Morphology, Life History and Colour Variability in the Endemic New Zealand isopod, *Isocladus armatus*

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Abstract

Research into colour polymorphism has been central to understanding the evolutionary mechanisms that maintain variation within populations. This is because colour polymorphism is quantifiable and often associated with differential selective pressures. Work on colour polymorphic organisms requires detailed knowledge of both the natural history of the focal organism and of the specific mechanisms that generate colour variation.

Isocladus armatus is a Sphaeromatid isopod crustacean endemic to New Zealand. This marine isopod is found in semi-sheltered shores across the country and is well known for its astonishing diversity of colour morphs. Although previous research into this species has been limited, *I. armatus* appears to have a considerably higher degree of colour polymorphism than most other isopod species.

In this study, I document the developmental life history, sexual dimorphism and reproductive behaviour of this species and I explore the potential genetic factors influencing the expression and maintenance of colour polymorphism. The research described here required the development of methods for breeding and maintaining isopods under laboratory conditions, making this the first comprehensive investigation of reproductive biology in *I. armatus.*

Because there have been no prior published studies on the *I. armatus* lifecycle or how and when females are sexually receptive, uncovering the reproductive biology of this species was a challenge. However, through trial and error and detailed observation of male and female morphology and development, this research describes the first successful multi-generation

reproduction of this species in captivity. In addition, through these generations, I demonstrate a clear genetic basis to colour variability in this species. The work in this thesis will inform future studies on this species, isopod biology in general and provides necessary insight into the wider question of how colour variation is maintained in natural populations.

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Acknowledgements

Firstly, a huge, huge thank you goes out to James (Jim) Dale for checking on me daily while New Zealand was in and out of COVID lockdowns to make sure I hadn't gone insane, for putting up with me knocking on your office door, my constant emails and the extensive zoom meetings at all times of the day and night. Thanks for always telling me it is going to be ok, even when I felt like the end would never be in sight.

And a massive thank you to Sarah Wells, for getting me started with the isopods for my Research in BioScience undergraduate project, and for showing me how to maintain them and breed them in captivity.

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Table of Contents

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Chapter 1

General Introduction

Maintenance of genetic variability within populations

A fundamental challenge in evolutionary biology is to resolve the mechanisms that maintain biological diversity in natural populations (Lewontin, 1974; Hedrick, 1986; Pamilo, 1988; Hartl, 1997; Kotiaho, 200; Futuyma, 2005). How genetic diversity is maintained is somewhat paradoxical because when genetic variants of a phenotypic trait co-exist, the general expectation is that Darwinian natural selection should simplify the variability down to a single most optimal state (Hartl et al, 1997; Futuyma, 2005).

However, there are numerous ways that genetic variability can persist, and these can be broadly categorised into two general types of evolutionary mechanisms: 1) Variation is non-adaptive and is comprised of a transient mix of forms that is attributable to regional stabilising selection. This causes a greater number of individuals in the population with non-extreme phenotypes and fewer numbers towards the extremes, but is counter-acted by gene flow and/or genetic drift which broadens the variation spectrum back out again (Lewontin, 1974; Endler, 1977; King & Lawson 1995; Bond, 2007; Gray & McKinnon, 2007; Merilaita, 2001). Or 2) Variation is an adaptive, stable equilibrium maintained by negative frequency-dependent selection where, when one colour morph becomes less common in the population than another, selection starts to favour the less frequent genes in the gene pool to help build the population of the low trait back up (Lank et al, 1995; Dale et al, 2001; Olendorf et al, 2006; Bond, 2007; Gray

& McKinnon, 2007;). In the most extreme cases of colour variability, variation can be so high that there are essentially unlimited numbers of forms.

Research into the intersection of genetic and phenotypic variation across different populations have provided much insight into the maintenance of biological diversity. For example, Haavie, et al (2000) investigated the genetic differentiation between three populations of the pied flycatcher (*Ficedula hypoleuca*) using microsatellite loci and mitochondrial DNA sequences compared to the colour variation of male plumage colour. They found that the analysis did not distinguish the Czech and Norwegian populations, while the Spanish population was clearly different to the first two. Strikingly the Spanish and Norwegian populations had a similar plumage variation whereas the Czech population had a more restrictive range of coloration and on average were lighter in colour (Haavie et al, 2000). Many other similar studies have been conducted such as on human skin colour diversity (Relethford, 2000) and guppy (*Poecilia reticulata*) male size and colour variation (Tripathi et al, 2009). However, our current understanding of the processes that maintain such massively polymorphic diversity remains incomplete.

To achieve a complete understanding of the maintenance of biological diversity, it is critical that patterns of gene flow and negative frequency-dependent selection are well understood as mechanisms maintaining biological diversity (Slatkin, 1987). However, few studies have combined a detailed investigation of population connectivity with an in-depth analysis of the naturally occurring selective variation associated with a massive amount of phenotypic polymorphism (Hoekstra et al, 2004; Dasmahapatra et al, 2012; Munoz et al, 2013). Analyses such as the above are especially needed in the marine environment, where there is a

lack of geophysics barriers to gene flow, resulting in a spatial scale of dispersal and population connectivity that can be considerably larger than terrestrial environments (Palumbi, 1994; Cowen et al, 2009).

Colour variability can be seen in many species of bird, snail, and other organisms (Reid, 1987; Mckinnon & Pierotti, 2010). Colour variability can be caused by environmental and genetic factors as well as gene-by-environment interactions. However, colour polymorphism specifically describes the condition in which individuals within a population display one or more distinct, genetically inherited colour variants (Roulin, 2003, Gray & Mckinnon, 2007). Colour polymorphism provides an effective way for researchers to understand the mechanisms that maintain genetic variation in natural populations (Roulin, 2003, Gray & Mckinnon, 2007): a key question asked over many decades of research in evolutionary biology (Barton, 1989; Pryke & Griffith, 2005; Mckinnon & Pierotti, 2010). In isopods, it generally appears that diversity in colouration is strongly genetically influenced (Roulin, 2003; Gray & Mckinnon, 2007; Shuster et al, 2014; Wellenreuther et al, 2014), although cases of environmental determined colour variability are also known (see Chapter 4).

The objective of this study is to investigate the biology of *Isocladus armatus*, an abundant endemic New Zealand marine isopod that exhibits massively polymorphic colour variation. My specific goals were to resolve the reproductive biology of *Isocladus armatus*, to develop and refine methodologies to breed them in captivity over multiple generations, and ultimately to evaluate whether colour variation has a genetic basis in this species. The outcomes of these objectives are critical to understanding *Isocladus armatus* in enough detail to contribute to its

ongoing development into a model system focused on looking at the evolutionary basis of colour polymorphism in general.

Isocladus armatus

New Zealand is internationally recognized as a world 'hotspot' for biodiversity. The contribution of the island country to overall global diversity is hugely significant, with an estimated 80,000 endemic species (Myers, et al, 2000; Environment Foundation, 2018). One of these endemic species is *Isocladus armatus*: a highly abundant but poorly known Sphaeromatid marine isopod that has an exceptionally dramatic phenotypic polymorphism.

Isocladus armatus is an endemic marine isopod found in intertidal rock pools and semi-sheltered shores throughout New Zealand. *Isocladus armatus* are strong swimmers who can control their position in a water column enabling them to be unrestricted by habitat and allowing for large amounts of dispersal within the population (Jansen, 1971; Wells & Dale, 2018).

Isocladus armatus was first described by Henri Miline-Edwards in 1840 and then again as *Isocladus springer* by James D Dana in 1853 (Hurley & Janson, 1977; Stephenson, 2002; Schotte, 2008). These two independent descriptions of *Isocladus armatus* were initially thought to be two different species belonging to the family Sphaeromatidae. However, in 1977 K. P. Jansen confirmed the conspecificity of these two descriptions in laboratory observations (Hurley & Jansen, 1977; Jansen, Unpubl. Data). On several occasions Jansen observed immature males of *Isocladus armatus* moulting into Dana's '*Isocladus springer*' form (Hurley & Jansen, 1977). After these initial observations of *Isocladus armatus* in 1977, little research on the biology of this

species has been undertaken until recently when J. Dale (Evolutionary Ecology Group, Massey University) implemented a research program into colour polymorphism using *Isocladus armatus* as a model species. As fundamental knowledge on the biology of this species was lacking, the main objective of the research described in this thesis is to uncover more information on *Isocladus armatus* to inform ongoing and future research into colour polymorphism.

The New Zealand Isopod crustacean *Isocladus armatus*, displays an astonishing degree of colour polymorphism in the form of (at least) five distinguishable colour morphs, but that also includes numerous gradations between morphs to the extent that no two individuals are alike (Morton & Miller, 1968; Jansen, 1971; Wells & Dale, 2018). Colour polymorphism in *Isocladus armatus* provides a tractable model system into research on the maintenance of genetic diversity because the species appears more diverse than other species of marine isopod and it is likely that colour polymorphism is under strong genetic control. (Wells & Dale, 2018; this study). The function of this extreme variation is unknown (Jansen, 1971). However, other studies in different species of isopod have shown that colour polymorphism is typically argued to be defensive against predation: it can function as a form of camouflage (Moreira, 1974; Jormalainen et al, 1995; Merilaita, 2001; Stevens & Merilaita, 2009).

The aim of the overall research program into *Isocladus armatus* is to explore the mechanistic and functional factors influencing the expression and evolutionary maintenance of colour polymorphism in the species. However, to help accomplish this overall goal, in my thesis there are four objectives that I address:

1) Document the developmental life history of *Isocladus armatus* to have a better understanding of the general biology of the species. Due to lack of research into the basic biology of *Isocladus armatus*, having life history knowledge will not only facilitate making the breeding of this species easier for future work, but also to help identify features that help differentiate between sexes during early developmental phases and to provide insights into the ontogeny and function of their sexual dimorphism.

2) Document the development of sexual dimorphism in *Isocladus armatus.* Having knowledge in identifying each sex as early as possible during development will aid significantly in breeding these animals and will aid in the exploration of colour polymorphism and its possible genetics.

3) Document the reproductive behaviour of *Isocladus armatus*, this study is the first to look at the reproductive behaviour of the species and having knowledge of their reproductive behaviour is essential to successfully breeding *Isocladus armatus* under controlled conditions.

4) Successfully breed *Isocladus armatus* in the laboratory and test for a genetic basic to colour variation. Having multi-generational information on *Isocladus armatus* will ultimately aid in determining the degree to which colour polymorphism is genetically or environmentally controlled in the species, and will assist in the identification of specific alleles associated with specific colour variations.

This thesis consists of four additional chapters in addition to this one. Chapter 2 focuses on the external morphology of *Isocladus armatus,* including the comparison of male and female body lengths at both the juvenile and adult stages, and it also explores the spatial distribution of *Isocladus armatus* at Stanmore Bay Beach, Auckland. Chapter 3 focuses on the development of

sexual dimorphism of *Isocladus armatus,* the growth stages of males and females, and the reproductive cycle. In addition, I describe for the first time the breeding behaviour of these animals in laboratory settings. Chapter 4 addresses the extensive colour polymorphism in *Isocladus armatus* to determine if there is evidence that it is genetically controlled. The data for this study was gathered by both breeding *Isocladus armatus* individuals in captivity, as well as from females that were fertilised in the field (from unknown sires) and that later released their young in captivity. Chapter 5 completes the thesis with a few final remarks and conclusions about the overall findings in this study.

Chapter 2:

Spatial distribution, morphology and body length of *Isocladus armatus*

Abstract

Understanding the basic biology of a species is essential for conducting detailed research on it. For example, basic knowledge of a species' general morphology, sexual dimorphism, and spatial distribution within its habitat is essential. In this chapter I endeavour to gather some basic biological information about the NZ endemic isopod *Isocladus armatus* from samples I collected at my field site at Stanmore Bay, Auckland, New Zealand. This chapter discusses the population samples of *Isocladus armatus* and the species spatial distribution on Stanmore Bay Beach. I found that isopods were most common in the mid-tide zones, supporting the only other previous study looking at this. After samples were brought into the laboratory for detailed analysis, I describe the general morphology and quantified the degree of sexual size dimorphism. I found that males were on average approximately 1.5 the size of females. The attributes discussed and described here provide the necessary framework for the more detailed observations discussed in chapter 3. This chapter also provides in detail the laboratory conditions that the isopods were housed under, for the studies presented in Chapters 3 and 4.

Introduction

Knowledge of a species basic biology is necessary to conduct more detailed research on specific questions relating to evolution and ecology. Detailed knowledge of even the basic biology of most of the world's approximately 10000 species of isopods is lacking. For Sphaeromatid isopods, ecological observations are either very general such as a study conducted by Menzies (1962), or more detailed but focused on a limited number of individual species (e.g. Jansen, 1971).

The biology of the abundant endemic NZ species *Isocladus armatus* is generally poorly understood. Therefore, the objectives of this study are to discuss the general nature of population samples of *Isocladus armatus* and to discuss the species spatial distribution on my study site at Stanmore Bay Beach, Auckland. Only one other study by K.P. Jansen (1971) has described the spatial distribution of *Isocladus armatus*. Replicating those findings at a different study site with a different population is important for more in-depth knowledge of these animals.

There are two main questions that I address in this chapter. The first main question is – what is the spatial distribution of *Isocladus armatus* within the tidal zone at Stanmore Bay Beach? On the rocky shores of New Zealand, the degree of wave action can vary greatly between the exposed shores and the more sheltered shores (Rasmussen, 1965). This influences the substrate and variation in temperature, moisture and salinity (Jansen, 1971). On the more sheltered shores at low tide, the summer temperatures can reach a high level and the danger of dehydration is greater (Jansen, 1971). The variation of salinity is also greater due to evaporation, rainfall and freshwater runoff from the land (Jansen, 1971). This contrasts with more exposed shores where sea spray and wave splash maintain these conditions within a narrower limit (Jansen, 1971). Having knowledge on the type of shores around New Zealand will help identify which ones *Isocladus armatus* are likely or not likely to be found, will help with the husbandry of the species in laboratory conditions, and also gives us insights on where the species is found spatially at different shores.

The second main question is how do individuals in the species *Isocladus armatus* display sexual dimorphism? To do this, external phenotypes and body length of male and female

Isocladus armatus individuals were measured after being collected. Having detailed knowledge of sexual dimorphism in this species is especially important for describing their life history and ultimately for breeding them in controlled laboratory conditions (as will be described in more depth in chapters 3 and 4).

To address these two questions the isopods were systematically collected from the study site at Stanmore Bay Beach, and then brought into the lab for detailed analysis. The spatial abundance of *Isocladus armatus* at the study site was noted as they were collected, and then morphological details were recorded in the lab. This chapter is important for creating a baseline of data on spatial distributions and sexual dimorphism of *Isocladus armatus* for ongoing research within (and outside of) this research system. Previous ecological studies on other Sphaeromatids describing their body length and spatial distribution have also been conducted on other species such as *Paracerceis sculpta* (Shuster, 1999) and *Dynamene bidenttata* (Holdich & Harrison, 1980). However, in total there are generally not very many studies on other species of isopod that have been published.

In addition, in this chapter, I also provide details of the conditions and the protocols the collected isopods were kept under in the lab, and these methodologies apply to the research described in chapters 3 and 4.

Methods

Study Site

Isocladus armatus are abundant in semi-sheltered shores also known as class IV shores (Rasmussen, 1965; Jansen, 1971). Rasmussen described in 1965 the class IV shore as ‘exposed

to light wave action at all levels of high tides, except in high gale-force winds when wave action is moderate'. The class IV shore is classed as flat with very shallow water, they are characterised by extensive platforms of smooth channelled siltstone where the channels are partly filled with sand mixed with small stones and shell fragments that are re-distributed by wave action (Rasmussen, 1965; Jansen 1971).

Rasmussen's definition of a class IV rocky shore describes Stanmore Bay Beach (36º37'06.6"S, 174º43'43.0"E), where *Isocladus armatus* was collected for this thesis, as an ideal habitat for the species (Figure 2.1).

Collection of Isocladus armatus

I examined the spatial distribution of *Isocladus armatus* life stages and colour morphs at Stanmore Bay beach. I began by establishing at low tide, a 100m transect parallel to the shore, that overlapped with a rocky intertidal region known to contain isopods. I then made two additional transects 12 metres apart at 7 and 19 metres from the eastmost point of the first transect. These two distances were randomly selected by a number generator. At these locations, the two additional transects ran perpendicular to the shore for 30 metres from the high tide line seaward to the low tide line as measured on the sampling date (Figure 2.2).

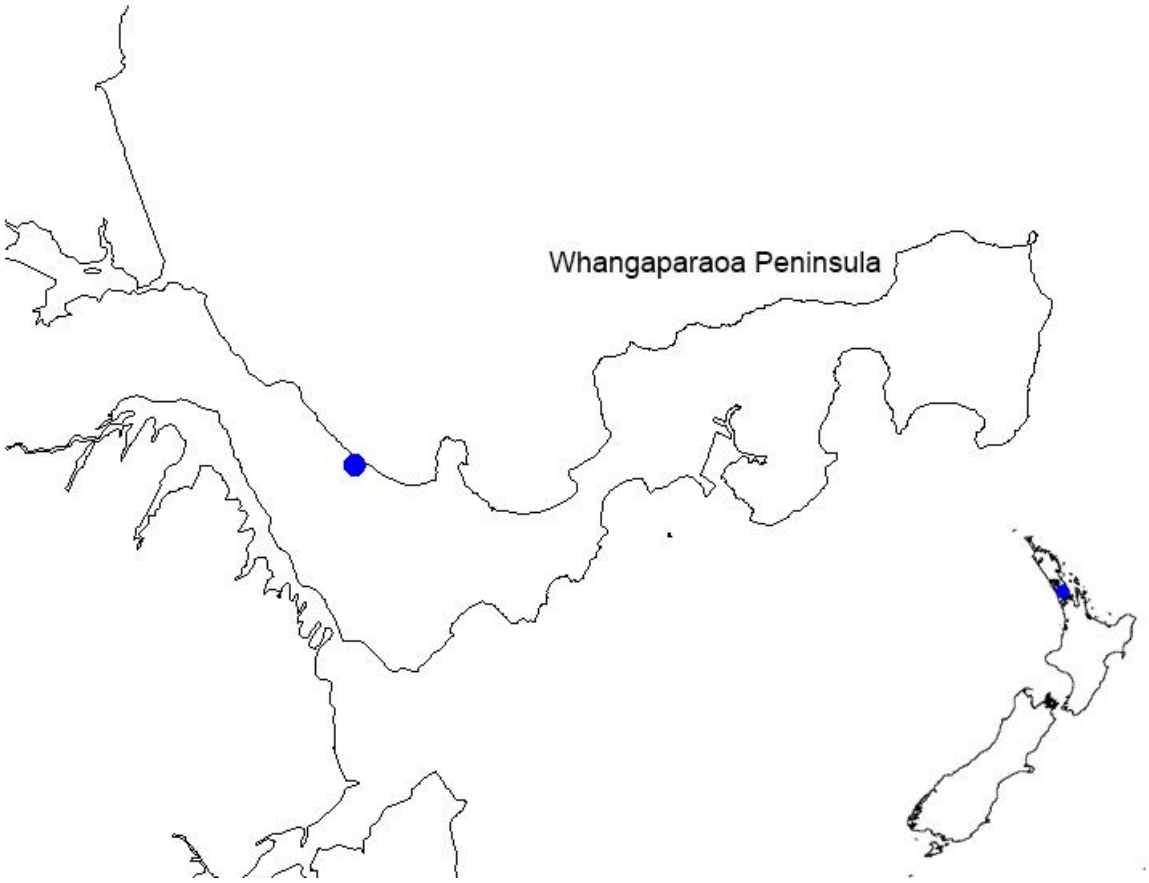

Figure 2.1: Map showing the location of Stanmore Bay Beach, on the Whangaparaoa Peninsula, Auckland Region, New Zealand.

Figure 2.2: Photo of the study site at Stanmore Bay beach with the first of two perpendicular transects measured out.

Beginning at the high tide line, I established a 1 m^2 quadrant using crossed metre sticks every five metres along each of the two perpendicular transects, heading towards the low tide line (25 metres). Once a quadrant was set, I collected every isopod I could find within the area using a hand net. If there was no rockpool at a collection point, the isopods were collected from the nearest rockpool typically within 1-2 metres adjacent to the marked collection point.

To standardise sampling intensity, samples were collected for 5 minutes at each collection point. Collected isopods were then placed into 700ml cups containing seawater, labelled with the transect number, distance from the high tide line and the minute they were collected in (e.g. Transect 1, Distance 10m, Minute 1 of 5).

From these transects and collection points, I collected a total of 308 individuals of *Isocladus armatus* from 10 different collection points, with 5 separate collections per point (i.e., 1 collection per minute across 5 minutes). All individuals collected were transported to the laboratory where they were separated into individual 700ml containers with 200ml of seawater, food and chalk (see Chapter 3 for more details). The isopods were separated into individual pottles to prevent cannibalism. The body length of each isopod was measured with a dissecting microscope, from the outermost mandible to the end of the pleotelson (to the nearest 0.2mm). The isopods' sex, reproductive condition and colour morph description were also recorded. Males were identified by their possession of a dorsal spine and/or the presence of penes located along the midline of the body on the last ventral pereonal segment (Figure 2.3). The dorsal pleonal spine of all identifiable males was measured to the nearest 0.2mm. Females were identified by their possession of oostegites which are small triangular sacs that hold live young

when a female is gravid (eggs are fertilised and develop into young within the oostegites). The oostegites are located at the base of each leg pair on ventral pereonal segments 2 - 5 (Figure 2.4).

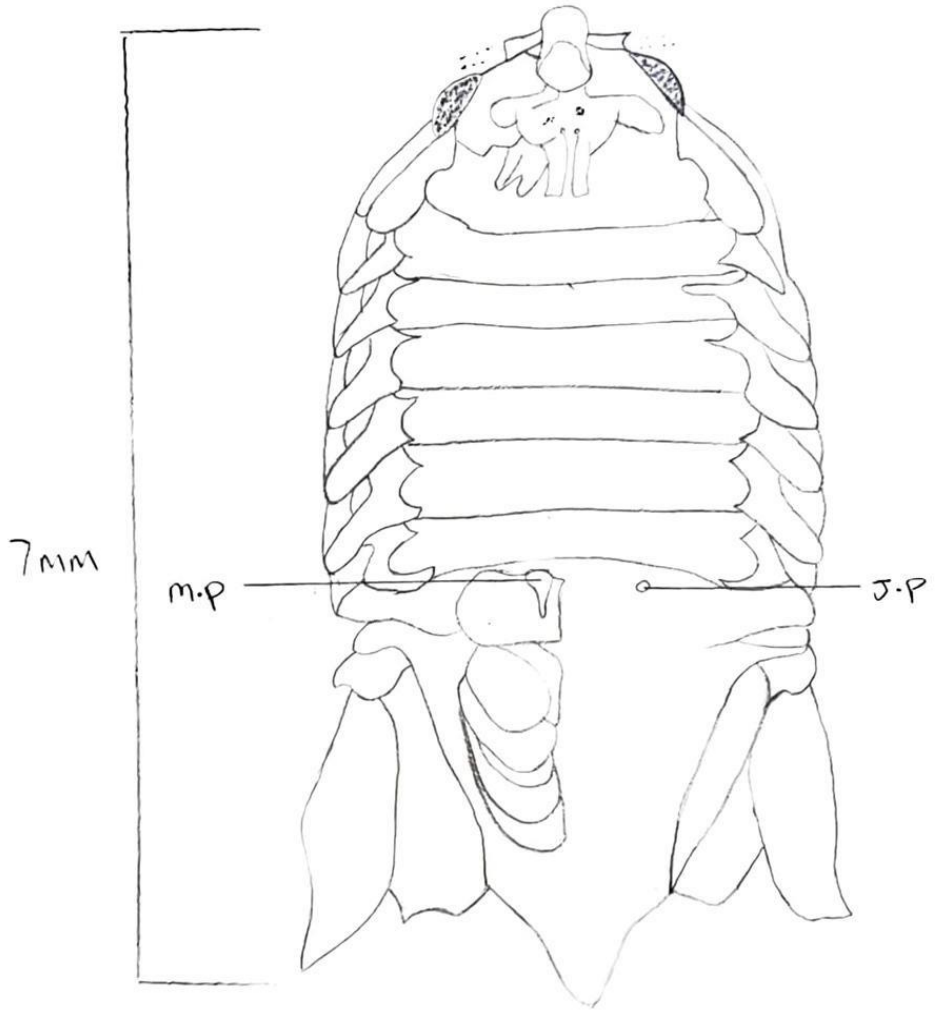

Figure 2.3: Ventral view of *Isocladus armatus*; left to right showing mature penes (m.p) with a large sigmoidal uropod and juvenile penes (J.P) with uropods that resemble the female uropod (Adapted from: Jansen, 1968).

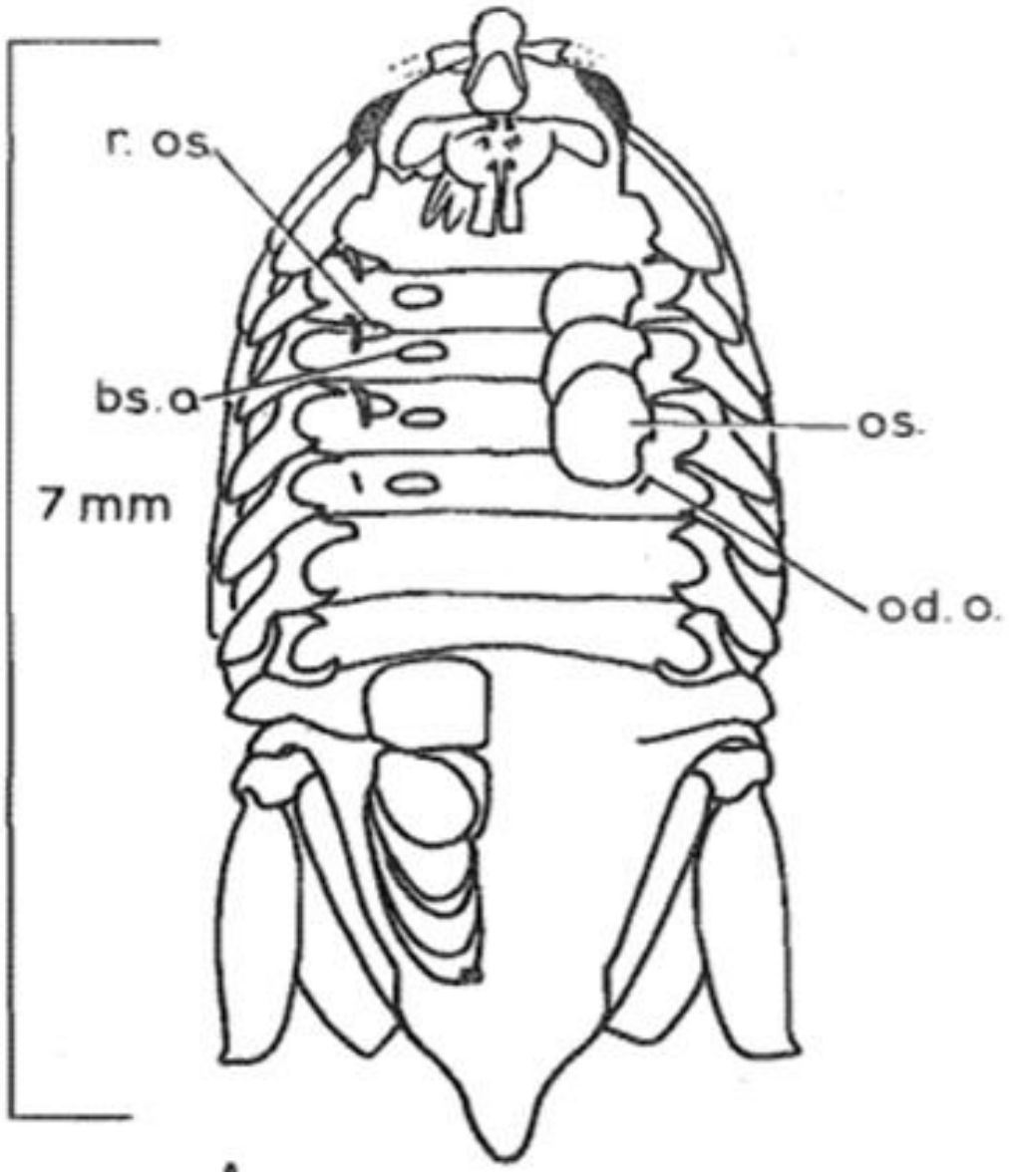

Figure 2.4: Ventral view of a female *Isocladus armatus* with mature oostegites (o.s) and rudimentary oostegites (r. os) (Retrieved from: Jansen, 1968).

Each female's reproductive condition was determined through observation under a dissecting microscope. First, their ventral side was dried by placing a female individual on a Kimtech delicate science wipe, where she could walk the water off onto the tissue. After she was dried the female was flipped onto her dorsal side and observed under the microscope to determine her oostegite state and the colour of her body cavity which was visible through the ventral pereon and could range from grey (not fertilised or gravid) to bright yellow (very gravid). In addition, any signs of mancas (baby isopods) were noted. This data was collected to conduct a separate study of the reproductive biology of this species and will be discussed further in Chapter 3.

After the initial processing of each individual isopod was completed, the isopods were fed and had their water changed thereafter twice each week. At each of these maintenance procedures, 200ml of fresh seawater from Stanmore Bay beach was placed in a clean, sterilised container. Once the water settled a small portion of chalk (around 5 mm x 5 mm) was added to provide the calcium carbonate that the isopods need to carry out their moults. Each isopod received one 5 mm x 5 mm shrimp flake and five 1 mm algae pellets to as food. It was critical to restrict the amount of food provided because too many of these algae pellets caused algae to grow on the isopod's exoskeleton.

All the isopods were examined over a period of three months. During this time, any observations made of each individual isopod were recorded and compared to any similar observations made on other individuals during this study. The similarities that were found within

and between both sex and age groups were recorded and compared (note that these data are evaluated in Chapter 3).

Results

Isopod abundance

The transects of *Isocladus armatus* collection at Stanmore Bay beach, indicated that they are most abundant between the High-water Neap and the Low-water Neap. Although I recorded the presence of *Isocladus armatus* individuals from 5 to 30 metres, they were most abundant between the 10 to 20 metre marks (Table 2.1).

This aligns with Jansen's findings in 1971 where he found that *Isocladus armatus* can be found from the High-water Spring to the Low-water spring on a class IV beach. However, in his work, they were also most abundant between the High-water Neap and the Low-water Neap (Jansen, 1971). This finding was notable because apart from the information Jansen found in 1971, there are no other descriptions of the spatial abundance of *Isocladus armatus*.

Table 2.1: Samples of individual *Isocladus armatus* collected from Stanmore Bay beach in February 2020, in relationship to distance from the high tide line.

Collections of *Isocladus armatus* from Stanmore Bay beach							
		Time (minutes)					
Transect (Horizontal metres)	Quadrat (Vertical metres)	1	2	3	4	5	Total Isopods
1	5	0	3	0	0	1	4
	10	10	6	1	2	1	20
	15	8	5	6	1	2	22
	20	5	6	8	4	5	28
	25	1	0	0	0	0	1
	30	1	2	0	0	0	3
Mean transect 1		4.2	3.7	2.5	1.2	1.5	78
2	5	0	1	0	1	0	2
	10	3	6	2	1	2	14
	15	10	4	7	2	4	27
	20	2	1	0	0	0	3
	25	0	0	0	0	0	0
	30	0	0	0	4	0	4
Mean transect 2		2.5	2.5	1.5	1.3	1	50

Body lengths

The body length of each isopod was measured and recorded after they were sexed, and their reproductive stage was determined. Juvenile *Isocladus armatus* leave the care of their mother when they are around 1 mm in length, the process of them leaving their mother takes between 2 and 7 days , once they have left the mothers brood pouches. The juveniles cannot be sexed until they have grown to around 3 mm in length, thus these individuals were excluded from the analysis.

Figure 2.5 shows the body lengths of sexed female individuals. The range and frequency of female body lengths demonstrated that the smallest female identified being 3 mm in length and the largest at 10.2 mm. However, this latter measurement is a very large female and should be considered as an outlier, because the next smallest female isopod was 1.2 mm shorter. The average length of a female *Isocladus armatus* individual was 5.88 mm and the average of a

sexually mature female was 6.12mm in length from a sample size of 117 individuals, 16 juvenile and 100 mature females.

Figure 2.6 outlines the range of male *Isocladus armatus* body lengths, with the smallest being 3 mm in length and the largest 10.8 mm in length. The average length of juvenile males was 6.24 mm long and the average of sexually mature males was 8.68 mm, taken from a total sample size of 160 male isopods, comprising of 101 juvenile males and 59 mature male *Isocladus armatus* males.

The average length of an adult female was very similar in length to the average juvenile male (Figure 2.4). The average length of a juvenile female was 4.26 mm (Figure2.4).

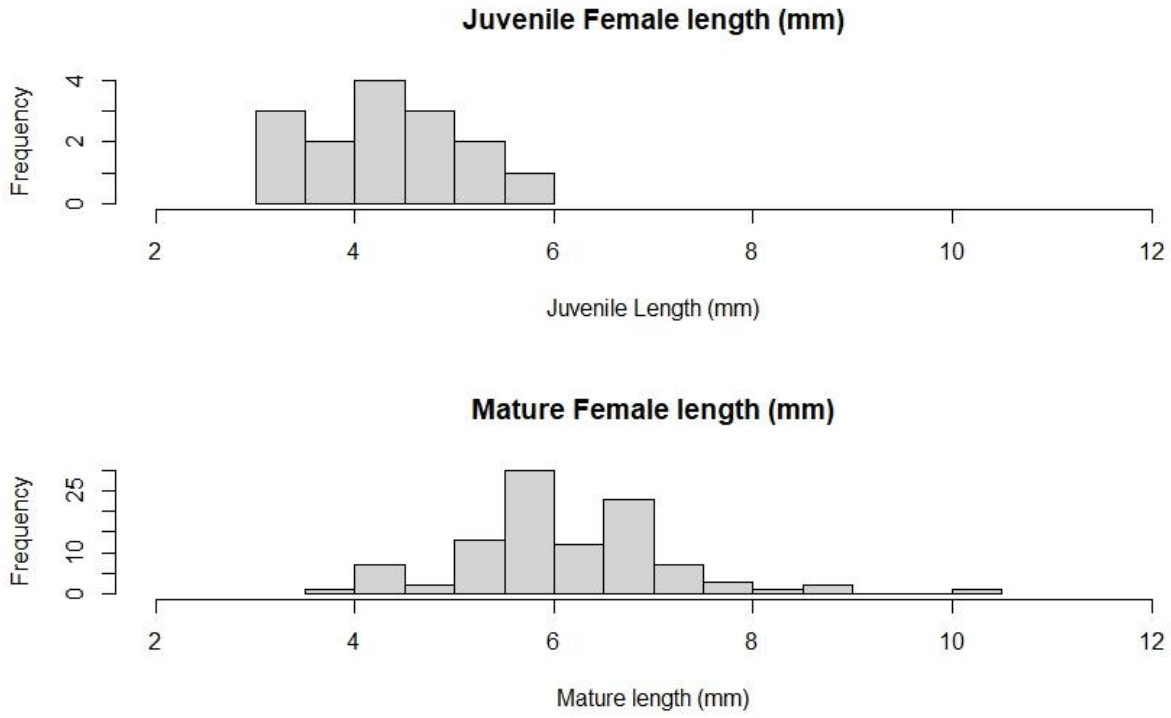

Figure 2.5: Histogram comparing the frequency and length of juvenile and mature *Isocladus armatus* females collected at the study site, Stanmore Bay Beach.

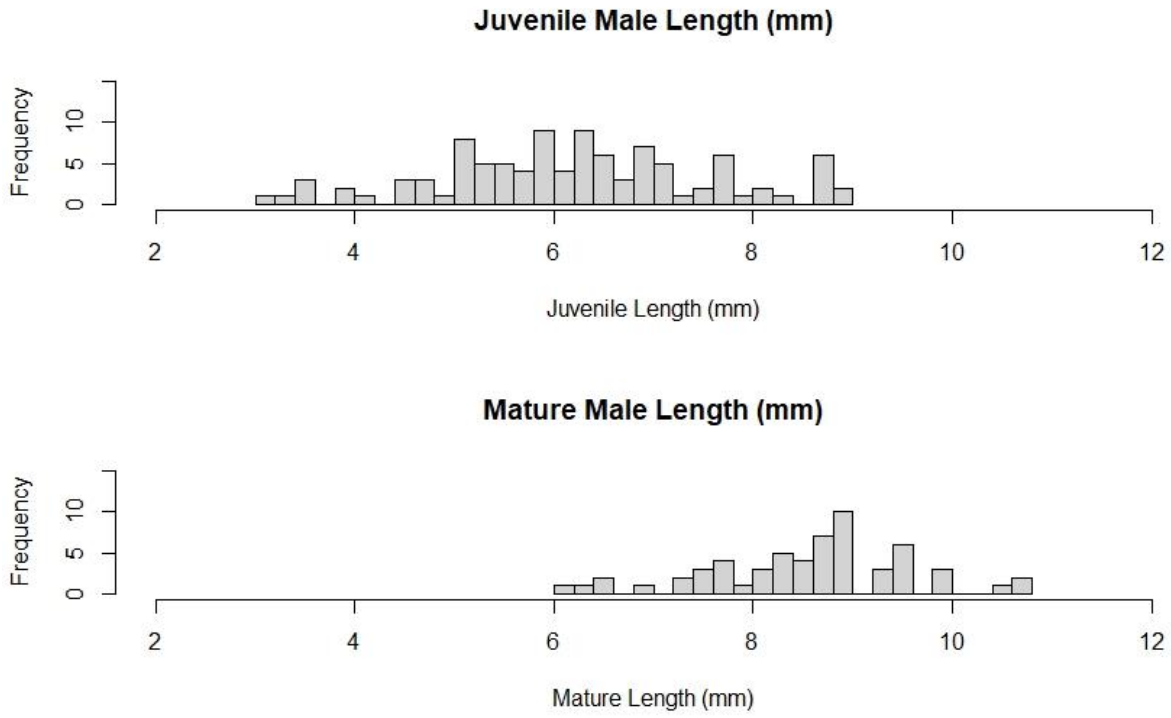

Figure 2.6: Histogram comparing the frequency and length (mm) of juvenile and mature *Isocladus armatus* male individuals collected at the study site, Stanmore Bay Beach.

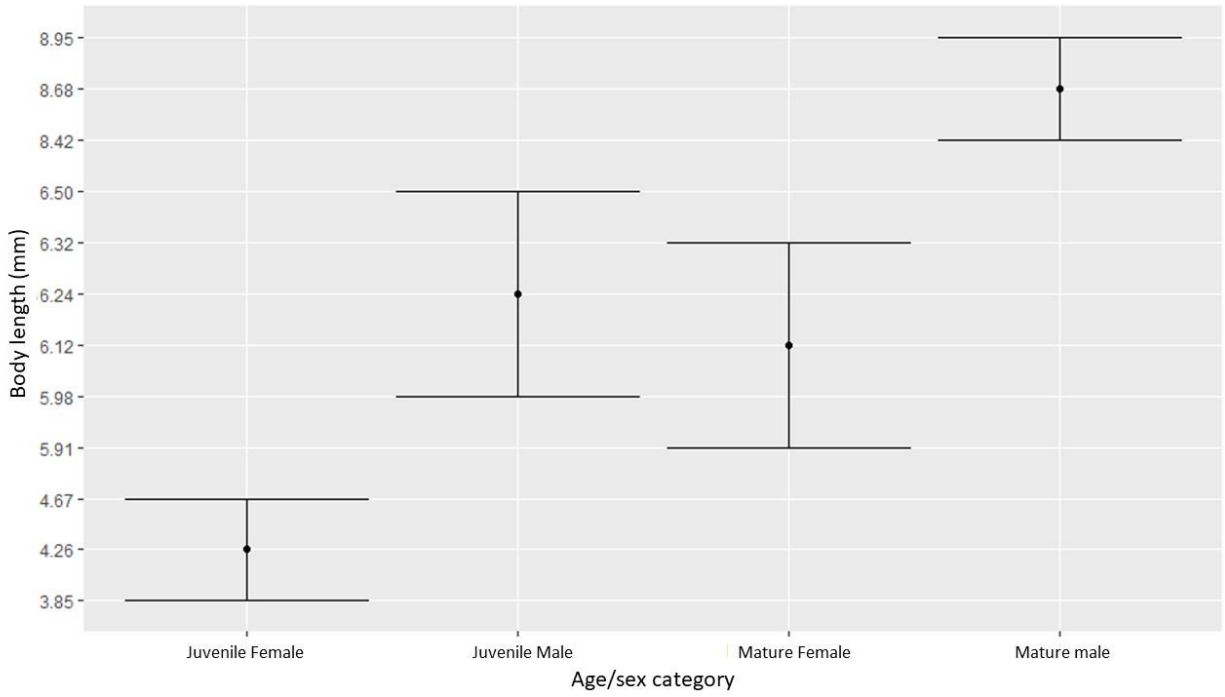

Figure 2.7: Plot showing the average length (mm) of juvenile and mature *Isocladus armatus* individuals of both sexes with a confidence interval of 95%.

Discussion

The population sample of *Isocladus armatus* collected on Stanmore Bay beach revealed similar patterns to what Jansen found in 1971 when he collected *Isocladus armatus* on the rocky shores of the Kaikoura peninsula. The upper limits of *Isocladus armatus* remain at the High-water Spring; however, the lower limits will rise up the beach with an increased wave action (Jansen, 1971). These findings were not only helpful in confirming what Jansen found at the Kaikoura Peninsula but also suggests that this spatial trend is followed by the species throughout New Zealand.

Isocladus armatus is a free-swimming isopod, therefore when the tide is incoming there is an upward movement of the population shoreward (Jansen, 1971; Wells & Dale, 2018). I observed that a greater proportion of the smaller and younger individuals will move up towards the high tide line. This would explain the results of transect 2, after the isopods were collected on an incoming tide the sample showed an apparent drop in the number of isopods from quadrat 20 to 30.

Another phenomenon that I noticed while out collecting isopods were that rock pools close to the high tide line were full of hundreds of tiny isopods that were the size of manca that looked like it was acting as some sort of nursery, however these observations were not represented in our two transects.

The average size of an *Isocladus armatus* individual is 6.36 +/- 1.82 mm in length, this included lengths of juveniles that were collected but were too small to sex. The average sexually mature male is 8.68 +/- 1.08 mm in length and the average length of a sexually mature female is 6.12 +/- 1.04 mm. The average juvenile male is 6.24 +/- 1.35 mm.

Figure 2.7. show the average sexually mature male is around 1.5 times the size of a sexually mature female *Isocladus armatus* individual, indicating that *Isocladus armatus* displays a large degree of sexual dimorphism. This information was important because apart from a brief discussion of sexual dimorphism by Jansen in 1971 there is no other information and no quantification of sexual size dimorphism in *Isocladus armatus* that has been published.

As expected, the average length of a juvenile female (average 4.12 +/- 0.83) and their confidence intervals fall well below any other sex or age group. This is the same for the average sexually mature male where their confidence intervals fall well above any other sex or age group.

The average sexually mature female falls within the upper 95% confidence interval of the average juvenile male (Figure 2.7). This could be due to the delayed maturation of the male isopod; this will be discussed further in Chapter 3 where I will compare in detail male and female individuals and deluge further into the sexual dimorphism of this species.

Chapter 3:

Reproductive biology and the development of sexual dimorphism in *Isocladus armatus*

Abstract

Understanding the sexual dimorphism and the reproductive biology of *Isocladus armatus* is a fundamental element needed for the study of their colour polymorphism. This first study of *Isocladus armatus* developmental life history outlines the five stages of female development and the four stages of male development that occurs until each individual becomes sexually mature. In addition, I describe the additional changes they undergo leading up to and after copulation. The new knowledge described in this chapter was essential for ultimately breeding *Isocladus armatus* in captivity (see Chapter 4). I show that females have a very short window of time that they are capable of being fertilised. Additionally, I demonstrate the *Isocladus armatus* can be produce multiple broods throughout their lifetimes, and this observation contrasts with life history patterns observed in some other species of marine isopods.

Introduction

Sexual dimorphism is the difference between male and female characteristics, and it is often caused by intersexual selection for a preferred trait in the opposite sex, or by intrasexual selection for weaponry and large size through competition with same-sex rivals (Darwin, 1871; Hedrick & Temeles, 1989). Sexual dimorphism strongly affects morphological, physiological and behavioural traits (Bertin, A., et al, 2002). A classic example of secondary sexual characteristics are the exaggerated tail feathers on the male peacock which are caused by sexual selective forces driven by female preference for elaborated traits in their prospective mating partners (Bertin, A., et al, 2002).

Isocladus armatus is a species of Sphaeromatid isopod crustacean that displays sexual dimorphism. These isopods are endemic to New Zealand and are found in semi-sheltered shores around the New Zealand coastline. Very little is known about the reproductive biology and life cycle of *Isocladus armatus*. Having knowledge of how *Isocladus armatus* reproduces is key to understand their evolution and to breed these animals in captivity. It is also important to aid in investigating their genetics and inheritance patterns (see Chapter 4)

In this chapter, the visual, external differences between male and female *Isocladus armatus* individuals are described in detail and contrasted, to inform clear differentiation between the sexes. Understanding the sexual dimorphism as described in this chapter is important for the comparison of external morphology of male and female individuals.

In addition, the five stages of female and four male stages that *Isocladus armatus* individuals undergo in their reproductive cycle are described in detail. Further, I provide an account, for the first time, of the mating behaviours of *Isocladus armatus* breeding pairs that were observed in the laboratory. This research was especially important to inform the captive breeding protocols and investigating the genetic basis to colour polymorphism in these animals (see Chapter 4). The descriptive accounts provided here represents the first detailed account of the reproductive differences in males and females of this species, in terms of both morphology and behaviour.

Methods/ Materials

Collection and care of isopods

The collection and care of *Isocladus armatus* individuals observed in this study is as described in Chapter 2.

Morphological descriptions

During the time that *Isocladus armatus* individuals were being cared for and having their water changed as described in Chapter 2, detailed records were kept of each isopod. This included sex, length and any morphological changes that occurred through development. During this time 118 females and 161 males were observed and had records taken down at each feeding or water change time.

If a moult was found during an observation time the date the moult was found was recorded. The isopod was then put under the dissection microscope and any morphological changes were observed, recorded and compared with other similar morphological changes of that same sex.

If a female was thought to be fertilised, she was monitored every second day where she was observed under the microscope and any morphological changes were noted down, this included behavioural changes and physiological changes in her ventral cavity.

Mating behaviour

I established male-female pairings consisting of six sexually mature males each set up with 3 females from each of 3 of 5 different stages of their reproductive life cycle. The three stages

(pre-moult, half-moult and post-moult) were observed for this study because not only did I want to see how males and females interacted but I also needed to establish where in those three stages females were sexually receptive. Each pairing was observed and changes in the behaviour was documented and compared to that male's behaviour towards the females of the other 2 reproductive conditions. All observations of a male with a specific female were completed over a 5-minute period.

The six Stage 3 males were selected based on their sexual maturity (See chapter 2). I ensured that male size would not contribute to the results by selecting a variation body of sizes so selected sexually mature males with varying body lengths, the sizes of the males selected were (ID number also provided); #090 (7.56 mm), #037 (8.16 mm), #109 (8.16 mm), #038 (8.52 mm), #105 (9.6 mm) and #115 (10.44 mm).

Three females from each of the three main reproductive stages of a female's cycle (Pre-moult, Post-moult (+) and half-moult (O)), with a similar body length were selected for each male, totalling 18 females.

Male #090 was allocated three females in the different reproductive stages with similar sized body lengths of 5.28 mm, male #105 was allocated females ranging between 5.04 mm and 5.40 mm, male #38's females were 6.00 mm, #109's females 6.36 mm to 6.60 mm, male #115's females 6.72 mm to 6.84 mm and male #090 had the largest rang with females 6.72 mm, 7.44 mm and 9.00 mm.

These females were allocated in this way so female size variation did not have an effect on how the male interreacted with a female.

Each of the six male was allocated an arena which was set up in a 700ml plastic cup with a layer of fine sand. Males were allowed to acclimate for 5 minutes. After 5 minutes he was introduced to his first female, and the male-female interactions were observed and recorded for a period of 5 minutes. After the 5-minute period, the female was removed back into her own cup, and the male was rested while the next male was observed with his allocated female for that round. This allowed each male to have a 25-minute break in between each of his female interactions.

The number of interactions that happened during the 5-minute time frames were noted with reference to whether it was male initiated or female initiated. The type of interaction that was also recorded for males, were 1) male bump where he would swim up to the female and 'bump' into her, and 2) mate guarding where he would grab the female and hold onto her (grabbing). The two main female interactions were 1) female bump and 2) climbing where the female would climb onto the male and position herself under the male's spine horizontally (horizontal guarding). The amount of time and number of times that each of the interactions occurred was recorded for each pairing.

Dissection methodology

I conducted two exploratory dissections on *Isocladus armatus* individuals, one male and one gravid female dissection. These were done under direct guidance and knowledge of Professor Steven Shuster. We performed the dissections in a dissection microscope where we took photos of any findings to help confirm some of my assumptions about morphology and the reproductive biology of this species.

Results

Sexual differences in *Isocladus armatus*

Female Isocladus armatus

Female *Isocladus armatus* are almond shaped and can range between 3 mm and 10.2 mm in length (Chapter 2). The female uropods (swimming legs) are sigmoidal in shape and taper into a pointed end – they are relatively small and can be difficult to see when stationary (Figure 3.1 & 3.2). This feature contrasts with male *Isocladus armatus* individuals as they have relatively large uropods and they appear to display their uropods frequently (Figure 3.2). During my observations I noticed that females tend to be brighter in colour than males; and that males, especially white males, tend to tinge green and their coloration dulls as they become older, however it is not known whether this is an artefact of being kept in captivity.

While observing the 118 *Isocladus armatus* female individuals I had in laboratory conditions, I found that female *Isocladus armatus* start to show their preliminary (rudimentary) oostegites when they are around 3 mm in length. These matured into a ‘flap’ that covered the brood pouch and acted essentially as a floor for embryo in the brood pouch (Figure 3.3). When the preliminary oostegites are developed the female has entered her reproductive cycle (Figure 3.4).

My observations of the 118 female individuals kept in separate containers and kept over several weeks, demonstrated that the female reproductive cycle consists of five stages, but only three stages were observed in the behavioural study. The whole cycle appears to occur over a

duration of 12 to 14 weeks in laboratory conditions starting from the initial sexual moult that produces preliminary oostegites (Stage 1) at around 3mm in length. The female will then go through a half-moult (Stage 2) where I have found that this is the stage where she is sexually receptive and can be fertilised. Stage 3 is after the second half of the female's moult. If the female has not been fertilised, she will then moult back into stage 1 (Pre-moult), however, if she has been fertilised she becomes gravid (Stage 4) and will release her mancas and become spent (Stage 5) where she will then moult back into stage 1. I observed that female *Isocladus armatus* individuals do not undergo moults between stages 3 and 5 and they can be up to 10.2mm in length. After a females mancas are released, the female goes through a final moult and returns back to the first stage, to repeat the cycle. (Detailed accounts are provided for each stage, below).

Figure 3.1: Female *Isocladus armatus* showing shape and absence of obvious uropods.

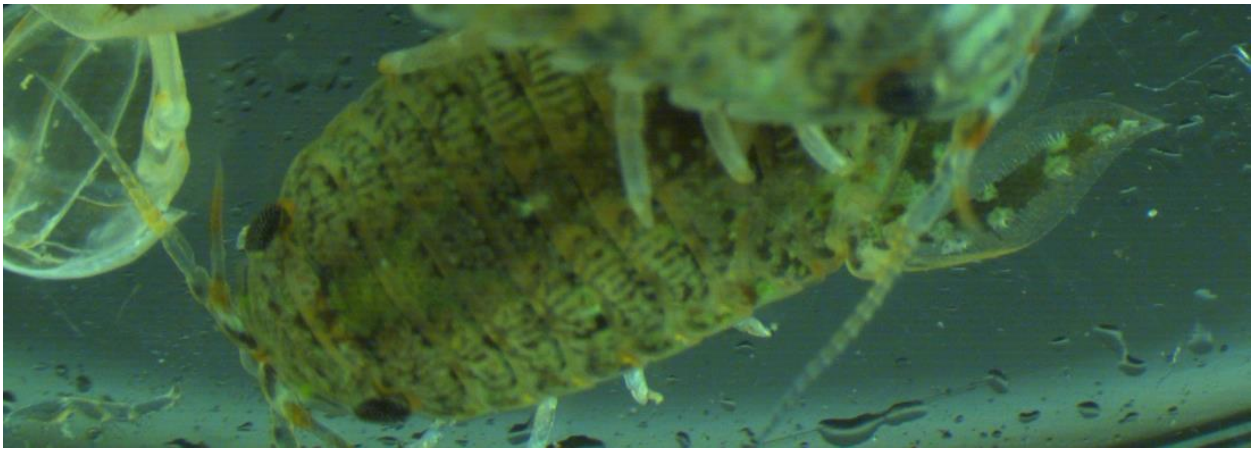

Figure 3.2: Active female with extended uropod.

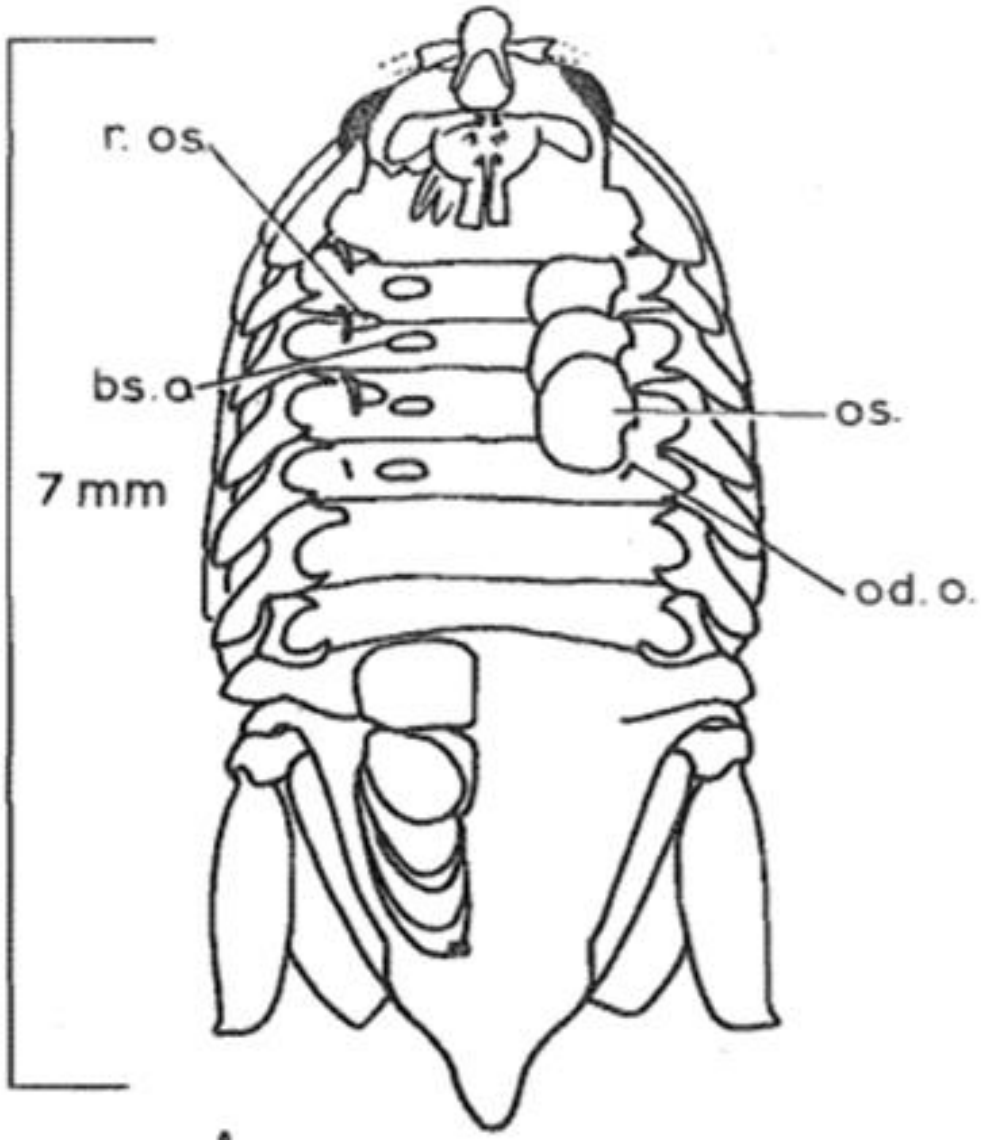

Figure 3.3: *Isocladus armatus* female showing the preliminary (rudimentary) oostegites (r. os), brood sacs and openings (bs .o), mature oostegites (os.) and position of oviductal opening (od.o.) (Retrieved from: Jansen, 1968).

Female reproductive system

The female reproductive system of *Isocladus armatus* consists of ovaries that run along the dorsal-most side of the female's cavity. The ovaries are connected to the exterior of the isopod via oopores (oviductal openings) that are found at the base of 5^{th} leg pair the oopore is where sperm enters the female's reproductive system.

Isocladus armatus females store their mancas (babies) in brood pouches as live individuals until they are ready to be dropped, and these brood pouches are located next to their oopores. There are five different stages to the female's reproductive cycle; Pre-moult, Half-moulted, post-moult, gravid, and spent (Figure 3.4).

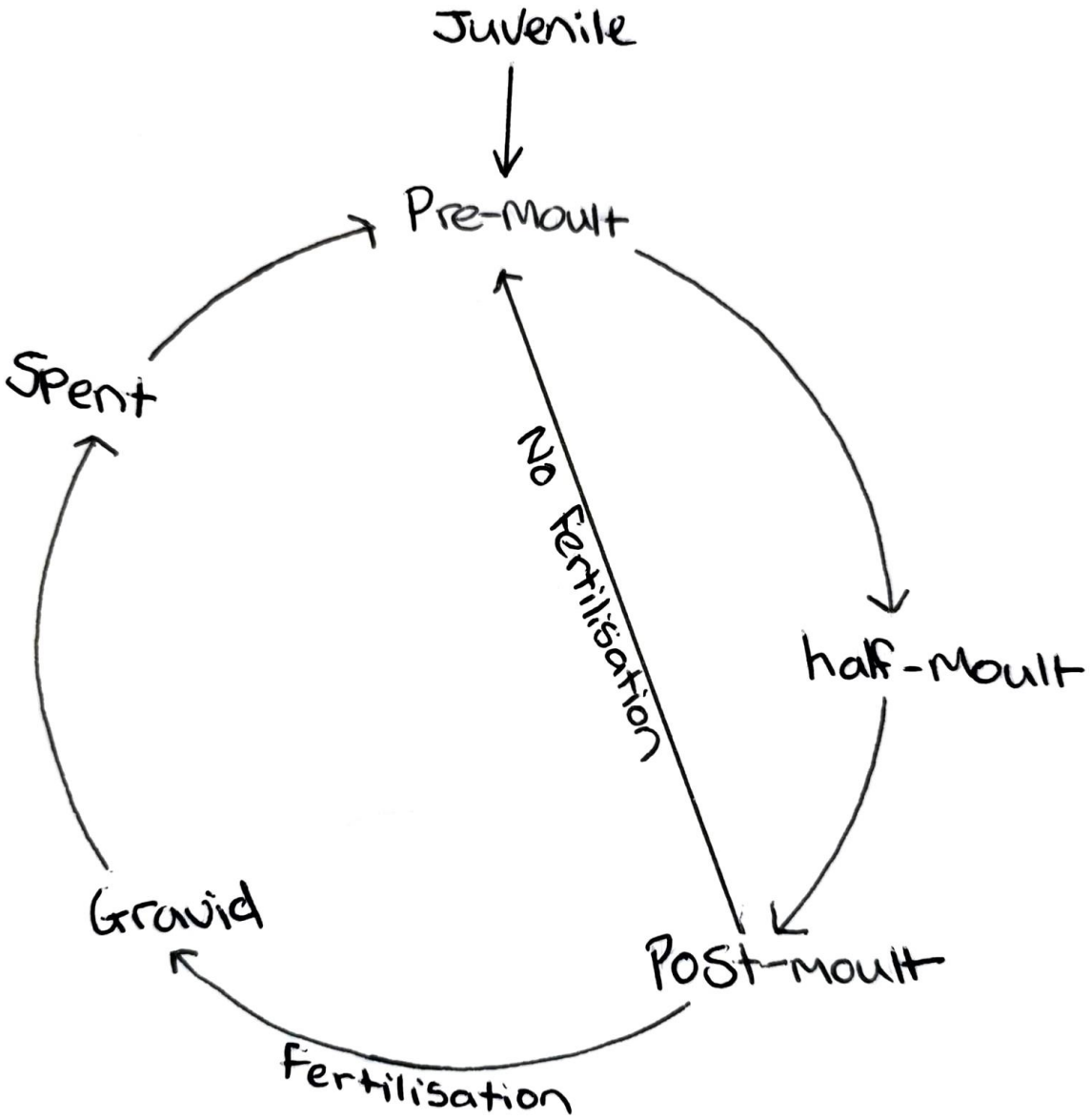

Figure 3.4: The reproductive cycle of female *Isocladus armatus* individuals goes through five stages from pre-moult (stage 1) to spent (stage 5).

Pre-moult females – Stage 1

Pre-moult females are sexually mature females with mature ovaries that have not undergone their reproductive moult. The ventral side of the pereon (underside cavity) of a premoult female is only slightly convex, with a transparent cuticle allowing the gut, ovaries and hepatopancreas to be seen easily.

Preliminary oostegites are translucent and are a 'spiked' triangular shape. They are visible on the ventral side of the pereon, underneath leg pairs two to four sets of legs.

There is no change in colour of a pre-moult female's ovaries, they remain translucent and the pereon remains grey. The females brood pouches are not visible until right before they undergo their biphasic sexual moult. Once visible, the brood pouches are unfilled and usually hyaline with a white tinge. I observed that a female will generally stay in this stage for 2-3 weeks before their biphasic moult.

Half-Moulted Females – Stage 2

A half-moult female is a sexually mature female that has shed the posterior portion of their cuticle, just above the 5th pereonite. This stage is very short, and the female will stay half-moulted for only 12 to 24 hours.

The textures of a pre-moult and half-moult female are easily identified because the posterior half of the half-moult female's body has expanded and is soft with a dull cuticle colour (Figure 3.5 & 3.6). The anterior half is constrained by the unshed portion of the cuticle which is hard and brightly coloured.

When a female is in their half-moult stage, she is sexually receptive and only during this short window of time. Copulation will take place if there are sexually mature males nearby. After copulation occurs, sperm is typically visible in the oviduct. (Figure 3.6).

My observations suggest that female *Isocladus armatus* do not possess sperm storage organs, therefore they need to undergo copulation each cycle. The female is inseminated directly into their oopores which are located at the base of legs 1-4. Each of the eight oopore's are inseminated individually, the male starts on one side and rocks the female to the opposite side each time an oopore is inseminated. The oopores enter directly into the brood pouch, this is where the nonmotile sperm will be found after copulation.

Figure 3.5: Half moulted female showing enlarged posterior and unshed anterior.

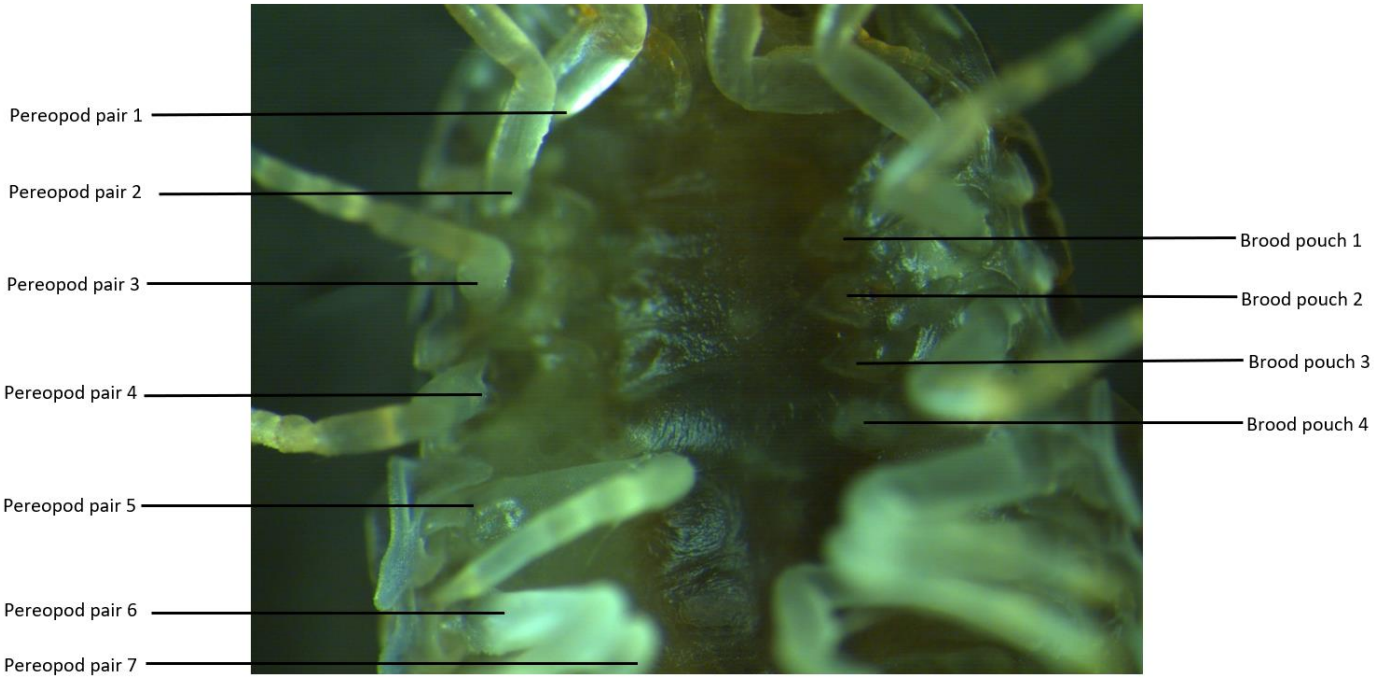

Figure 3.6: Ventral side of half-moult female showing pairs of hyaline brood pouch pairs (brood pouch 1 to 4), brood pouch pairs 1, 2 and 3 at the anterior end contain preliminary oostigetes. Brood pouch pair 4 are contains mature oostegites at the posterior end.

Post-Moult Females (Stage 3)

Post-moult females have completed a full moult and have shed the anterior portion of their cuticle.

During this stage of female development ova is transported along the oviduct and into the eight brood pouches, containing sperm from copulation. This is where the ova will become fertilised if the female has undergone copulation in her half-moult stage.

As the yellow ova fills the brood pouch, its colour will become more vibrant until the density of ova causes the brood pouch to appear bright yellow in colour.

Eventually the female's whole ventral pereon cavity will fill with ova and become convex. The full brood pouches take up all available space in the body, with an exception to the cephalon (head). The hepatopancreas and gut are pushed anteriorly to make more room for the ova.

The embryo will remain in the brood pouch until they are released (dropped) as mancas. Right before the mancas are dropped, the brood pouches protrude out into the ventral cavity so there are eight triangular bumps on the surface of the pereon.

Gravid Females (stage 4) and embryo development

A gravid female is a female that has been fertilised and is carrying embryos or mancas. She usually carries between 60 and 100 mancas during any one cycle.

Similar to *Paracerceis sculpta* Shuster (1991), *Isocladus armatus* have three distinguishable stages of embryo development in gravid females.

In the first stage of embryo development, the embryos are elongated with a slight curve, they are still bright yellow in colour with a slight white/opaque border around the outer edge (Figure 3.7 & 3.8, retrieved from female dissection). At this stage embryos lack both eyespots and segmentation.

Embryos in the second stage of development look similar to first stage embryos and are still yellow in colour, however they have developed dark eyespots. These eye spots make the yellow cavity of the female isopod look speckled.

In the third stage of embryo development the female now has fully developed mancas in her brood pouches. Mancas contain no yellow yolk when fully developed, and the pigmentation of their cuticle is usually visible through the female's transparent cuticle. Mancas located within the brood pouches are well formed, clearly segmented and can be seen moving (Figure 3.9).

Females usually stop eating 1 to 2 days before releasing offspring. Mancas begin to slip out of the brood pouches through an opening in the cavity wall underneath the oostegites and cling to the ventral side of the female’s cavity for some time (up to 48 hours) before being released. Gravid females usually release their mancas gradually over a 1 to 2 week period. The delayed release of manca could also suggest that fertilization is delayed, however more observations are needed to make a conclusion on this.

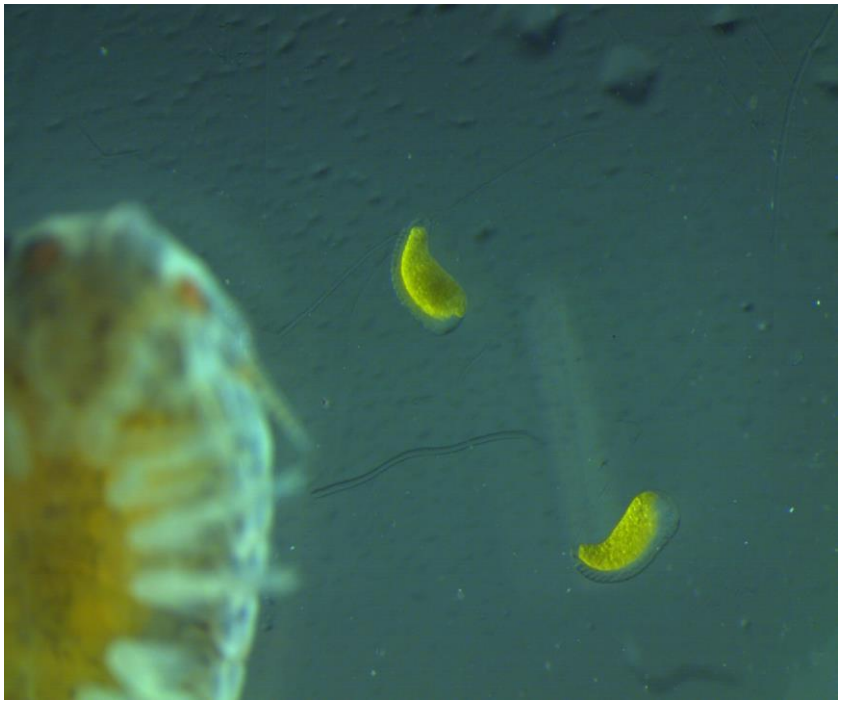

Figure 3.7. Yellow embryo in stage 1 of development

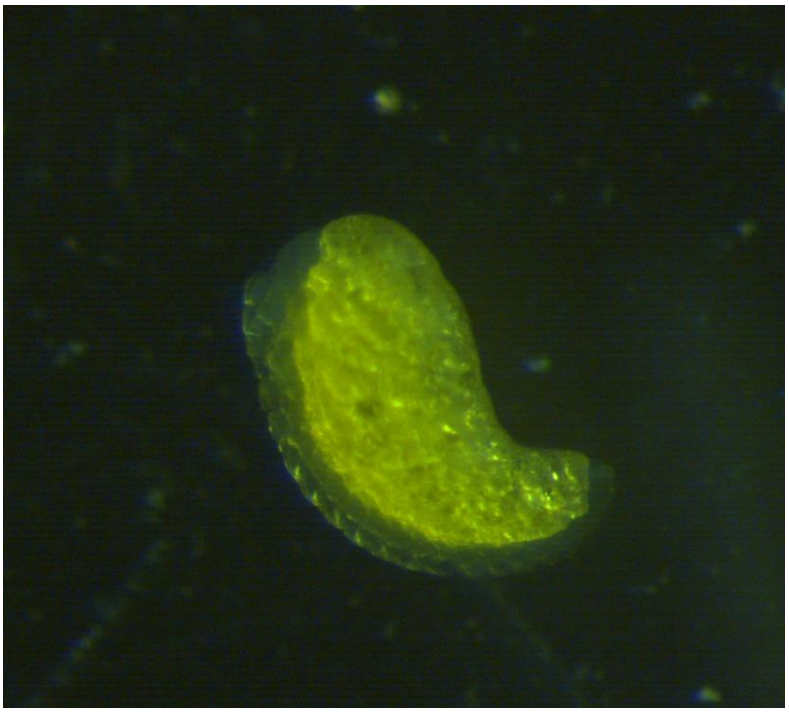

Figure 3.8. Close-up of yellow embryo in stage 1 of development

Figure 3.9: Gravid female with fully developed mancas in cavity, almost ready to be released. The cuticle colour of mancas can be observed with a white dorsal stripe.

Spent Females (stage 5)

Spent females are females that have released all their young. At this stage, their ventral cavity looks concave and empty. The hepatopancreas and gut are still pushed up towards the dorsal side of the cavity. Females usually stay in this stage for 2 to 3 weeks before moulting back into their pre-moult stage. During this two-week period the hepatopancreas and gut will drop back into the pre-moult position.

Female survivability after manca release

Isocladus armatus females, unlike some other groups of isopods, do not lose their functional mouthparts during gestation as they maintain their metamorphized mouthparts. I observed this during my time documenting the female *Isocladus armatus* lifecycle. This contrasts strongly with other species of isopod (semelparity) because it means they can repeat the reproductive cycle multiple times (iteroparity). I observed that most females can go through their breeding cycle up to three times before dying of senescence, and each cycle of the 15 crosses that were followed through from start to finish lasted around 12 to 14 weeks. Interestingly, 7 of these females were not fertilised at the time of their sexual moult following releasing their mancas and they all died within 10 days of that final sexual moult suggesting that it was unlikely for a female to survive their cycle if they have not been fertilised in their following half-moult stage.

Juvenile Isocladus armatus

On their first days out of their mother's brood pouch, *Isocladus armatus* manca (Juvenile Isopod) are typically around 1.5 mm in length and are almond shaped (Figure 3.10). All manca resemble small females and sexes cannot be differentiated at this stage of development.

In the space of 2 to 3 weeks, manca go through many growth moults at a fast rate, until they are around 5 mm in length. These translucent moults are less than 1 mm in size, which makes the moults very hard to see. These small moults can be easily missed when changing the isopods water.

Male manca mature faster than female manca. Once the male manca's length is around 5 mm they start showing signs of budding genitalia, this was observed by watching the broods and observing them under the microscope every other day until the geniltalia was able to be seen. Males tended to be sexed and separated into a cup before the female isopods of the same brood. Juvenile males start to show signs of penes on their ventral side. These signs consist of small rounded 'buds' on the 7th perionite where penes will eventually develop and elongate. This is the only known way to tell that an isopod is male at this stage (Figure 3.13).

Female manca take longer to show their preliminary oostegites, which develop as eight small 'spikes', four on each side of the body. The preliminary oostegites are transparent and are located under the base of legs 2-5.

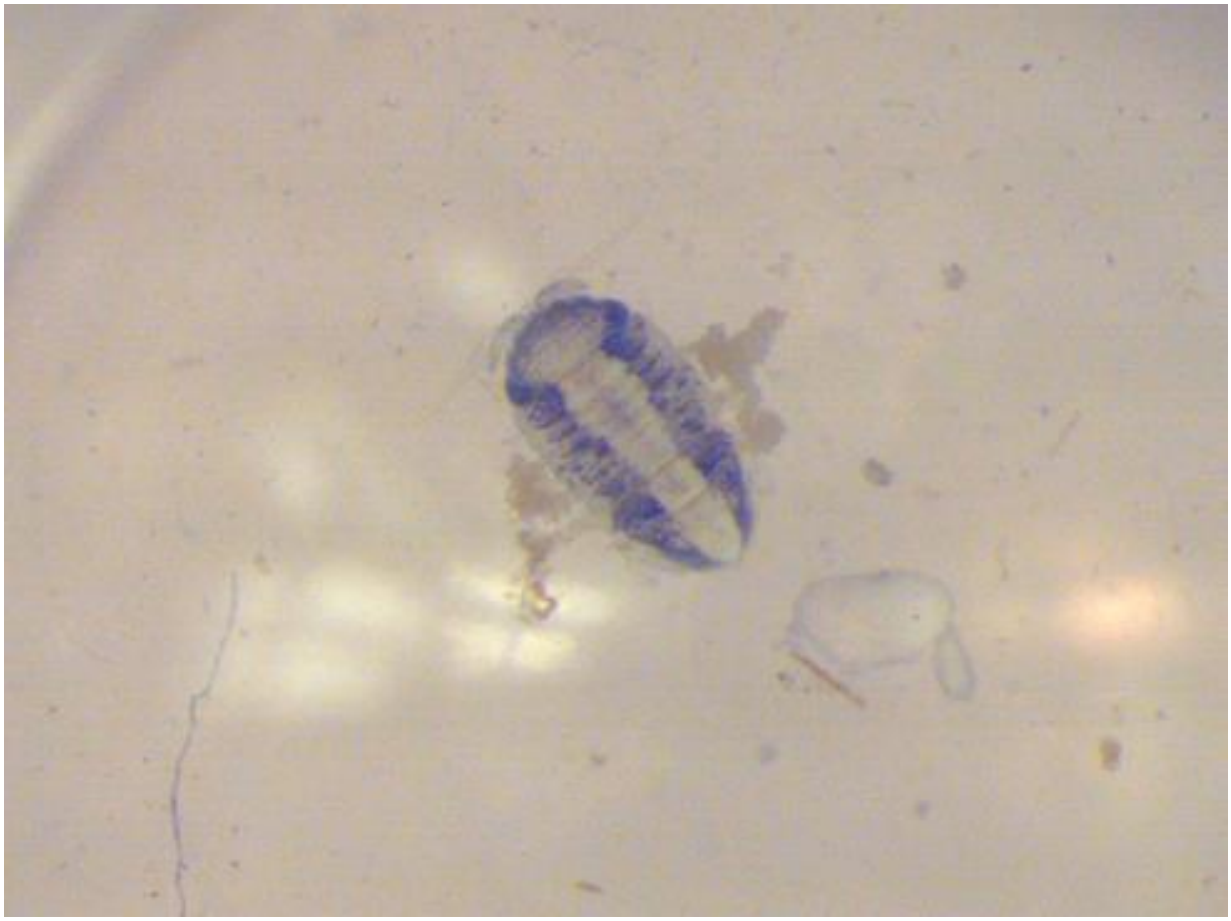

Figure 3.10: Juvenile *Isocladus armatus* under dissection microscope, showing almond shape.

Male Isocladus armatus

Isocladus armatus males mature faster than females and grow from around 3 mm to around 10.8 mm in length, which is around 1.5 times the size of the average (Chapter 2) female individual. Male uropods are sigmoidal in shape and taper into a point at the end just like a female uropod, however unlike the female uropods, the male uropods are very large in size and are held in a peacock-like fashion. It is unknown,if the males are preforming a display as more research needs to be conducted to conclude what the purpose of fanning out their uropods is.

Mature males possess an elongated spine protruding from the dorsal side of perionite 7, with a small tooth on either side of the spine (Figure 3.11). Once identifiable as male, the isopods go through 4 stages until they are sexually mature. During three of the four stages the males are cannibalistic (at least in captivity) and will eat any females they are partnered with or other males they may be placed with.

Figure 3.11: Male *Isocladus armatus*. Spine is protruding from perionite 7. Large uropods can be seen while stationary.

During my observations in morphological changes related to moults in males, I discovered that their development consists of a series of four stages. There are three major growth moults (Juvenile to Stage 1, Stage 1 to Stage 2 and Stage 2 to Stage 3) and one spinal growth (Stage 3 to Stage 4) that a male undergoes to morph from a juvenile *Isocladus armatus* individual to a sexually mature individual.

Stage 1 Males

When a juvenile has undergone enough moults to reach a body length of around 3 mm they can be sexed. A male will display two small rounded ‘buds’ on the 7th perionite where penes will eventually develop and elongate (Figure 3.12). There is no spine or teeth protruding from perionite 7 at this stage and he cannot be told apart from a female when looking at his dorsal side. This is classed as a Stage 1 male and is the earliest stage that a male can be sexed.

Stage 2 males

Stage 1 males will go through a growth moult into a stage 2 male, and these males are an average of 5.88 mm in length taken from a sample of 57 individuals. They have underdeveloped penes and a slight raise on the anterior side of the cuticle at pereonite 7, where their spine will eventually develop (Figure 3.13). This male is now classed as a stage 2 male.

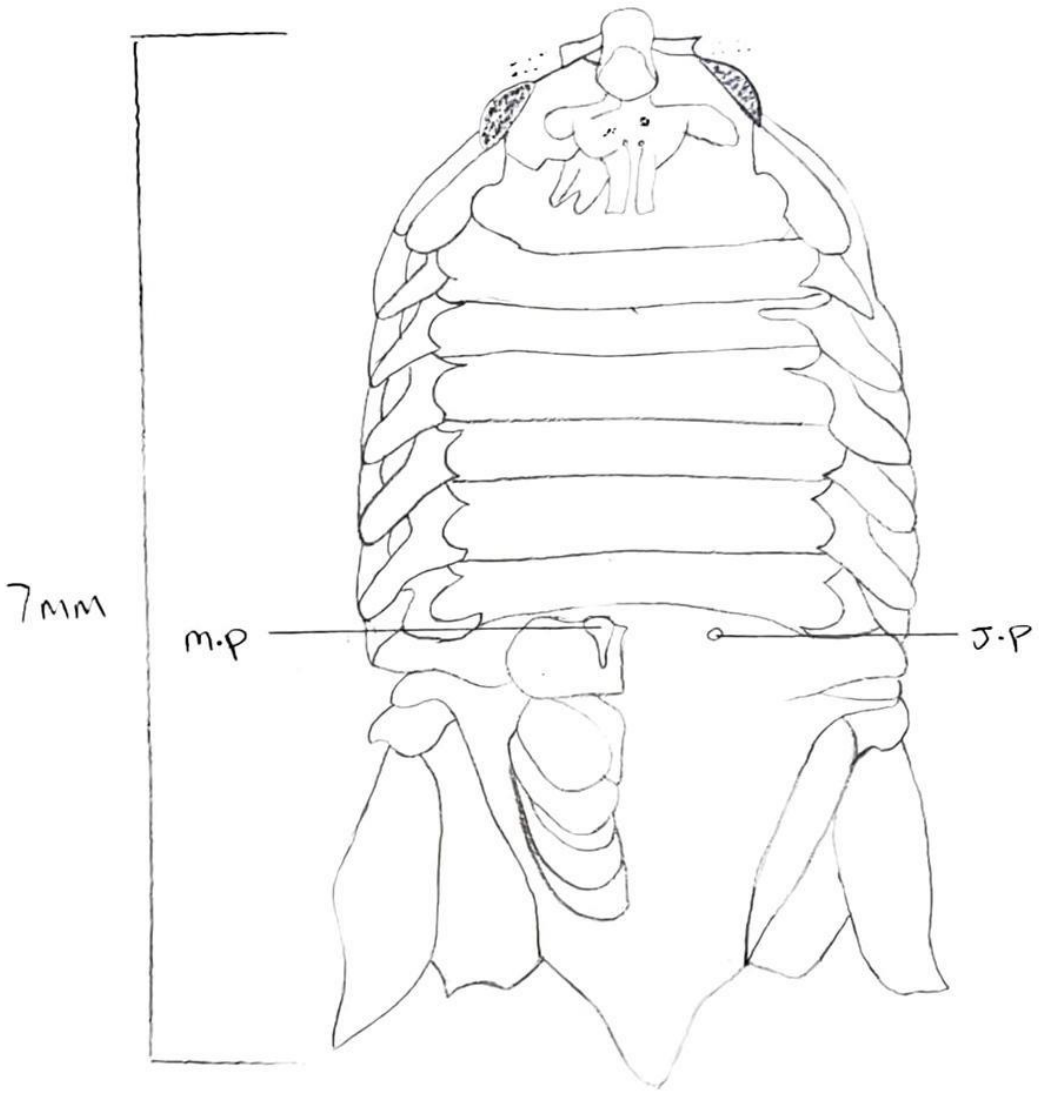

Figure 3.12: Showing Male penes. J.P resembles the pene of a Stage 1 male. M.P resembles the penes of a Stage 4 male (Adapted from: Jansen, 1968).

Figure 3.13: Stage 2 male, showing a small stub on the 7th perionite where the spine will later develop.

Stage 3 males

A stage 2 male will go through a third and final moult, and their body length has reached the final length that they will be as a stage 4 male because they do not undergo another growth moult.

In this moult, their penes grow to full length however, and their spine is only around three quarters of the length of the pleotelson (Figure 3.14).

If a stage 2 male was paired with a half-moult female, he is not interested in mating and generally the soft and vulnerable female will be eaten, this was observed during trial and error when I was learning the stages of males and how they interacted with females. Once I determined the stages of the males I resolved the trends in what stage males ate the females they were paired with for breeding attempts.

Figure 3.14: Late stage 3 male, spine has not quite grown to the end of the pleotelson.

Stage 4 males

Stage 4 (adult) males are an average of 8.62 mm in length taken from a sample of 39 indeviduals. Stage 4 males are on average 1.5 times larger than a fully mature female. These males possess an elongated spine protruding from the dorsal side of pereonite 7 along the entire length of the pleotelson towards the posterior end of the isopod. There is a small tooth on either side of the spine (Figure 3.15).

Stage 4 males are sexually mature and if paired with a half-moult female they will attempt to copulate.

Figure 3.15 Stage 4 male with fully developed spine.

Male Genitalia

Papillae

The penile papillae or ‘penes’ are projections from the ventral side of the 7th pereonite (Figure 3.12). The penes of an *Isocladus armatus* male can be found in the centre of the ventral cavity and bear the external openings of the vas deferens. Unlike other species of isopod that have fused penile structures (Wilson, 1991) *Isocladus armatus* penes are two separate, triangular structures that sit flush with the ventral side of the pereion pointing towards the ventral end of the pleotelson (Figure 3.12). I used this knowledge of the developing penes to sex male *Isocladus armatus* prior to them developing their spine.

Appendix masculinae

The appendix masculina is a rod-like structure found on the second pleopod. These along with the first pleopod act as a funnel-like extension of the penes (Figure 3.16) (Wilson, 1991). This ‘funnel’ aids with the transfer of the elongate spermatophores to the females oopores (Wilson, 1991).

Isocladus armatus have immobile sperm that look like a very fine wound-up piece of cotton. It is white in colour and is produced in a three-branch sperm duct. I located the sperm duct towards the cephalon of the isopod during my male dissection. (Figure 3.17)

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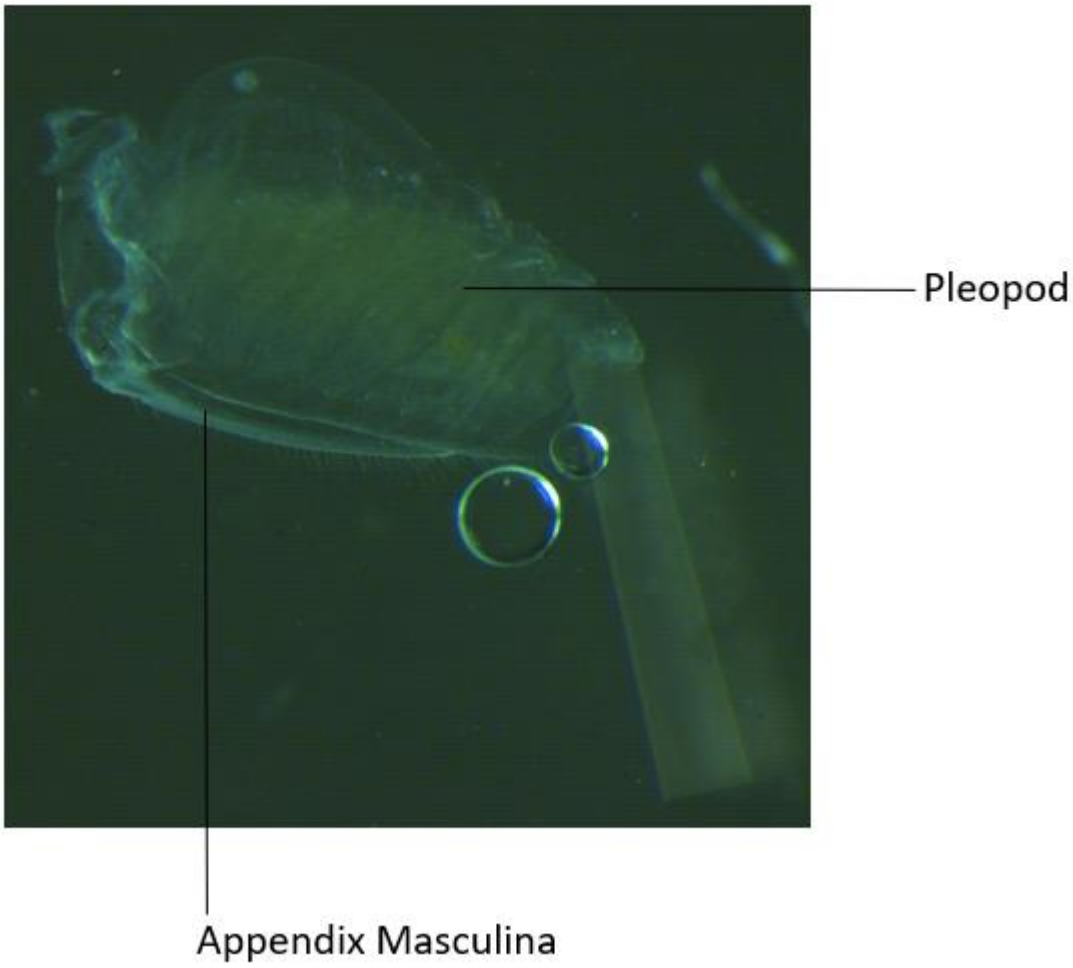


Figure 3.16: Second Pleopod with attached rod-like appendix masculina sitting along the base of the pleopod, photo taken at time of the dissection.

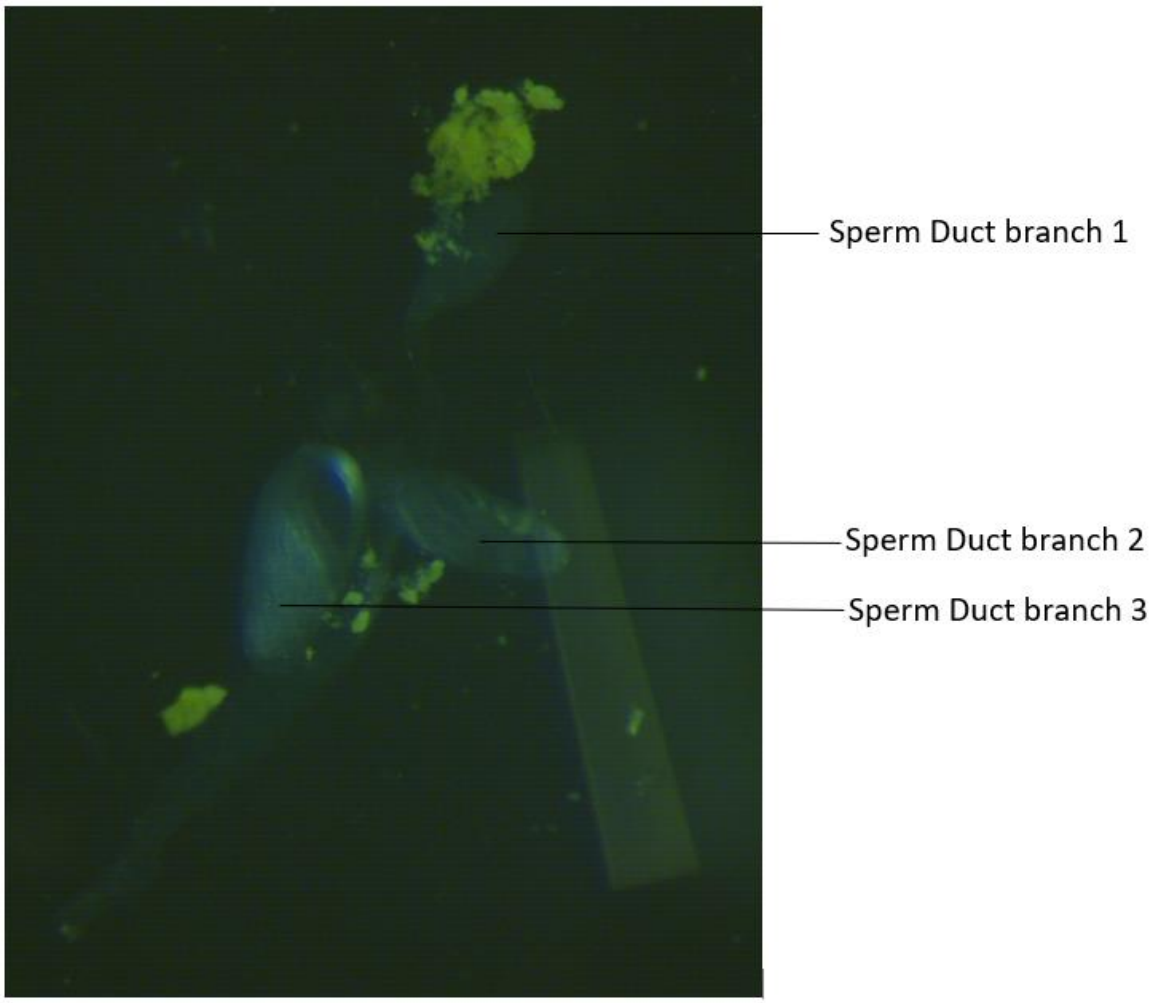


Figure 3.17: Three branched sperm ducts from a male *Isocladus armatus* individual that was dissected in the lab.

Mating behaviour

In the presence of females, I observed males holding their uropods fanned out in a peacock-like fashion giving the isopod an enlarged silhouette whilst swimming around the cups, however I am unsure whether this behaviour is an act of aggression towards other males or threats, or if it is a display to attract female *Isocladus armatus* individuals. More research will be needed to determine the function of this behaviour. (Figure 3.18).

Prior to mating, *Isocladus armatus* males and females display what I have called mate guarding where they attempt to clasp onto a female (see below). There were two instances I observed during attempted mate guarding where the female struggled to get away, or the male chased the female around the cup trying to grab her (both of these two females were half-moulded and were in their sexually receptive stage). There were three instances where there was no interaction at all between the male and female, these were male #109 and his pre-moult female, #090 and his post-moult female and #090 and his pre-moult female. Male #090 also did not interact with his half-moult female however - the female sat on him in horizontal guarding for the duration of the 5 minutes.

The other females seemed to be placid whilst mate guarding and during interactions. The two types of mate guarding that I observed, ‘traditional’ and ‘horizontal spinal’ are shown in Figure 3.19. Traditional mate guarding is displayed by the male holding the female’s dorsal side to his ventral pereon whereas horizontal mate guarding is when the female climbs onto the male and sits horizontally over the males pleotelson directly between his dorsal cuticle and his spine. I

observed mate guarding occurring up to 12 hours before copulation and lasting right up until the female has dropped her mancas.

Controlled mating observations

The results of the controlled behaviour observations show that sexually mature males have very little interaction with pre-moult females, however there are more interactions of pre-moult females towards the sexually mature males with a total of 10 seconds of male initiated interaction and 47 seconds of female-initiated interactions (Table 3.1).

Sexually mature males interacted the longest with post-moult females with a total interaction time of 268 seconds. There were 6 instances of mate guarding and 11 instances of bumping and a total of 22 male-initiated interactions.

Half-moult females interacted with sexually mature males frequently, with a total of 240 seconds of interactions towards males. Males also interacted with half-moult females frequently with 162 seconds.

Table 3.1: Interactions of males with three different females at the three main stages of their reproductive cycle, Pre-moult, half-moult (O) and Post-moult (+).

	No interactions	Male initiates	Male bump	Mate guarding	Time	Female initiates	Female bump	Climbing	Time
Pre-moult	3	6	0	1	10	6	4	2	47
O	0	30	19	11	162	2	1	1	240
+	1	22	11	6	268	2	2	0	0
Total interactions	**4**	**58**	**30**	**18**	**440**	**10**	**7**	**3**	**287**

When copulation occurred, I observed that it can last up to one hour. In the traditional guarding position, the male will rotate the female, so they are face to face (Figure 3.20). In this position sperm is transferred to the female along the appendix masculina. The rotation motion from guarding to facing happens eight times.

The sperm of *Isocladus armatus* is pseudoflagellae - this means the sperm does not move so where it is placed during copulation is where it will stay. Due to this, I believe that the fertilisation of each egg (ova) occurs in the brood pouch as it arrives from the ovaries.

After copulation, the male guards the female for extended periods of time, in some cases right up until the female releases her manca.

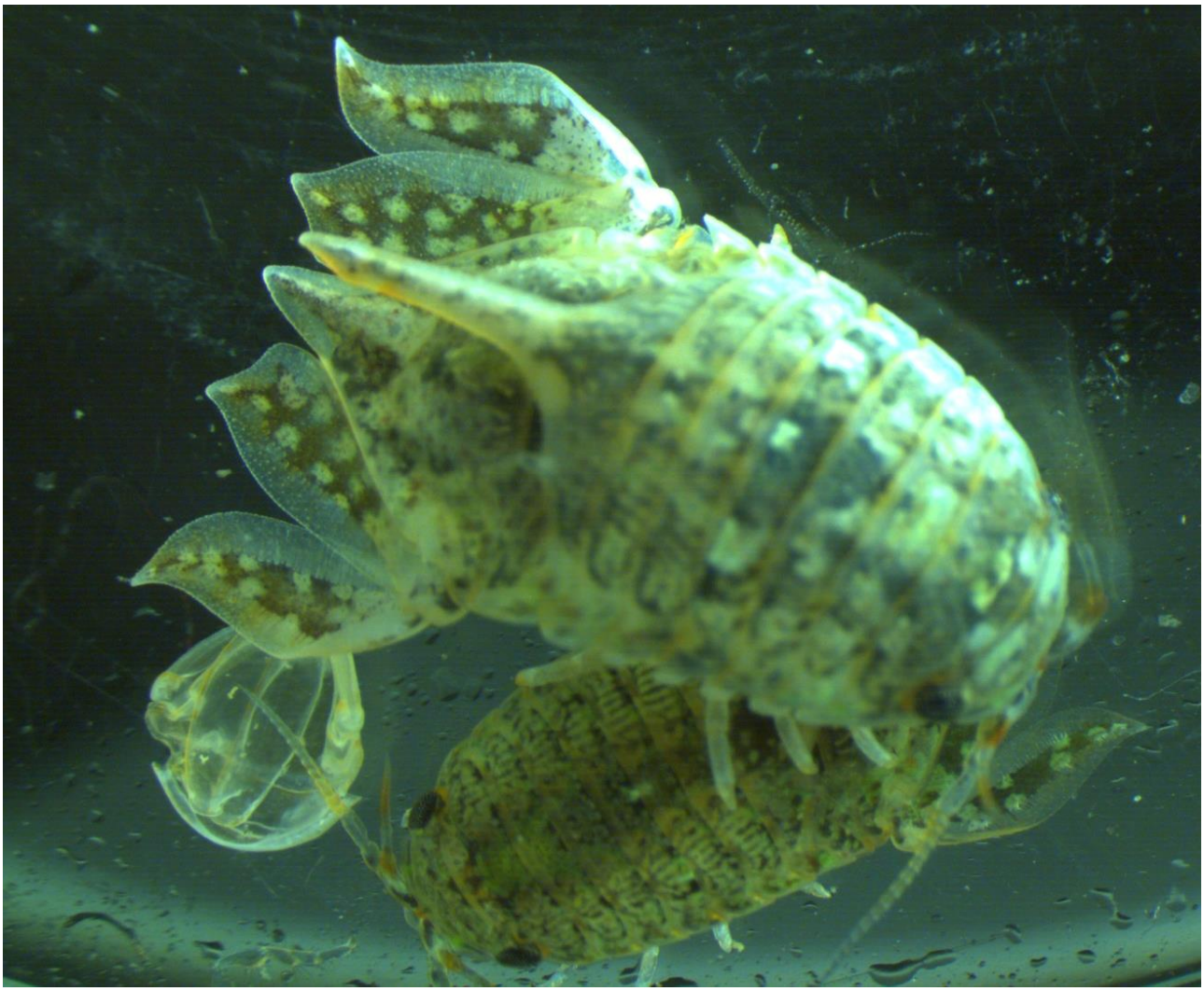
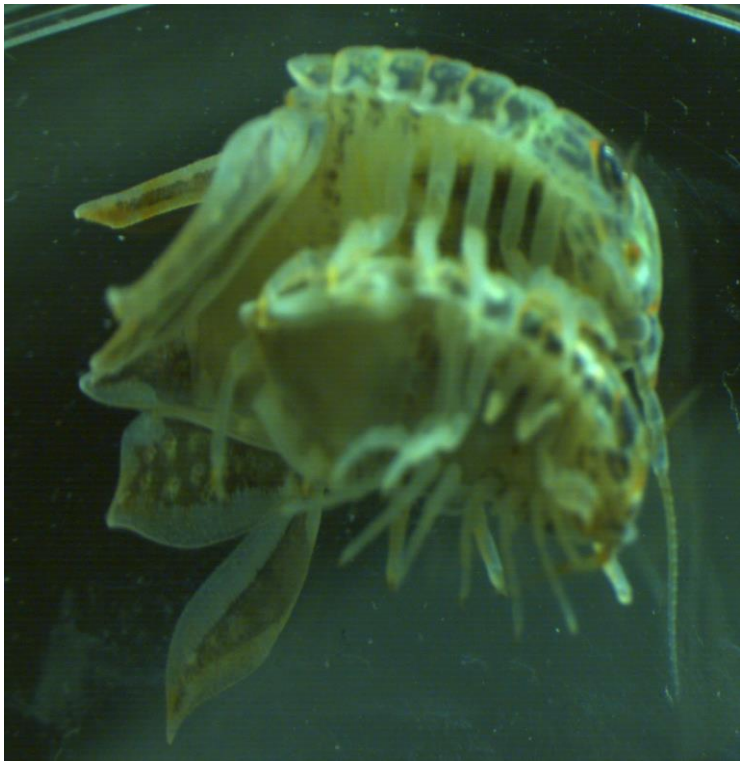


Figure 3.18: Male interacting with half-moulted female, uropods on display.



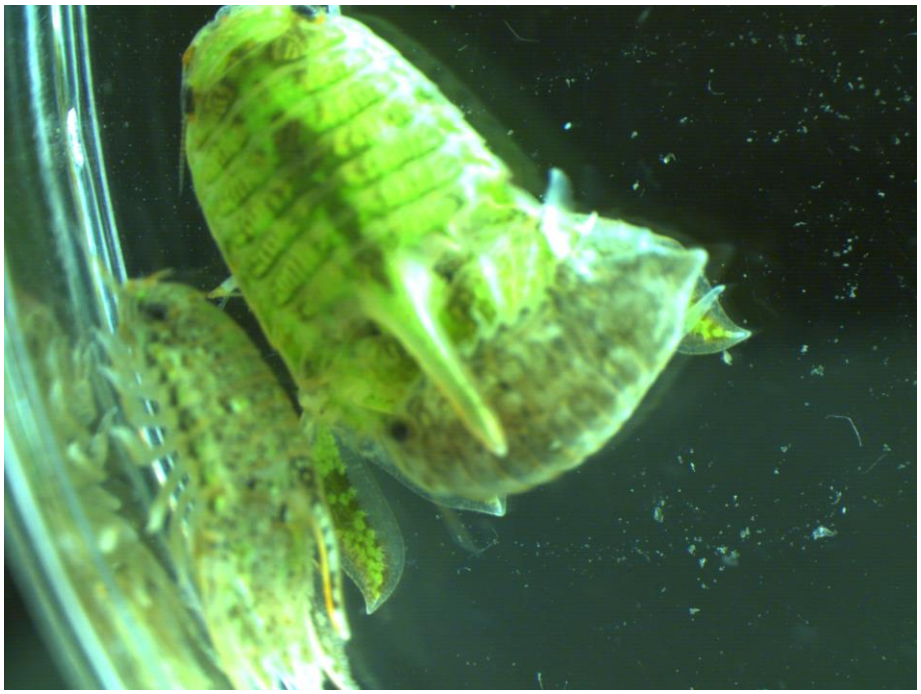


Figure 3.19: Top: Male and Female displaying ‘Traditional' form of guarding. Bottom: Male and Female displaying ‘Horizontal’ guarding.

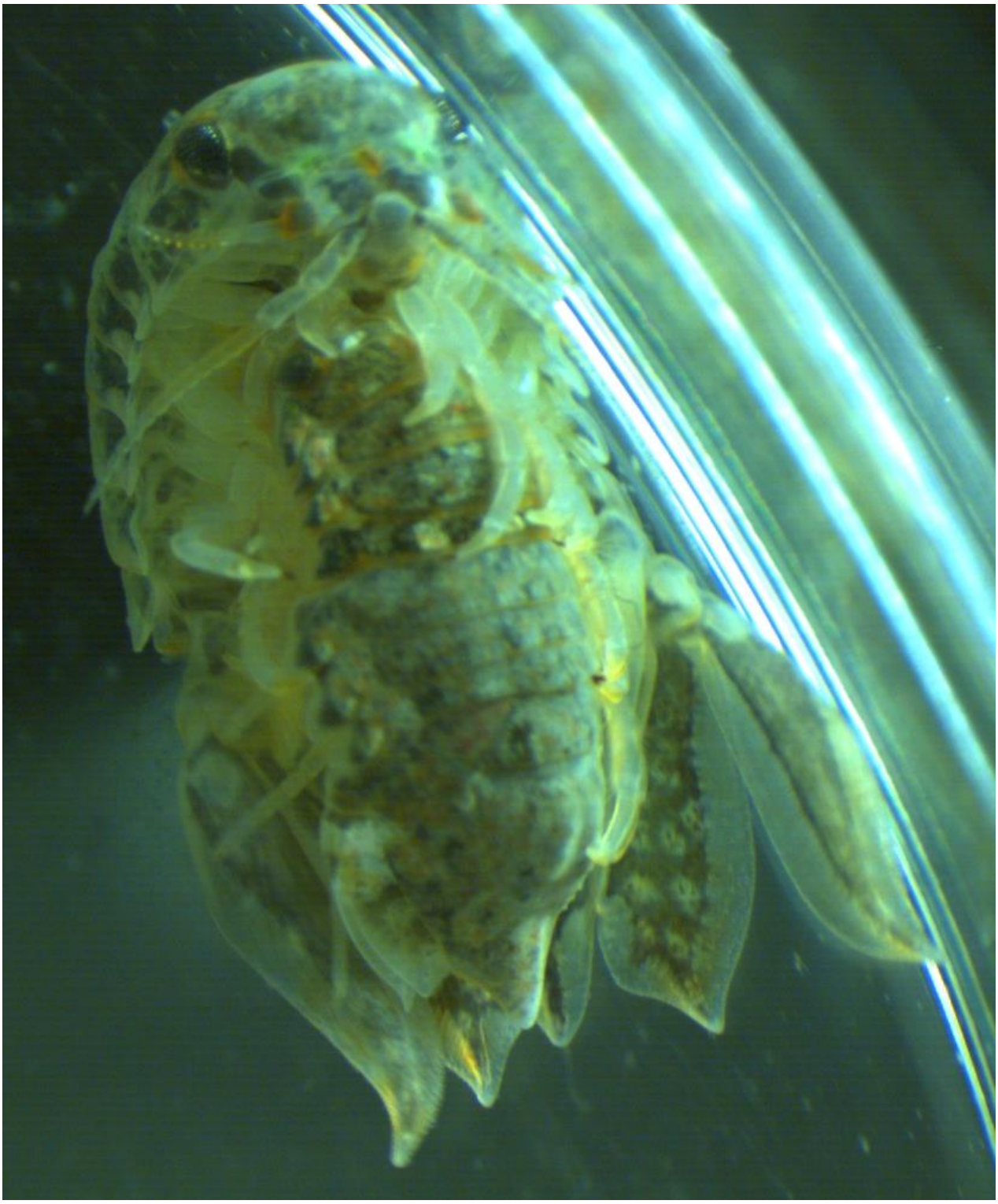


Figure 3.20: Male and female undergoing copulation with female facing male.

Discussion

In this study, I observed and describe for the first time the reproductive stages that both male and female *Isocladus armatus* individuals undergo in their lifetime. Females have five stages in their reproductive cycle, pre-moult, half-moulted, post-moult, gravid and spent. Males have four stages until they are sexually mature, involving three moults and one spinal growth moult and are observed to be cannibalistic in the first three stages under laboratory conditions.

The mating behaviour of this species has also been described for the first time. This involves two types of mate guarding, traditional and horizontal. I assume that mate guarding of pre-moult and half-moulted females ensures that the male is present during the females very short half-moulted stage where she is sexually receptive. Copulation was observed in the laboratory for the first time, it consisted of eight rotations for the female isopod and lasted up to an hour. The sperm of *Isocladus armatus*, like other isopods, is pseudoflagellae and does not move, therefore males possess an appendix masculina to act as a funnel to transfer the sperm into the females oopores.

During interactions between mature males and females in different stages of their reproductive cycle, males initiated more interactions with half-moult females than post-moult females which is what I expected because females are sexually receptive in their half-moult state. However, to my surprise male-initiated interactions lasted longer when a female was in her post-moult state. None of these interactions took into account any copulation that may have occurred. Females were typically not very interested in initiating any interaction with the males, however it

is interesting to note that pre-moult females initiated the most interactions with the males than any other stage female.

Male #090 did not seem interested in any of his females he was paired with, he did have a large range in female sizes, but two of the three females were all smaller than him so I can rule out larger females discouraging him. I may have categorised him wrong and he could have been a Stage 3 instead of a stage 4 male.

Isocladus armatus exhibits clear sexual dimorphism, like many other sphaeromatid isopods (Iverson, 1982). Males are on average larger than females, and possess an elongated spine protruding from perionite 7, dorsally along the length pleotelson towards the posterior end of the isopod. This spine has a small tooth on either side. The male uropods are large with a strongly sigmoidal shape. The uropods are lanceolate, where the apex is acute and turned outwards (Figure 3.2 & 3.20).

Females, unlike the males, do not possess the teeth or a spine on peronite 7. Their uropods however, do resemble the same shape as male uropods but are much smaller in comparison and are not displayed. The female uropods are usually tucked under the body and are only visible from the dorsal side whilst swimming.

I observed females to be typically brighter in colour than males with the males dulling out and gaining a green tinge as they become older (Most noticeable on white males) - however it is uncertain if males only become green due to being in captivity, possibly caused by the algae pallets they were being fed or if this is a naturally occurring colour change that happens during the ageing of wild male isopods.

As this is a preliminary study there are still many things that are unknown about the sexual dimorphism and development of this species. Future studies could focus on determining the number of growth moults a juvenile *Isocladus armatus* individual undergoes before they reach 3mm in length and start showing signs of genitalia. Determining the use of the males spine and the purpose of having fanned out uropods - is it ornamental or is it used for fighting and warding off other males or predators? - would be fascinating lines of future research.

Chapter 4:

Captive breeding and inheritance of colour polymorphism in *Isocladus armatus*

Abstract

Colour polymorphism is defined as when individuals display two or more distinct, genetically inherited colour variants within a single interbreeding population. It is commonly seen in many animals and plants throughout the world. *Isocladus armatus* is a species of marine isopod that has astonishingly diverse colouration patterns - however it is not known whether this colour variability is genetically determined, and hence whether it constitutes a genetic polymorphism. Although this New Zealand endemic marine isopod has among the highest amount of colour variation compared to other isopod species, there are only a few published studies on the biology of this species. Having knowledge on whether the colour variability that *Isocladus armatus* displays is genetically controlled, is essential knowledge needed to inform whether using this species as a model population will help answer wider questions looking into how genetic variation is maintained in natural populations. The aim of this study was to breed *Isocladus armatus* in laboratory conditions and to test if the colour variability was genetically determined. Although the results of my breeding experiments cannot resolve the specific genetic mechanisms, they demonstrate a clear and strong genetic base to colour variability in this species, consistent with patterns of Mendelian inheritance, indicating that the colour variation does represent a genetic polymorphism.

Introduction

When individuals in a population display two or more genetically based colour variants within a single interbreeding population it is defined as colour polymorphism (Roulin, 2003; Gray & Mckinnon, 2007). Colour polymorphism is genetically inherited and relatively easily quantified (Roulin, 2003; Gray & Mckinnon, 2007). Colour polymorphism can provide a

tractable system for scientists to understand how genetic variation is maintained in the natural population of a target species (Gray & Mckinnon, 2007). The ways in which genetic variation is maintained in a population has been a key question asked by evolutionary biologists over many years (Barton, 1989; Pryke & Griffith, 2005; Mckinnon & Pierotti, 2010). Colour polymorphism can be seen in many species of bird, snail and other organisms in not only New Zealand but also across the world (Reid, 1987; Barton, 1989; Pryke & Griffith, 2005; Mckinnon & Pierotti, 2010).

Isocladus armatus is a species of isopod crustacean that has extraordinarily high levels of colour variability. As such, having knowledge of the putative genetic basis to colour variants is critically needed to validate the potential for research on the maintenance of genetic diversity within these isopod populations (Wells & Dale, 2018). However, the hypothesis that the colour variations in *Isocladus armatus* are genetically controlled has not yet been tested. These tests are necessary because it is also possible that colour variation is environmentally determined (see below).

In isopods, diversity in colouration is often strongly genetically influenced (Table 4.1; Roulin, 2003; Gray & Mckinnon, 2007; Shuster et al, 2014; Wellenreuther et al, 2014). Colour polymorphism is relatively common in littoral isopods (Naylor, 1972; Enckel, 1980). A previous study that was conducted on Sphaeromatid isopods, found that the colour polymorphisms are controlled by dominant Mendelian alleles, at a low frequency within a population (Shuster et al, 2014). In their study, Shuster et al (2014) indicated that this mechanism for colour polymorphism inheritance is typical of many isopod crustaceans (Shuster et al, 2014). Another study conducted on *Idotea baltica* found that the variation of colour polymorphism frequencies may indicate frequency dependent selection (Jormalainen & Merilaita, 1994). A third study was interested in

the terrestrial isopod *Porcellio dilatatus*, this study provided evidence that the inheritance patterns of colour morphs can be very simple, such as being controlled on a single locus (Sassaman & Garthwaite, 1980).

I reviewed the basis to colour variation in a sample of six species of marine isopods (Table 4.1). A number of isopod species have been shown to have environmentally controlled coloration, for example in two Dynamene species, *Dynamene bidentata* and *Dynamene magniorata,* individuals have a background coloration caused by to pigments in the epidermis and cuticle derived from the algal food they consume. This environmentally caused variation results in 5 major colour morphs for *D. bidentata* and 9 main colour morphs or *D. magniorata* (Table 4.1) (Arrontes, 1969; Holdich, 1969; Holdich, 1976; Schotte, 2008; van der Land & Schotte, 2008).

Those results however contrast with other species. The number of major colour morphs for *Idotea baltica, Sphaeroma rugicauda* and *Venezillo evergladensis* are 6, 5 and 6 respectively, and the colour morphs for these species have been shown to be genetically controlled (See Table 4.1) (West, 1964; Bishop, 1969; Marsdon, 1976; Khazaeli & Heath, 1979; Johnson, 1984; Jormalainen et al, 1995; Kotta et al, 2000; Orav-Kotta & Kotta, 2004; van der Land et al, 2009; Claus et al 2019; Taiti, 2016).

Finally, Cymodoce emarginata has 4 major colour morphs, however it still unknown how their colour morphology is controlled (Table 4.1) (Arrontes, 1991; Schotte, 2008; van der Land et al, 2019).

Table 4.1: Detailing different species of isopod, what environment they live in, the number of colour morphs the species possesses and if research has found the colour morphs genetically for environmentally controlled.

Species Name	Family	Environment	Number of major colour morphs	Genetic or environmentally conrolled
Idotea baltica	Idoteidae	Benthic	6	Genetic
Sphaeroma rugicauda	Sphaeromatidae	Intertidal	5	Genetic
Dynamene bidentata	Sphaeromatidae	Intertidal	5	Environmental
Dynamene magnitorata	Sphaeromatidae	Intertidal	9	Environmental
Cymodoce emarginata	Sphaeromatidae	Intertidal	4	Unknown
Venezillo evergladensis	Armadillidae	Terrestrial	6	Genetic

Isocladus armatus are a species of Sphaeromatid marine isopod, endemic to intertidal rock pools and semi-sheltered shores around New Zealand (Wells & Dale, 2018). Unlike other species of marine isopod, *Isocladus armatus* have a highly unusual and extreme degree of colour variability. These colour variations are visible on the dorsal side of their hard exoskeletons (Jansen, 1971; Wells & Dale, 2018). Within their large degree of colour variation, *Isocladus armatus* have (at least) five distinguishable colour morphs that can be distinguished (Wells & Dale, 2018). These colour morphs can be categorised as “White”, “Striped”, “Spotted”, “Variegated” and “No Pattern” (Figure 28) (Wells & Dale, 2018). Comparatively little is known about this species due to this abundant New Zealand endemic isopod species being largely overlooked by biologists (Jansen, 1971; Wells & Dale, 2018).

Figure 4.1: Colour polymorphism in *Isocladus armatus*. (Top: Left to Right) variegated, striped, No Pattern, White (Bottom: Left to Right) No pattern, Variegated, Striped, Spotted. (Retrieved from: Wells & Dale, 2018).

In this chapter, I test whether there is a genetic basis to colour variability in *Isocladus armatus*. I hypothesize that colour variation in *Isocladus armatus* has a genetic basis. Specifically, my hypothesis is that colour polymorphism in *Isocladus armatus* is controlled by Mendelian inheritance. The main question I attempt to answer is: Does colour polymorphism in *Isocladus armatus* follow Mendelian inheritance and do the alleles that are coding for the expression of colour morphs become inherited in the traditional Mendelian style?

Other studies that have looked at isopod colour polymorphism have suggested that the most common mode of allele inheritance within these crustaceans is dominant mendelian alleles, with low frequencies in the population (Shuster, 1989).

Methods/ Materials

Collection and care of isopods

See chapter 2 for collection methods and care methods of *Isocladus armatus.*

Captive breeding

Wild, female *Isocladus armatus*, who conceived in the wild, released offspring in the lab, this became generation F1. Virgin isopods from generation F1 were used as breeding stock for this study once they reached sexual maturity. The sex and maturity of the isopods released in the lab were observed and distinguished by the development of penes and oostegites on the ventral cavity surface (Chapter 2).

I established crosses by pairing two individuals of the same morph together to try and get the offspring as close to being homozygous for their one colour morph as possible. Due to the isopods all maturing at different times, in some cases it was not possible to pair an isopod with one of the same colour morph - in these cases the isopod was paired with a No Pattern individual, or another individual at the same stage of development.

Once the isopods were paired up (one male and one female), each pair of isopods were given a code relating to their generation number and the colour morph of the Dam and Sire. The code components used were Wh (White), Var (Variegated), NP (No Pattern), SP (Spotted), and ST (Striped). An example of a code would be F1STST1 meaning this pairing was made up of

two F1 generation isopods (F1), The isopods were both striped (STST) and this is the first Striped x Striped pairing for the F1 generation (STST1).

Males were left in the cups with the females until the female had undergone her half-moult stage and was in her post-moult stage (Chapter 2). Once the female isopod was in her post-moult stage, the male was removed from the cup and put into a male-only pot. The male's colour was recorded and photographed for identification later.

I observed *Isocladus armatus* providing some parental care to their offspring where the offspring stay attached to the mother (Chapter 3) and are dropped periodically over one to two weeks. Once the offspring had been dropped by the Dam, the mancas were observed under a dissection microscope and the colour morph for each individual manca was recorded. The frequencies of each morph in each brood were then calculated.

A total of 49 captive pairings were set up, but the female died before releasing mancas in 25 of these pairings. In eight of the remaining 24 pairings the female underwent her moult cycle back to a pre-moult female without releasing any mancas, leaving a total of 15 successful breedings.

Data Analysis

I had two types of genetic results, those obtained from gravid females collected from the field (Dam phenotype known, Sire phenotype unknown) and those from controlled crosses in which both parental phenotypes were known. To broadly ascertain whether there was a genetic basis to the colour variability in *Isocladus armatus*, I tabulated the number of offspring of each morph as a function of the Dam morph and as a function of the Sire morph (when known). I

predicted that there if there was no genetic basis to colour variability, then the morphs in the offspring of all broods would be similar to the morph distribution found in the wild (based on random sampling). Alternatively, if there is a strong genetic basis to colouration then I expected the morphs of offspring to be the same as the morphs of the Dams and Sires respectively. Patterns of mendelian inheritance would be inferred through particulate inheritance, namely broods of offspring having 1 or 2 morphs present in ratios consistent with heterozygosity coupled with dominant alleles for particular morphs over others.

In the Appendix I have included comparisons of subsets of the broods with brief discussions of possible genetic mechanisms behind the observed patterns (lumped by the morphs of the parents) and analysed with heterogeneity G-tests (Shuster et al, 2014). In general these analyses indicate consistency with Mendelian patterns of inheritance.

Results

Wild Isocladus armatus colour morphs

The frequency of colour morphs from the wild female *Isocladus armatu*s sampled at random are provided in Table 4.2. No Pattern had the largest presence with 173 individuals, followed by the next largest being Variegated with 82 individuals, Striped had 25 individuals, White had 16 individuals and Spotted having just 3. There were other colour morphs that were also collected from the field - they are included in this table but categorised into the closest of the five colour morphs this study is focussing on. Green colour morphs were mainly seen in 19 male individuals collected, and there was one example in the female category and one in the juvenile category, these individuals have been placed into the No Pattern category. Three males collected

were a green striped colour morph, these were placed in the striped category. Finally, 4 orange morphs were collected and categorised as a No Pattern morph, this colour was found only in females twice, and juveniles twice.

Knowing the frequencies of each morph in the wild will help us understand the frequencies of colour morphs I gained from breeding captive *Isocladus armatus* individuals.

Table 4.2. The frequencies of colour patterns from wild *Isocladus armatus* individuals collected from Stanmore Bay beach. Note that a small number of additional putative morphs (e.g "Orange") beyond the five described earlier are included in this table (see text).

	Colour Morph					
Sex	No Pattern	Varigated	Striped	White	Spotted	Total
Male	89	32	18	7	1	147
Female	66	32	7	6	2	113
Juvenile	18	18	0	3	0	39
	173	82	25	16	3	299

Breeding crosses

Some females collected from the wild that were gravid dropped their mancas in the laboratory. Others that were not gravid were crossed in the laboratory following controlled methods (Chapter 2). Table 4.3 is a collation of the 25 successful breedings and manca releases that I observed in the laboratory. Crosses where the Sire is unknown are females that copulated in the wild and females where the males colour morph is known are ones that were crossed in captivity (Table 4.3).

I compared offspring colour morphs versus Dam colour morphs (Table 4.4). Eight dams that were No Pattern had a total of 371 No Pattern, 161 Variegated, 36 white and 29 striped

juveniles (Table 4.4). Ten Striped dams produced a total of 351 striped, 259 No Pattern, and 166 White juveniles (Table 4.4). Four Variegated Dams produced 86 Variegated, 72 No Pattern, 27 Striped, and 11 White juveniles (Table 4.4). Two White Dams produced 56 White, 27 No Pattern and 19 Striped juveniles (Table 4.4).

One white Dam produced 39 No pattern juveniles (28 of these were an Orange morph and categorised as No Pattern, although they are likely a new previously unrecognized morph), 21 Spotted and 17 White juveniles. No other dam of another colour morph produced spotted juveniles.

Table 4.3: Summary showing the Dam, Sire and mancas morph of crosses that were observed during this study.

	Parental phenotypes		Offspring phenotypes					
cross	Dam	Sire	NP	STR	VAR	WHI	SPO	Total
1.3	NP	unkn	38	0	0	36	0	74
1.4	NP	unkn	29	0	42	0	0	71
1.5	NP	unkn	42	0	37	0	0	79
1.9	NP	unkn	92	0	0	0	0	92
2.2	NP	NP	13	0	11	0	0	24
2.3	NP	unkn	25	29	0	0	0	54
2.4	NP	VAR	59	0	48	0	0	107
2.5	NP	VAR	19	0	23	0	0	42
1.1	STR	unkn	35	41	0	0	0	76
1.2	STR	unkn	28	33	0	0	0	61
1.14	STR	WHI	49	35	0	96	0	180
2.1	STR	STR	28	58	0	0	0	86
2.7	STR	NP	41	45	0	0	0	86
2.8	STR	STR	24	25	0	37	0	86
2.9	STR	STR	25	56	0	0	0	81
2.11	STR	STR	3	10	0	0	0	13
2.12	STR	STR	14	32	0	0	0	46
2.13	STR	STR	12	16	0	33	0	61
1.1	VAR	unkn	4	0	10	11	0	25
1.7	VAR	unkn	21	0	35	0	0	56
2.14	VAR	NP	41	0	41	0	0	82
2.15	VAR	VAR	6	27	0	0	0	33
2.6	WHI	NP	9	0	0	15	0	24
2.16	WHI	STR	18	19	0	41	0	78
1.08	SPO	unkn	39	0	0	17	21	77
			714	426	247	286	21	**1694**

Table 4.4: Dam colour morph vs offspring colour morph (number of broods indicated in the parantheses).

	Dam morph				
Offspring morph	No Pattern (8)	Striped (10)	Variegated (4)	White(2)	Spotted(1)
NP	317	259	72	27	39
STR	29	351	27	19	0
VAR	161	0	86	0	0
WHI	36	166	11	56	17
SPO	0	0	0	0	21

Table 4.5: Sire colour morph vs offspring colour morph (number of broods indicated in the parantheses).

	Sire morph				
Offspring morph	No Pattern (4)	Striped (7)	Variegated (3)	White(1)	Spotted(0)
NP	104	124	84	49	0
STR	45	216	27	35	0
VAR	52	0	71	0	0
WHI	15	111	0	96	0
SPO	0	0	0	0	0

I completed a second comparison, and this was that of Sire colour morph versus juvenile colour morphs produced (Table 4.5). No Pattern sires produced 104 No Pattern, 52 Variegated, 45 Striped and 15 White juveniles (Table 4.5). Striped sires produced 216 Striped, 124 No Pattern and 111 White juveniles (Table 4.5). Variegated sires produced 84 No Pattern, 71 Variegated and 27 Striped juveniles (Table 4.5). Finally, White sires produced a total of 96 White, 49 No Pattern and 35 Striped juveniles. There were no successful crosses with Spotted sires however, no other colour morph sire produced any Spotted juveniles.

Conclusion

The results of this study strongly suggest there is genetic control of colour polymorphism in *Isocladus armatus* and that it is clearly heritable. Table 4.4 and 4.5 show that if a parent is one colour than it will most likely produce the most offspring of that same colour morph, although they frequently produce one or two other morphs as well, indicating Mendelian inheritance.

For the No Pattern offspring, there was a tendency for such morphs to be present in all the observed broods, however they were much more common in broods where at least one of the parents was a no pattern. Striped offspring also had a presence in most broods (except for Spotted broods), however Striped offspring were most common in broods where at least one of the parents was a Striped pattern. Both of the No Pattern and Striped morphs display possible allele dominance over the other colour morphs especially when one of the parents is either Striped or No Pattern.

Variegated offspring were a little more exclusive, only being present in broods where a parent was either No Pattern or Variegated but had a larger presence when the Dam was No Pattern, or the Sire was Variegated. Table 4.3 shows that the No Pattern dams that produced Variegated offspring were either paired with a sire that was Variegated or an unknown sire (that was possibly a Variegated wild sire). With one exception, in cross 2.2 (Table 4.3) where both parents of the brood were No Pattern, they produced almost a 50/50 split of No Pattern and Variegated offspring.

White offspring, interestingly was much more common when a Dam was Striped. This is demonstrated in cross 1.14 (Table 4.3) where the Dam was Striped BUT the sire was White and the majority of offspring produced (96) were White. This trend was also repeated in cross 2.16 (Table 4.3) where the Dam was White and the sire was Striped producing the highest number of

offspring White (41). There is clear inheritance shown here, however I think this is more complex than with the other colour morphs.

Finally, the Spotted morph was the most exclusive among the offspring and was only observed with Spotted parents. This clearly indicates that a Spotted parent was needed to produce Spotted offspring and therefore is highly heritable and possibly a recessive trait.

Some of the observed patterns may not be as straightforward as what was originally hypothesised due to the discovery of three different forms of possible epistasis (see appendix) between the Striped, White and Variegated colour morphs. These results suggest that there may be a link between the striped and White colour morphs, as well as a possible form of epistasis. However more research and possible genetic testing may be needed to confirm the form of inheritance these colour morphs are undergoing.

There was also one cross that is difficult to explain (Cross 1.08), where a Spotted dam from the wild was crossed with an unknown sire. The results of this cross were difficult to interpret, because the juvenile colour morphs that the Spotted dam released were 11 No Pattern, 17 White, 21 Spotted and 28 Orange mancas (a previously undescribed morph). This make it hard to conclude what colour morph the sire was (for the purpose of table 4.3 and this study the Orange mancas were added into the No Pattern category making this cross have a total of 39 No Pattern juvenile morphs. Given that this brood was the only one to have Orange mancas, it strongly suggests that this morph has a specific allele associated with it.

Cross 2.8 had white and no patterned individuals with pink undertones. This could also be explained by superimposition with one colour morph over another, as seen in the Hawaiian happy face spider (Oxford & Gillespie, 1995; Oxford & Gillespie, 1996), a mangrove snail

Littoraria filose (Reid, 1987) and many more organisms. A study done on *T. grallator* found that a combination of a red line morph and a red front and back morph gives rise to a red ring morph, which is one morph overlaid on the second morph (Oxford & Gillespie, 1996). What was observed in *T. grallator* could be what is being seen in the STWH cross and the STST cross where there are many White and No Pattern morphs that seem to be overtaking the Striped morphs. In future studies, crosses need to be conducted where the exact genotypes of the parents are known -this would give us a larger insight into the dominance of the White and Striped phenotypes and how they affect inheritance. The results of my work however, strongly indicate that colour variation in *Isocladus armatus* is based on a number of alleles that are inherited in a Mendelian fashion.

Limitations of the study included the number of female isopods of each morph that I was able to be breed - it would have strengthened my results and conclusions if I had access to more of the 'rare' morph and to more of the Spotted and Variegated morphs as little data was gathered from these varieties. Another limitation was the fact that the isopods were dying or there was cannibalistic eating of each other. This could be due to the isopods missing a vital aspect of their natural environment that they are not receiving under laboratory conditions.

This study was the first research project that has successfully bred *Isocladus armatus* under laboratory conditions. This is important as it has given us a strong baseline to help understand the genetic variation and inheritance of these animals and how this variation is maintained in *Isocladus armatus* populations and their natural environment.

Chapter 5:

Summary and Concluding Remarks

Isocladus armatus is a New Zealand endemic isopod that displays huge amounts of colour variability. In Chapter 2, I compared the spatial distribution of *Isocladus armatus* individuals residing at Stanmore Bay Beach, Auckland to the study that was conducted by Jansen in 1971 on the Kaikoura Peninsula. The results from the Stanmore Bay Beach transects complemented the results that Jansen got in 1971 – specifically that *I. armatus* are most abundant between the neap low tide and neap high tide lines. Body lengths between juveniles, and sexually mature males and females were compared and contrasted. These measurements indicated there was a relatively pronounced sexual size dimorphism with sexually mature males being and approximately 1.5 times the length of females. These results indicate that there has likely been strong sexual selection occurring on males of this species, although future research is needed to resolve specific details about this aspect of their biology.

I investigated the sexual dimorphism in this species further in chapter 3, with a focus on the morphological and developmental differences between males and females. Males display their large uropods in a peacock-like fashion whereas the female uropod is not displayed and the only time they are seen is when the female is swimming.

Isocladus armatus individuals are almond shaped, females are smooth on the dorsal side of their exoskeleton unlike the male that possesses a dorsal spine (see below).

The female reproductive cycle consisted of 5 stages; pre-moult, half-moult, post-moult, gravid and spent. Female *Isocladus armatus* individuals have 8 brood pouches, oostegites and oopores.

Male *Isocladus armatus* are also almond shaped but they possess a spine and teeth that protrude from perionite 7, males also have 2 triangular shaped penes with pesudoflagulate sperm. Males go through four stages before they are sexually mature, in the first three they are cannibalistic (at least in captivity).

Chapter 4 focused on breeding of *Isocladus armatus* and observed patterns in the mancas colour polymorphism. Broods that were produced in this study suggest that colour polymorphism in these animals is genetically controlled, based on alleles that assort in a Mendelian fashion, although the exact Mendelian mechanisms were not identified.

As this species has not been extensively studied, there is ample opportunity for further research. This includes things such as the function for the male spine, and how many moults juveniles undergo before developing genitalia. There is a need for a deeper look into the genetic control of colour polymorphism - now we know that the colour variability is in fact genetically controlled. Other things that are unknown and still need to be addressed include if the colour polymorphism functions as a camouflage from predators or if it plays a part in mate selection in this species. Having knowledge of the above are all things that will enhance our understanding of *Isocladus armatus* as a model organism and also our understanding of the wider question of how colour polymorphism is controlled in natural populations.

References

Arrontes, J. (1991). Colour Polymorphism in relation to spatial distribution in some intertidal isopods in northern spain. *Journal of the Marine Biological Association of the United Kingdom, 71*, 749-758

Barton, N. H. (1989). Evolutionary quantitative genetics: How Little do we know? *Annual review of genetics 23*, 337-370

Bertin, A., David, A., Cezilly, F., & Alibert, P. (2002). Quantification of sexual dimorphism in *Asellus aquaticus* (Crustacea: Isopoda) using outline approaches. *Biological Journal of the Linnean Society, 77,* 523-533

Bishop, J. A. (1969). Changes in Genetic Constitution of a population of *Sphaeroma rugicauda* (Crustacea: Isopoda). *Evolution, 23*(**4**), 589-601

Bond, A. B. (2007). The evolution of colour polymorphism: crypticity, searching images, and apostatic selection. *Annual Review of Ecology, Evolution and Systematics, 38,* 489-514

Cartier, J., Rudolph, J., & Stewart, J. (2001). The nature and structure of scientific models. *The National Center for Improving Student learning and Achievement in Mathematics and Science (NCISLA),* 1-8

Claus, S., Schotte, M., & Boyko, C. B. (2019, February 24). *Sphaeroma rugicauda* Leach, 1814. https://www.marinespecies.org/aphia.php?p=taxdetails&id=146936

Collins, A., & Stewart, J. H. (1989). The knowledge and structure of mendelian genetics. *The American Biology Teacher, 51*(**3**), 143-149

Cowen, R. K., & Sponaugle, S. (2009). Larval dispersal and marine population connectivity. *Annual Review of Marine Science, **1**,* 443-466

Dale, J., Lank, D. B., & Reeve, H. K. (2001). Signalling individual identity versus quality: A model and case studies with ruffs, queleas and house finches. *American Naturalist, 158,* 75-86

Darwin, C. (1871). The descent of man and selection in relation to sex, John Murray

Dasmahapatra, K. K. (2012). Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. *Nature, 487,* 94-98

Endler, J. A. (1977). Geographic Variation, Speciation and Clines. *Princeton University Press: Princeton*

Environment Foundation. (2018, April 10). *Environment guide.* New Zealand's Biodiversity. http://www.environmentguide.org.nz/issues/biodiversity/

Futuyma, D. J. (2005). *Evolution.* Sinauer Associates Inc.

Gillespie, R. G., & Tabashnik, B. E. (1989). What makes a happy face? Determinants of colour pattern in the Hawaiian happy face spider *Theridion grallator* (Araneae, Theridiidae). *Heredity 62,* 355-363

Gray, S. M., & McKinnon, J. S. (2007). Linking color polymorphism maintenance and speciation. *Trends in Ecology and Evolution 22*(**2**), 71-79

Hartl, D. L., Clark, A. G., & Clark, A. G. *Principles of population genetics.* Volume 116. Sinauer Associates Inc.

Haavie, J, et al. (2000). Discrepancies in population differentiation at microsatellites, mitochondrial DNA and plumage colour in the pied flycatcher – inferring evolutionary processes. *Molecular Ecology 9*, 1137-1148Hedrick, A. V., & Temeles, E. J. (1989). The evolution of sexual dimorphism in animals: hypotheses and tests. *Tree, 4*(**5**), 136-138

Hedrick, P. W. (1986). Genetic Polymorphism in heterogeneous environments: a decade later. *Annual Revision of Ecological, Evolution and Systematics, **17**,* 535-566

Hoekstra, H. E., Drumm, K. E., & Nachman, M. W. (2004). Ecological genetics of adaptive color polymorphisms in pocket mice: geographic variation in selected and neutral genes. *Evolution, 58,* 1329-1341

Holdich, D. M. (1969). Polychromatism in the genus Dynamene (Crustacea: Isopoda). *Pubblicazioni Della Stazione Zoologica di Napoli, 37*, 18-27

Holdich, D. M. (1976). A comparison of the Ecology and life cycles of two species of Littoral Isopod. *Journal of Experimental Marine Biology and Ecology, 24,* 133-149

Holdich. D. M., & Harrison. K. (1980). The isopod genus *Dynamene* from Australian waters, with description of a new species from coral reefs. *Memoirs oth the Queensland Museum, 20*(**1**), 163-170

Hurley, D. E., & Jansen, K. P. (1977). The marine fauna of New Zealand: Family Sphaeromatidae (Crustacea Isopod: Flabellifera). Universal Printers.

Iverson, E. W. (1982). Revision of the isopod family Sphaeromatidae (Crustacea: Isopoda: Flabellifera) I. subfamily keys with diagnoses and keys. *Journal of Crustacean Biology 2*(2), 248-254

Jansen, K. P. (1968). A comparative study of intertidal species of Sphaeromidae (*Isopoda falabellifera*). *PhD*. New Zealand: University of Canterbury.

Jansen, K. P. (1971). Ecological studies on intertidal New Zealand Sphaeromatidae (Isopoda: Flabellifera). *Marine Biology 11*, 262-285

Johnson, C. (1984). Geographic variability in the genetic polymorphism of the Isopod, *Venezillo evergladensis. Journal of Natural History, 18,* 465-474

Jormalainen, V., & Merilaita, S. (1944). Differential predation on sexes affects colour polymorphism of the isopod *Idotea baltica* (Pallis). *Biological Journal of the Linnean Society 55,* 45-68

Jormalainen, V., Merilaita, S., & Tuomi, J. (1995). Differential predation on sexes affects color polymorphism of the isopod *Idotea baltica* (Pallas). *Biological Journal of the Linnean Society,* 55, 45-68

Khazaeli, A. A., & Heath, D. J. (1979). Colour Polymorphism, selection and the sex ratio in the Isopod *Sphaeroma Rugicauda* (Leach). *Heredity 41*(**2**), 187-199

King, R. B., & Lawson, R. (1995). Color-pattern variation in Lake Erie water snakes: the role of gene flow. *Evolution, 49,* 885-896

Kotiaho, J. S., Simmons, L. W., & Tomkins, J. L. (2001). Towards a resolution of the lek paradox. *Nature, **410**,* 684-686

Kotta, J., et al. (2000). Major Changes in Macroalgae Community Composition Affect the Food and Habitat Preference of *Idotea baltica. International Review of Hydrobiology, 85*(**5-6**), 697-705

Lank, D. B., Smith, C. M., Hanotte, O., Burke, T., & Cooke, F. (1995). Genetic polymorphism for alternative mating behaviour in lekking male ruff. *Nature, 378,* 59-62

Lewontin, R. C. (1974). The Genetic Basis of Evolutionary Change. *Columbia University Press: New York*

Marsdon, I. D. (1976). Effect of Temperature on the Microdistribution of the Isopod *Sphaeroma rugicauda* from a Saltmarsh Habitat. *Marine Biology 38,* 117-128

Mckinnon, J. S., & Pierotti, M. E. R. (2010). Colour polymorphism and correlated characters: genetic mechanisms and evolution. *Molecular Ecology 19,* 5101-5125

Merilaita, S. (2001). Habitat heterogeneity, predation and gene flow: Colour polymorphism in the isopod, *Idotea baltica. Evolutionary Ecology, 15,* 103-116

Moreira, P. S. (1974). Cryptic protective coloration in *Serolis polaris* (Isopoda: Flabellifera). *Crustaceana (Leiden),* 27, 1-4

Muñoz, M., et al. (2013). Divergence in coloration of reproductive isolation in the *Anolis marmoratus* species complex. *Molecular Ecology, 22,* 2668-2682

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature, 403,* 853-858

Olendorf, R., et al. (2006). Frequency-dependent survival in natural guppy populations. *Nature, **441**,* 633-636

Orav-Kotta, H., & Kotta, J. (2004). Food and Habitat choice of the isopod *Idotea baltica* in the northeastern Baltic Sea. *Hydrobiologica, 514,* 79-85

Oxford, G.S., & Gellespie, R. G. (1995). The effects of genetic background on the island-specific control of a colour polymorphism in *Theridion grallator* (Araneae: Theridiidae), the Hawaiian happy-face spider *76,* 257-266

Oxford, G. S., & Gillespie, R. G. (1996). Genetics of a colour polymorphism in *Theridion grallator* (Araneae: Theridiidae), the Hawaiian happy-face spider, from Greater Maui. *Heredity 76,* 238-248

Palumbi, S. R. (1994). Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics, 25,* 547-572

Pamilo, P. (1988). Genetic variation in heterogeneous environments. *Annales Zoologici Fennici,* 99-106

Pryke, S. R., & Griffith, S. C. (2005). Red dominates black: agonistic signalling among head morphs in the colour polymorphic Gouldian finch. *Proceedings of the royal society B 273*(1589), 949-957

Rasmussen, R. A. (1965). The intertidal ecology of the rocky shores of the Kaikoura Peninsula. *PHD Thesis, Christchurch: Department of Zoology, University of Canterbury.*

RDocumentation. (2019).

Reece, J. B., Meyers, N., Urry, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V., Jackson, R. B., & Cooke, B. N. (2015). Mendel and the Gene idea. *Campbell Biology*, 10, 267-291

Reid, D. G. (1987). Natural selection for apostasy and crypsis acting on the shell colour polymorphism of a mangrove snail, *Littoraria filosa* (Sowerby) (Gastropoda: Littorinidae. *Biological Journal of the Linnean Society 30*, 1-24

Relethford, J. H. (2000). Human Skin Colour Diversity is highest in sub-Saharan African Populations. *Human Biology 72*(**5**), 773-780

Roulin, A. (2003). The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. *Biological Reviews, 79*, 815-845

Sassaman, C., & Garthwaite, R. (1980). Genetics of a pigment polymorphism in the isopod *Porcellio dilatatus. The Journal of Heredity 71*(158), 158-160

Schotte, M. (2008, January 03). *Dynamene bidentata* (Adams, 1800). https://www.marinespecies.org/aphia.php?p=taxdetails&id=256988

Schotte, M. (2008, January 03). *Isocladus armatus.* http://www.marinespecies.org/aphia.php?p=taxdetails&id=258365#sources

Shuster. S. M. (1989). Male Alternative reproductive strategies in a marine Isopod Crustacean (*Paracerceis sculpta*): The use of genetic markers to measure differences in fertalisation success among α-, β-, and γ-Males. *Evolution, 43*(**8**) 1683-1698

Shuster, S. M. (1991). Changes in female anatomy associated with the reproductive moult in *Paracerceis sculpta,* a semelparous isopod crustacean. *The Journal of Zoology, **225,** **365-379***

Shuster, M. S.,et al. (2014). The inheritance of autosomal and sex-linked cuticular pigmentation patterns in the marine isopod, *Paracerceis sculpta.* (Isopoda: Sphaeromatidae). *Journal of Crustacean Biology 34*(4), 460-466

Shuster, M. S., & Guthrie, E. E. (1999). Effects of temperate and food availability on adult body length in natural and laboratory populations of *Paracerceis sculpta* (Holmes), a Gulf of California isopod. *Journal of Marine Biology and Ecology, 233*, 269-284

Slatkin, M. (1987). Gene flow and the geographic structure of natural-populations. *Science, 236,* 787-792

Stephenson, B. (2002, November 22). *Isocladus armatus.* https://www.aucklandmuseum.com/collections-research/collections/record/am_naturalsciences-object-247151?k=%22Armoured%20Isopod%22&ordinal=12

Stevens, M., & Merilaita, S. (2009). Animal camouflage: current issues and new perspectives. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 364, 423-427

Taiti. S. (2016, October 12). *Venezillo evergladensis* Schultz, 1963. https://www.marinespecies.org/aphia.php?p=taxdetails&id=887843

Tripathi. N., et al (2009). Genetic linkage map of the guppy, *Poecilia reticulata*, and quantitative trait loci analysis of male size and colour variation. *Proceedings of The Royal Society B*, **276**, 2195-2208

van der Land, J., & Schotte, M. (2008, January 03). *Dynamene magnitorata* Holdich, 1968. https://www.marinespecies.org/aphia.php?p=taxdetails&id=118942

van der Land, J., Schotte, M., & Boyko, C. B. (2019, June 24). *Cymodoce emarginata* Leach, 1818. https://www.marinespecies.org/aphia.php?p=taxdetails&id=118929

van der Land, J., Schotte, M., & Poore , G. (2009, February 20). *Idotea balthica* (Pallas, 1772). https://www.marinespecies.org/aphia.php?p=taxdetails&id=119039

Wellenreuther, M., Svensson, E. J., & Hansson, B. (2014). Sexual selection and genetic colour polymorphisms in animals. *Molecular Ecology, 20(***22***)*, 5398-5414

Wells, S. J., & Dale, J. (2018). Contrasting gene flow at different spatial scales revealed by genotyping-by-sequencing in *Isocladus armatus*, a massively colour polymorphic New Zealand marine isopod. *PeerJ,* DOI 10.7717/peerj.5462

West, D. A. (1964). Polymorphism in the Isopod *Sphaeroma Rugicauda*. *Evolution, 18*(**4**), 671-684

Wilson, G. F. (1991). THIRTEEN. Functional Morphology and Evolution of Isopod Genitalia. *In Crustacean sexual biology*, 228-245. Columbia University Pres

Appendix

Heterogeneity G-tests were used to evaluate the inheritance patterns of each morph compared to the expected values based on specific Mendelian hypothesis, testing whether the observed frequencies of offspring deviated from expected values. These tests were conducted in R studio, Version 1.1463 (RDocumentation, 2019). Specific crosses were pooled for each analysis based on the specific Mendelian hypothesis and the morphs of the parents (including putative morphs for unknown sires). 1) Gi measures the deviation within individual crosses of observed phenotypic frequencies from Mendelian expectation; 2) GTotal measures the deviation of the sum of all Gi values from their expected magnitude given the number of tests of the genetic hypothesis 3) GPooled measures the deviation of the observed from expected phenotypic frequencies, when observed frequencies are pooled across all tests of the genetic hypothesis; and 4) GHeterogeneity measures the difference between GT and GP, thereby revealing whether observed frequencies within individual tests of the genetic hypothesis contribute disproportionately to the value of GT (Adapted from Shuster et al., 2014).

In Table A.1 the females that were classed as being crossed with an unknown sire shows that these crosses are consistent with Mendelian genetics when compared to cross 2.7 (G= 0.19) which displays clear mendelian genetics with all G scores in Table A.1 being less than 1. These crosses also suggest that the No Pattern colour morph is dominant over the Striped colour morph in this table.

Table A.1: Striped (STR) X morph other than striped

cross	Parental phenotypes Sire	Dam	Offspring phenotypes STR	NP	VAR	WHI	SPO	Total	Gi	P	Hypothesis
1.1	unkn	STR	41	35	0	0	0	76	0.47	0.49, ns	+||+ x STR||+
1.2	unkn	STR	33	28	0	0	0	61	0.41	0.52, ns	+||+ x STR||+
2.7	NP	STR	45	41	0	0	0	86	0.19	0.66, ns	+||+ x STR||+
2.3	unkn	NP	29	25	0	0	0	54	0.30	0.58, ns	STR||+ x +||+
			148	129	0	0	0	277			

		P
Gtotal[df=4]	1.37	0.84, ns
Gpooled[df=1]	1.30	0.25, ns
Gheterogeneity[	0.07	0.995, ns

The crosses with two (apparent) Striped heterozygous parents also display Mendelian genetics with a low G score (Table A.2). This supports the information I gained from table A.1. There are two crosses with juveniles that may be misclassified that also fit this hypothesis. This is cross 2.10 (G = 2.47) which had 2 juveniles classified as No Pattern that were a possible White colour morph, the G score for this cross does not support the hypothesis: STR||+ x STR ||+. Cross 2.12 is the second cross which has one juvenile classified as a No Pattern which was a possible white however, the G score for this cross 0.69 and therefore fits within the hypothesis however, they may not suit the hypothesis because they raise the total G pooled score (1.30) has significantly deviated from the Mendelian expectations (especially cross 2.10). G-heterogeneity is not significant suggesting that the crosses are consistent in their results (Table A.2).

Table A.2: Striped (STR) X Striped

cross	Parental phenotypes Sire	Dam	Offspring phenotypes STR	NP	VAR	WHI	SPO	Total	Gi	P	Hypothesis	
2.9	STR	STR	56	25	0	0	0	81	1.42	0.23, ns	STR||+ x STR||+	
2.11	STR	STR	10	3	0	0	0	13	0.03	0.86, ns	STR||+ x STR||+	
2.10	STR	STR	58	28	0*	0	0	86	2.47	0.12, ns	STR||+ x STR||+	* 2 possible white progeny; scored as NP
2.12	STR	STR	32	14	0*	0	0	46	0.69	0.55, ns	STR||+ x STR||+	* 1 possible white progeny; scored as NP
			156	70	0	0	0	226				

		P
Gtotal[df=4]	4.61	0.37, ns
Gpooled[df=1]	4.10	0.043*
Gheterogeneity[	0.51	0.44

The cross with one Variegated parent and one unknown parent in Table A.3 are consistent with the Mendelian genetics of the no pattern cross Variegated crosses 2.4 and 2.5. The sire in cross 2.2 died, he is possibly not the true sire, it could have had an unknown Variegated parent. The other results suggest that Variegated is dominant over No Pattern, this could be apparent wild type colours (Table A.3).

Table A.3: Varigated (VAR) x No pattern (NP) Plus three possible "wild type" (UNKN) sires.

	Parental phenotypes		Offspring phenotypes									
cross	Sire	Dam	NP	VAR	STR	WHI	SPO	Total	Gi	P	Hypothesis	
2.2	NP	NP	13	11	0	0	0	24	0.17	0.68, ns	+||+ x +||+	possible unkn VAR sire
2.4	VAR	NP	59	48	0	0	0	107	1.13	0.29, ns	VAR||+ x +||+	
2.5	VAR	NP	19	23	0	0	0	42	0.38	0.54, ns	VAR||+ x +||+	
2.14	NP	VAR	41	41	0	0	0	82	0.00	1.00, ns	VAR||+ x +||+	
1.5	unkn	NP	42	37	0	0	0	79	0.32	0.57, ns	VAR||+ x +||+	
1.4	unkn	NP	29	42	0	0	0	71	2.39	0.12, ns	VAR||+ x +||+	
1.7	unkn	VAR	21	35	0	0	0	56	3.54	0.06, ns	VAR||+ x +||+	
			224	237	0	0	0	461				
			P									
	Gtotal[df=7]	7.93	0.34, ns									
	Gpooled[df=1]	0.37	0.54, ns									
	Gheterogeneity[	7.56	0.27, ns									

A Variegated cross such as cross 2.15 in Table A.4 displays that the results that we see in the previous tables is consistent and the cross itself displays Mendelian inheritance.

Table A.4: Varigated (VAR) x Varigated

	Parental phenotypes		Offspring phenotypes								
cross	Sire	Dam	STR	NP	VAR	WHI	SPO	Total	Gi	P	Hypothesis
2.15	VAR	VAR	27	6	0	0	0	33	0.88	0.35, ns	VAR||+ x VAR||+
			27	6	0	0	0	33			

Cross 1.9 in Table A.5 displays the offspring phenotypes of an unknown colour morph cross a No Pattern colour morph. 100% of the offspring were no pattern suggesting the unknown

sire had a No Pattern colour morph. Therefore, suggesting that both parents were homozygous for No Pattern. This result also supports the information from the previous tables.

Table A.5: No pattern (NP) - possible "wild type"

	Parental phenotypes		Offspring phenotypes									
cross	Sire	Dam	NP	VAR	STR	WHI	SPO	Total	Gi	p	Hypothesis	
1.9	unkn	NP	92	0	0	0	0	92	0.00	1.00 ns	+||+ x +||+	possible unkn sire
			92	0	0	0	0	92	0.00			

Cross 1.3 (Table A.6) with an unknown sire is consistent with the Mendelian inheritance in cross 2.6 both crosses suggest that the No Pattern colour morph is a ‘wild type’.

Table A.6: White (WHI) x No pattern (NP) - possible "wild type"

	Parental phenotypes		Offspring phenotypes								
cross	Sire	Dam	NP	WHI	STR	VAR	SPO	Total	Gi	P	Hypothesis
2.6	NP	WHI	9	15	0	0	0	24	1.52	ns	+||+ x WHI||+
1.3	unkn	NP	38	36	0	0	0	74	0.05	ns	WHI||+ x +||+
			9	15	0	0	0	24			
	Gtotal[df=2]	1.57	0.46, ns								
	Gpooled[df=1]	1.52	0.22, ns								
	Gheterogeneity[	0.05	0.82, ns								

Both crosses in Table A.7 display a possible epistasis of the White colour morph over the Striped colour morph. This suggests that there is an independent assortment of Striped and White with white epistatic over Striped.

Table A.7: White (WHI) - possible epistasis over STR

	Parental phenotypes		Offspring phenotypes								
cross	Sire	Dam	NP	STR	WHI	VAR	SPO	Total	Gi	P	Hypothesis
1.14	WHI	STR	49	35	96	0	0	180	3.14	ns	WHI||+ +||+ x +||+ STR||+
2.16	STR	WHI	18	19	41	0	0	78	0.23	ns	+||+ STR||+ x WHI||+ +||+
			67	54	137	0	0	258			
	Gtotal[df=2]	3.37	0.19, ns								
	Gpooled[df=1]	2.39	0.12, ns								
	Gheterogeneity[	0.98	0.32, ns								

The results of Table A.8 are interesting, both cross 2.8 and 2.13 should be consistent with the results in Table A.2 but the ratios of Table A.8 show a possible epistasis of the Striped colour morph over the White colour morph regardless of the White colour morph frequency being inflated among progeny consisting with the crosses in Table A.7.

Table A.8: Striped (STR) - possible epistasis over WHI?

	Parental phenotypes		Offspring phenotypes								
cross	Sire	Dam	NP	STR	WHI	VAR	SPO	Total	Gi	P	Hypothesis
2.8	STR	STR	24	25	37	0	0	86	1.70	ns	WHI||+ x STR||+
2.13	STR	STR	12	16	33	0	0	61	0.98	ns	STR||+ x WHI||+
			36	41	70	0	0	147			
	Gtotal[df=6]	2.68	0.26, ns								
	Gpooled[df=1]	0.66	0.42, ns								
	Gheterogeneity[	2.02	0.16, ns								

The results of Table A.9 display a possible epistasis of the colour morph White over the Variegated colour morph. Suggesting that the unknown sire had a White colour morph gene, with a possible White cuticle colour.

Table A.9: White (WHI) - possible epistasis over VAR

	Parental phenotypes		Offspring phenotypes								
cross	Sire	Dam	NP	VAR	WHI	STR	SPO	Total	Gi	P	Hypothesis
1.10	unkn	VAR	4	10	11	0	0	25	3.02	ns	WHI||+ x VAR||+
			4	10	11	0	0	25			

Cross 1.08 in Table A.10 shows strange results. The Spotted dam that has been crossed with an unknown sire has produced offspring with a range of colour morphs. The information the cross has given us does not help us make a hypothesis on how these frequencies have originated; however, this cross gives us something to research further on.

Table A.10: Unexplained cross - Spotted (SPO) x UNKN sire

	Parental phenotypes		Offspring phenotypes							
cross	Sire	Dam	NP	WHI	ORA	SPO	Total	Gi	P	Hypothesis
1.08	unkn	SPO	11	17	28	21	77	3.02		
			11	17	28	21	77			