other zooplankton, the relationship between prey size and selectivity holds true. Despite the dominance of calanoid copepods in the diet, both species negatively selected them based on the proportional abundances in the 150 µm mesh net, but neutrally selected them based on the proportional abundances in the 500 µm and 1 mm mesh nets. This indicates that a large abundance in the environment does not guarantee positive selectivity. The high number of neutrally selected prey groups, particularly in *M. asperum*, is hypothesised to be characteristic of opportunistic feeding by predating on what is available (Pakhomov et al. 1996, Bernal et al. 2015). This could indicate that *M. asperum* has adopted a more opportunistic or generalised feeding strategy in the Perth Canyon compared to other regions (Watanabe et al. 2002, Sassa & Kawaguchi 2004), with neutrally selected prey constituting (by MNi,%) 40–85% of its diet.

Similar to the Ivlev’s analysis, euphausiid adults are not positively selected based on their abundance in the 1 mm mesh net, hypothesised to be a result of their larger proportional abundance in this net. Instead, poecilostomatoid copepods are indicated as the only prey positively selected by *M. asperum* and no positive selections by *M. phengodes*. While poecilostomatoids were somewhat prevalent in the diet, they were also common in the 150 and 500 µm mesh net samples, which might explain why they were only neutrally selected based on their larger proportional abundance in those net sizes. The presence of poecilostomatoids in the 1 mm mesh net is only a fraction of their environmental abundance which may have created a bias in the analysis resulting in their positive selectivity (Van Noord et al. 2013).
The diets of adult myctophids in the Mediterranean Sea showed similar selectivity to this study using the Chesson’s selectivity index and incidentally the zooplankton abundances in the Mediterranean Sea are similar to those in the Perth Canyon (de Puelles et al. 2007, Bernal et al. 2015). Their findings indicated that the two most prevalent prey groups in the diet (calanoids and euphausiids) were consumed in similar quantities to that of this study, with euphausiids positively selected by most species and calanoids being negatively, and sometimes, neutrally selected (Bernal et al. 2013, Bernal et al. 2015). As prey abundances and fish diet vary based on region, it is useful to have a selectivity index, such as the Chesson’s index, for which values are independent of prey abundance, as this has the potential to indicate species-specific preferences in other regions too (Kremers 1984). Compared to the Ivlev’s analysis, which assigned a high number of positive selections, the Chesson’s index appears stricter in its allocation of positive selection and indicates a more neutral feeding strategy. A larger samples size should be used to determine the prey selectivity for interspecific size classes to better understand the ontogenetic relationship to diet and prey selectivity.

4.4 Other methods of dietary analysis

Determining the diet by prey weight allows access to metrics such as mean proportion by weight and index of relative importance (Chipps & Garvey 2007), although, weight is not necessary when determining prey selectivity. An advantage of using prey weight is that it mitigates the numerical bias of small prey such as copepods, which are often consumed in large quantities but contribute only a small amount of nutrients compared too much larger prey items.
Measuring the isotopic niche of organisms is a useful alternative to conventional means of dietary analysis and the basic concept is that an organisms chemical composition will directly reflect what it has consumed (Newsome et al. 2007, Cherel et al. 2010). Stable isotope analysis uses a mass spectrometer to extract isotopes of carbon and nitrogen from the tissue of the organisms. Organisms take on nitrogen from their food and stable nitrogen isotope measurements thus serve as indicators of a consumers trophic position (Hobson et al. 1994). This analysis allows for the quantification of trophic levels in order to compare taxonomically related and unrelated species in an environment (Hobson et al. 1994, Cherel et al. 2010). Stable carbon signatures pass along the food chain and are predominantly used to indicate the foraging habits of predators and thus the original source of carbon (Cherel et al. 2010). Most recently, the trophic position of myctophids based on stable isotopes was assessed and concluded that myctophids in the equatorial and tropical Atlantic Ocean typically occur between trophic levels two (fully herbivore) and four (secondary carnivore) (Olivar et al. 2018).

While isotopes are a good indicator of the trophic level of a predator, it cannot typically determine the species composition in the diet. Fatty acids on the other hand are extracted from the predator’s tissues and are used to accurately determine what the predator has consumed (Iverson et al. 2002). As fatty acids pass from prey to predator up the food chain they provide indications of key food sources in the diet going back several months (Iverson et al. 2002, Iverson 2009). They are primarily used qualitatively to infer trophic levels and spatial and temporal variations in diets, but can be reliably used to trace the origins of an organism’s diet as it is capable of accurately identifying individual species and provides quantitative estimates of a predator’s diet (Budge et al. 2002, Iverson et al. 2004, Iverson 2009). For example, the fatty acid
signatures of myctophids have been used to show their importance in the diet of king penguins and their chicks during fattening periods, indicating that the majority of nutrients came from myctophids (Raclot et al. 1998).

Genetic identification of gut contents is particularly useful as it is not limited to undigested or hard remains (Deagle et al. 2005). Genetics can be used to identify the gut contents to species level and, in some cases, even ontogenetic stage regardless of how many prey items are present (Symondson 2002). Polymerase chain reaction methods of identifying diet have emerged as a major tool in diet studies and due to its sensitivity is able to identify even trace quantities of DNA in a predator's diet (Rosel & Kocher 2002, O’rorke et al. 2012). DNA sequencing can also detect the presence of digested gelatinous prey which are not typically detected in dietary studies due to their rapid digestion or were typically grouped into broad taxonomic levels (Clarke et al. 2018).

The first use of DNA sequencing to determine the diet and trophic position of myctophids was carried out in the Southern Ocean in 2016 (Clarke et al. 2018). The study found that the diet of several myctophid species were dominated by amphipods, euphausiids and copepods, similar to the diets of both *Myctophum* species from this study (Clarke et al. 2018). Clarke et al. (2018) also concluded that DNA based diet analysis highlighted the importance of gelatinous prey items in the diet of myctophids, an aspect missing from this current study possibly due to their rapid digestion. DNA sequencing could substantially improve the knowledge of mesopelagic fish diets and determine the role of mesopelagic fish and their trophic position to aid in the development of ecosystem models and food webs (O’rorke et al. 2012, Clarke et al. 2018).
4.5 Importance of myctophids

Myctophids are currently of little economic importance, used typically as fish feed or crop fertiliser. However, due to the overfishing of other species in many parts of the world and the significant proportion of marine fish biomass that myctophids represent, they are being increasingly viewed as a potentially untapped food resource (Gjøsaeter & Kawaguchi 1980, FAO-Fisheries 1997, Priede 2017). Myctophids have a short lifespan and low fecundity making them susceptible to overfishing (Gjøsaeter & Kawaguchi 1980, Karuppasamy et al. 2008), in particular *M. asperum* is suggested to have a lifespan of only 1 year (Hayashi et al. 2001). While myctophids are of little direct importance to the diet of humans, they are important in the diet of commercially significant species consumed by humans around the world as well as ecologically important species. The role of myctophids in the oceanic food web is not simply linear, and is better described as a complex network of interactions at different trophic levels. Myctophids contribute to the biological pump of nutrients and facilitate the link between the deep ocean and the surface waters. Their large biomass means they are vast contributors to the diet of many predators and influence the dynamics of regional food webs (Bernal et al. 2015). Variations in predator populations often have a cascading effect across the food web, with implications surrounding the community structure and ecosystem function (Worm & Myers 2003). In this way, they are key components in oceanic food webs, influencing many of the world’s fisheries (Pauly & Christensen 1995).

5.0 Conclusion

This study involved the investigation of three species of myctophids, in conjunction with the assessment of prey abundance in the Perth Canyon. Examination of the larval
Diaphus sp. indicated a significant lack of feeding at night, hypothesised to be a result of the absence of rod photoreceptors in their underdeveloped eyes restricting their feeding to photic conditions. The large abundance of calanoid copepods in the environment is reflected by their dominance in the diet of both M. asperum and M. phengodes making up the majority of each species diet and occurring in almost every fish examined. It is evident that calanoids are clearly an important prey source, however, their level of selectivity is less clear. It is likely that calanoids are neutrally selected by both species as their large abundance in the environment makes them easy to capture and would not need to be actively sought out. It is likely that M. asperum and M. phengodes are highly selective towards euphausiids adults as they are one of the larger prey groups present in the canyon. Euphausiids adults are hypothesised to contribute less than 1% of the overall zooplankton abundance in the Perth Canyon (based on the 150 and 500 µm mesh nets) but were the only species to be positively selected by both species and contributed 15% and 25% to the diets of M. asperum and M. phengodes, respectively. This is backed up by the analysis of fish and prey morphology, which revealed a quality over quantity feeding strategy, with both Myctophum species consuming larger prey over ontogenetic development. While both species are thought to be highly selective towards euphausiid adults it is hypothesised that M. asperum demonstrated a more generalised feeding strategy by expanding its diet to include a greater variety of prey groups and was the only species to exhibited piscivory, a typically opportunistic behaviour in mesopelagic fish.

Research in the Perth Canyon is important with the canyon recently declared a Commonwealth Marine Park and the existence of a gap in the knowledge of mesopelagic food webs in the canyon. Similar to other oceanic food webs around the
world myctophids are likely to play a key role in the distribution of nutrients in the Perth Canyon and the whole of the Southeast Indian Ocean. Further research is required to cement our understanding of myctophids in the Perth Canyon food web. Implementation of isotope, fatty acid and DNA analysis will greatly enhance not only our knowledge of their diet in the Perth Canyon but also the ability to compare this information to other studies around the world in an effort to gauge their global significance. Additionally, this project will form a baseline for future research in the Southeast Indian Ocean, leading into the research efforts during the second International Indian Ocean Expedition (Hood et al. 2015) specifically in May and June of 2019 when the 110'E line will be repeated.
6.0 References

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7.0 Appendix

Appendix 1

Figure 1: Hypothetical myctophid showing morphology and arrangement of various photophores. Sourced from Paxton (1972).

Photophore terminology definitions

AOₐ – Anal Organs (anterior)
AOₚ – Anal Organs (posterior)
Br – Branchiostegals
Bu – Buccal
CP – Cheek Photophores
Dn – Dorsal nasal organ
INGL – Infra- and infracaudal organ(s)
LT – Luminous Tissue
Op – Operculars
PLO – Pectoral Lateral Organ
PO – Pectoral Organs
Pol – Posterior lateral organ
Prc – Precaudals
PVO – Pectoral Ventral Organs
SAO – Supra Anal Organ
So – Suborbital organ
SUGL – Supracaudal organ(s)
VLO – Ventral Lateral Organ
Vn – Ventral nasal organ
VO – Ventral Organ