The Effect of Drawdown on the Movement of Groundwater Invertebrates

Tracy White

Faculty of Science and Engineering

Supervisor: Grant Hose

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Abstract

Groundwater abstraction is a global problem limiting water resources and impacting dependent ecosystems. Groundwater invertebrates provide important services in their ecosystems. This study aims to clarify the role of sediment characteristics, invertebrate traits and drawdown rate on the response of groundwater invertebrates to water level changes. Column experiments using one of three sediment types and two groundwater invertebrate species were used with three drawdown rates. Hypotheses tested are: 1) coarse sediments and slower rates best facilitate the downward movement of invertebrates, 2) copepods and syncarids will differ in their downwards movement and 3) coarse sediment and fast rates will best facilitate the downwards movement of dead copepods. It was found that: 1) the proportion of copepods and syncarids stranded was not significantly different for the drawdown rates or sediment sizes, 2) copepods and syncarids were not found to differ significantly in their ability to move downwards and 3) sediment size and drawdown rate had no significant effect on the distance travelled by dead copepods. 11 % of copepods in the drawdown columns were stranded across all sediment types and rates, while 5 % of syncarids were stranded in the large sediment across all rates. While no statistically significant results were found the results here suggest that stranding does occur despite some capacity for invertebrates to follow a declining water table. The number of invertebrates stranded here under these conditions suggests that management of groundwater abstraction is needed to protect groundwater biodiversity and groundwater ecosystems.

Keywords

groundwater, invertebrate, rate of drawdown, sediment type, species traits

Chapter 1 - Literature Review
The Effect of Drawdown on the Movement of Groundwater Invertebrates

1.1 Groundwater abstraction

Groundwater is a globally important resource providing water for human consumption (Wada et al. 2010). Groundwater also provides baseflow to rivers and contributing to the function of groundwater dependent ecosystems (GDEs), such as lakes, ponds, wetlands and aquifers by providing water for vegetation and animals (Nevill et al. 2010). Groundwater also provides water for vegetation outside GDEs (Custodio 2000).

Intensive groundwater use can provide many socioeconomic benefits especially in developing countries where it provides water for drinking and irrigation, in the process improving health, malnutrition and helping to alleviate poverty (Llamas & Martinez-Cortina 2009). However, because aquifers are a common resource they may be expected to succumb to the *tragedy of the commons* (Hardin 1968). Aquifers may be exploited to such an extent that the costs outweigh the benefits. Problems such as groundwater depletion, declining water quality and subsidence may lead to social conflict and inequality.

The resources provided by groundwater are threatened by increasing demand worldwide due to an expanding population and improving technologies which enable more efficient pumping of groundwater (Wada et al. 2010). Wada et al. (2010) estimate that from 1960 to the year 2000 abstraction has increased in sub humid and arid areas from 312 km^3 to 734 km^3 resulting in an increase in groundwater depletion from 126 km^3 yr^{-1} to 283 km^3 yr^{-1}. Groundwater abstraction is more intense in arid, highly populated areas with a reliance on groundwater Wada et al. (2010). There are cases of massive depletion of groundwater in China and Spain with 101 m and almost 300 m of drawdown respectively since pre-development (Werner et al. 2013). Groundwater use has increased significantly in Australia (Eamus 2006), with the highest concentration of groundwater use in the Murray Darling Basin, where it is mostly used for agricultural purposes (Harrington & Cook 2014). Excessive groundwater abstraction can lead to many environmental problems including, deterioration of water quality, subsidence (Shen & Xu 2011), seawater intrusion (Werner 2010), the intrusion of other pollutants (Gun & Lipponen 2010) and sea level rise (Konikow 2011). GDE's can also be effected by excessive pumping, which may cause changes to vegetation communities (Pfautsch et al. 2015) and invertebrate communities (Carmignani & Roy 2017) as a result of water loss.

Groundwater abstraction results in falling groundwater levels (drawdown) and this can lead to invertebrate desiccation and mortality if invertebrates become stranded within the unsaturated zone (Stumpp & Hose 2013). This can lead to invertebrate community changes due to the disappearance or reduction of some species (Imberger et al. 2016). This in turn can lead to changes to ecosystem function (Vander Vorste et al. 2016) as groundwater invertebrates provide services such as water purification and improve the hydraulic conductivity of the soil matrix through burrowing activities.

It is known that some groundwater species are more susceptible to stranding in certain hydrological conditions (Stumpp & Hose 2013). Sediment pore size (affected by the sediment size, shape and packing) is also known to effect rates of stranding with smaller sediment pore sizes leading to higher rates of groundwater invertebrate stranding compared to large sediment pore sizes (Korbel, Stephenson & Hose unpublished). A previous study also found that drawdown rate had no effect on groundwater copepod stranding (Stumpp & Hose 2013). This study aims to further examine determinants of invertebrate stranding to assist in the management and protect the biodiversity of groundwater invertebrates.

1.2 Hydrological effects of drawdown

Under natural conditions groundwater is in a constant state of movement through the soil matrix, however, this is much slower than the movement of surface water, rates of 0.3 m per day may be considered high for groundwater (Alley, Reilly & Franke 1999). Regional groundwater flow was first described by Hubbert (1940). Groundwater moves from higher to lower levels of pressure which coincides with higher and lower levels in the landscape and recharge to discharge areas. Part of this system involves water travelling upwards from the valleys. Flow is primarily driven by the geology and the topology of the system and the flow typically follows a subdued mirror of the topology (Freeze & Cherry 1979). Groundwater recharge areas occur where the saturated net flow is traveling away from the water table, while the discharge areas occur where the saturated net flow is travelling towards the water table (Freeze & Cherry 1979). Groundwater recharge includes precipitation, or it may also come from surface water bodies that are losing water. Discharge occurs at surface water bodies that are gaining water or may also be due to evaporation.

On a regional scale pumping may alter groundwater flow, cause declines in groundwater storage and alter recharge (surface water entry to subsurface) and discharge (subsurface water exit to the surface) patterns. The effects of pumping on the direction of groundwater flow and the changes to the recharge and discharge patterns may be illustrated by a hypothetical example given by Alley, Reilly & Franke (1999). In this example, a well is placed adjacent to a stream. When the pumping rate becomes high enough, groundwater flow changes direction and instead of moving to the stream it moves toward the well, thereby also changing the discharge area to a recharge area, where water moves away from the water table.

Theis (1940) also described the changes that occur over a regional scale because of pumping using the water budget concept. The water budget explains the flow of water in terms of inputs and outputs to the system (Aeschbach-Hertig & Gleeson 2012). In a natural system prior to development the amount of recharge should be approximately equal to the amount of discharge as the system is in a dynamic equilibrium with fluctuations balancing out over the long term, for example, because of seasonal changes (Alley, Reilly & Franke 1999). Pumping groundwater changes the groundwater flow system by increasing recharge, removing water from storage and decreasing discharge or a combination of these (Theis 1940). For example, the distance to the recharge/discharge areas from the area of pumping affects where the water flowing to the well comes from within the aquifer. If the well is close to a recharge area then pumping will increase the recharge, if the well is placed so that it is far from recharge, then pumping will not increase recharge, in this case the water may come from storage or discharge areas (Theis 1940). A system undergoing pumping may reach a new equilibrium over time with new rates of recharge and discharge (termed capture) balancing out and the storage remaining stable. However, if abstraction is too great water will continue to be removed from storage and groundwater levels will decrease (Alley, Reilly & Franke 1999).

1.3 Sustainable pumping

Pumping can result in considerable drawdown, for example, areas in Spain are thought to have had almost 300 m of drawdown since pre-development times (Werner et al. 2013). The Murray Darling Basin and Pilbara regions can also have 10 m or more of drawdown per year (Earth Tech Earth Tech Engineering 2003, Cook et al. 2017). To avoid groundwater depletion and adverse environmental consequences, safe and sustainable pumping rates were developed, however, these were felt to be based on confusion and misunderstandings of groundwater (Zhou 2009). In the past, it was thought that the pumping rate was safe if the pumping rate was less than recharge. However, this can still result in groundwater depletion and environmental damage, as even if the pumping rate is less than the recharge, it may still have induced recharge and decreased discharge, taking water from groundwater dependent ecosystems (Zhou 2009). The idea of sustainable pumping was created in the 1980's and took a more holistic view of the hydrological system. Sustainable pumping sought to cause no harm to the ecosystem over the long term and expanded concern to include reduced flows to groundwater dependent ecosystems and the impact of capture and the cone of depression (Zhou 2009). The problem with sustainable pumping is that it is poorly defined and hard to determine what no harm means. Our ideas of environmental protection continue to evolve as we learn more about groundwater and its connection to the biosphere.

1.4 Australian and international drawdown rates (Long term and Short term)

Drawdown rates differ markedly between aquifers and use in the long term (see table 1). Drawdown rate is dependent on many highly variable factors including the volume and duration of pumping, distance of monitoring point from bore and hydrological and geological controls as well as other environmental factors such as drought.

Table 1 – Long term groundwater drawdown rates in Australia from various aquifer uses.

Location (state)	Main Uses	Rate (m/y)	Reference
Gippsland Basin	Irrigation, petroleum	1	(Varma & Michael 2012)
Swain Coastal Plain	Domestic Water	2.2 (between 1990 - 1991)	(Groom, Froend & Mattiske 2000)
Pilbara Region	Mine-dewatering	10	(Cook et al. 2017)
Murray-Darling Basin	Irrigation	10-20 (between 2001 - 2003)	(Earth Tech Engineering 2003)

Short term groundwater drawdown rates world-wide

Short term drawdown rates differ greatly for each aquifer and for each use (see Table 2). The above also applies to aquifers in the short-term. The last value on this table was at a domestic well where a decline of 1 m in four hours is not uncommon (Coppola et al. 2005). Short term rates were chosen to get a clearer picture of instantaneous rates of drawdown or as close to instantaneous as possible for more realistic data for my experiment. For this study I calculated the maximum daily rate to set the upper limits for my study.

Table 2 – World-wide short-term groundwater drawdown rates for aquifers under various uses. Short term drawdown rates were estimated from graphs or tables showing declines in groundwater levels over months or days. The values represent the maximum daily rate.

Location	**Groundwater use**	**Maximum Daily Rate (cm/d)**	**Reference**
Pilbara	Iron ore mining, de-watering	13	(Cook et al. 2017)
New Jersey	-	28	(Jones 2006)
California	Domestic supply	55	(Cooper et al. 2015)
New Jersey	Domestic supply	600	(Coppola et al. 2005)

1.5 Ecological effects of drawdown

1.5.1 Effects of drawdown on GDEs and invertebrates

Groundwater drawdown diverts water from rivers (Nevill et al. 2010), impacting the base flow, the physical and chemical composition and the distribution of biota. For example, groundwater drawdown in some parts of the Murray Darling Basin is excessive (Murray Darling Basin Commission), it is estimated that many rivers of the Murray Darling Basin are reduced to less than 60% flow (Kingsford & Thomas 2004) impacting all biota that rely on this water (Finlayson et al. 2013). Water is also diverted from other water bodies by drawdown, impacting ponds, wetlands and woodlands etc. A study by Groom, Froend & Mattiske (2000) found that Banksia woodland vegetation suffered substantial mortality around a bore as a result of declining water tables due to pumping and drought in the Gnangara Mound.

Drawdown can result in invertebrate stranding if invertebrates are unable to navigate the pore spaces to the saturated zone or they are unable to burrow into fine sediment. Desiccation and mortality can then result. Animals may be more likely to desiccate on gravels rather than finer sediments with organic matter (Poznanska et al. 2015). Laboratory studies show stranding of invertebrates with a declining water level (Stumpp & Hose 2013, Vadher et al. 2017) and field studies illustrate the disappearance or reductions of some species due to water level declines

(Imberger et al. 2016). Mass mortalities can also result from large water-loss events leading to long exposures. For example, Imberger et al. (2016) found that some species of amphipod were sensitive to drought and saw declines and disappearances of species in Australian streams. The ecological response to drawdown will depend on the timing, duration, frequency and amplitude of the water level decline (Wantzen et al. 2008), with some invertebrates more vulnerable than others. Changes in invertebrate community abundance, richness, composition, distribution (Carmignani & Roy 2017) and finally ecosystem function (Vander Vorste et al. 2016) could be expected.

1.5.2 Effect on community structure

Studies have shown a reduced invertebrate abundance, diversity and distribution with water level loss. Declines in abundance have been noted to occur as a result of pumping (Castella et al. 1995), dewatering (Haxton & Findlay 2008) and drought (Boulton 2003). The diversity of invertebrates also declines with the degree of water level reductions (White et al. 2011, Carmignani & Roy 2017), with a shift towards species with a higher tolerance to drying or disturbance (White et al. 2011, Imberger et al. 2016). White et al. (2011) found that species with a higher, faster mobility increased in lakes subjected to anthropogenic declines while species that were less mobile and slower decreased compared to reference sites. Half of the species were absent from reservoirs subjected to more than 3 m of drawdown, families such as Elmidae and Hydrobiidae were absent while species such as Chironomidae and Naididae were present in higher numbers (White et al. 2011). Species such as oligocheates and amphipods tend to be present in high numbers at sites with water level fluctuations illustrating their tolerance (Kaster & Jacobi 1978, Smagula & Connor 2008) at least in some situations. Species distributions have also been noted to decrease with declining water levels as the available habitat is reduced (Carmignani & Roy 2017). Groundwater invertebrates usually prefer to reside at the top of the water table and hence will need to migrate downwards. Their ability to survive depends upon finding new habitat with the appropriate resources.

1.5.3 The effect of drawdown on ecosystem services and function

Groundwater fauna perform important ecosystem services including water purification, destroying pathogens, nutrient recycling and alleviating floods through increasing hydraulic conductivity (Griebler & Avramov 2014). It is thought that groundwater invertebrates are especially involved in increasing the sediment permeability through their movement and burrowing activities. Even low densities of invertebrates may increase the flow through creating preferential flow paths for water (Hose, pers comm). A study by Nogaro et al. (2006) found that tubificid worms were able increase the hydraulic conductivity of fine sediments because of their deep burrowing activity, whereas chironomid larvae did not increase the hydraulic conductivity because they create U shaped burrows on the surface.

Drawdown has been shown to alter groundwater communities by changing the community composition or causing species disappearance which would have flow on effects for the ecosystem services provided. White et al. (2011) found that immobile species declined with water loss, leading to changes in the feeding groups present. Less filterers were present, as these were mostly clams, and more collector-gatherer and parasite groups were present. While this study did not examine the effect of this on ecosystem function, alterations like this would be expected to alter the functioning of the ecosystem. Vander Vorste et al. (2016) manipulated conditions in mesocosms to stimulate drying and the subsequent increase of water table depth on amphipods. Drying reduced the amphipod population as they had difficulty burrowing the required depth to the water table and this reduced leaf litter decomposition in the mesocosms, an important ecosystem function. The reduction or disappearance of species of groundwater invertebrates due to drying could also be expected to reduce or eliminate important groundwater ecosystem services, although the importance of individual species of groundwater invertebrates on ecosystem functioning is poorly known (Boulton et al. 2008).

1.6 The role of sediment characteristics

The ability of animals to seek refuge is complex and depends on many sediment characteristics including the size and shape of the sediment grains and the packing and heterogeneity of sediments as these affect the porosity of the sediment or the interstitial voids or pores (Gayraud & Philippe 2003). Animals seek refuge by moving into voids or burrowing to create voids where conditions allow (Stubbington, Wood & Reid 2011), and different species can move into different sediments (see below). For invertebrates to seek refuge the shape, volume and arrangement of individual pore spaces are important.

The sediment size is important as it affects the size of the sediment pores, with larger spaces allowing invertebrates to crawl or swim through. The size of individual pores are thought to be important rather than the total porosity of the soil matrix (Hose et al. 2017). Arya & Paris

(1981) showed how the sediment size relates to the sediment pore size. Many field and laboratory studies have shown that that invertebrates are excluded from small pore spaces (Hose et al. 2017, Korbel, Stephenson & Hose unpublished) and studies have shown that animals prefer grains similar to their body size when given a choice between smaller grains (Korbel, Stephenson & Hose unpublished). The porosity of sediments also decreases with increasing heterogeneity of grain sizes because the addition of smaller grains fills up the void space. Two or more grain sizes will decrease the porosity of the system depending on the proportion of each size (Gayraud & Philippe 2003).

The shape of sediment grains also has an influence on the shape, size and volume of pore spaces. Fraser (1935), found that angular grains increase the porosity, with needle like structures having the highest porosity, however, Gayraud & Philippe (2003) did not find any difference in porosity for different shapes of uniform sediments. This could be a result of heterogeneity of sediments masking the effect (Gayraud & Philippe 2003) or the lack of sufficient difference between the sediment shapes.

Graton & Fraser (1935) studied spheres of the same size to determine the effects of packing on interstitial spaces. Packing is concerned with the arrangement of the grains and their layers within the matrix. Some arrangements have a looser configuration and others a tighter one, thereby affecting the void space shape, volume and arrangement (Graton & Fraser 1935), which can control the movement of animals. For example, uniform spheres packed in a tight arrangement have less than half the pore volume of loosely packed spheres (Graton & Fraser 1935). The influence of packing increases with depth due to the compaction weight of the sediments above (Gayraud & Philippe 2003). Hose et al. (2017) found that animals could live in small sized sediments if they were not packed tightly.

The texture of the sediment would also be expected to affect the movement of groundwater invertebrates. Harder, larger sediment grains would allow animals to move through the voids, and the matrix would be more stable. Smaller, soft sediments grains with high water content would need to be disturbed or burrowed into.

1.7 The role of species traits

Studies have examined how certain traits or characteristics help animals persist under varying environmental conditions. While little information is available on which groundwater invertebrate traits help them survive drawdown, studies have examined traits that favour disturbance from drought or drying in different environments: see Bonada, Doledec & Statzner (2007) and Carmignani & Roy (2017). Table 3 (following) from Bonada, Doledec & Statzner (2007) has been modified to apply to groundwater invertebrates within interstitial spaces. Traits favouring survival with drawdown include small body size, fast mobility, ability to burrow, drought resistant forms, a preference for larger sediment sizes and active directed migration.

1.7.1 Body size, shape and mobility

Groundwater invertebrates range in size and shape and have different modes of locomotion. These traits affect the ability of invertebrates to move downwards into the sediments and fit within the pore spaces (Korbel, Stephenson & Hose unpublished). For example, bigger animals would be expected to be restricted more by sediments than smaller animals, rendering them more prone to stranding unless they can dig into the sediment. Amphipods at around 4000 µm long and 610 µm wide are much larger than cyclopoid copepods at around 400 µm long and are restricted to larger sediments (Korbel, Stephenson & Hose unpublished). Regarding mobility faster moving animals are less likely to become stranded with drawdown if they can follow the water downwards and reach the saturated zone quickly. Movement also depends on the substrate with some animals better able to move through some sediments than others. Some bivalves, including ostracods (Dole-Olivier et al. 2000) and gastropods are relatively immobile (Samad & Stanley 1986), while copepods can move reasonably fast (Korbel, Stephenson & Hose unpublished). Some harpacticoid copepods move by using their thoracic limbs and by worm-like undulations of the body (Faulkes 2013). These specialisations are particularly effective and provide momentum and give them speed (Faulkes 2013).

1.7.2 Burrowing or digging capacity

Burrowing or digging capacity differs between species, for example, oligocheates and amphipods are good burrowers (Carmignani & Roy 2017). In contrast, **c**alanoid copepods prefer swimming in open water (Korbel, Stephenson & Hose unpublished). Also, different sediment types required different abilities to move into them. Movement into sand requires entry via a shallow angle or liquefication, possible with very fast moving appendages (Faulkes 2013), while entry into finer sediments requires the creation and lengthening of cracks via force from muscular contractions and expansions and is mostly restricted to soft bodied animals (Dorgan 2006). There is also evidence that some animals lack the physical ability to burrow long distances and only burrow to shallow depths. For example, Palmer, Bely & Berg (1992) found that despite stream invertebrates having enough time (6 mins – 5.5 h) to burrow the necessary 30 cms into the sediment to avoid a flood, 50 – 90 % choose only to burrow 1.5 – 3.5 cms and were washed downstream. However, this study did not report the size of the animals only the average size of the sediment (1 mm). The inability of animals to move into sediment may not be directly related to their burrowing ability but indirectly related to the lack of oxygen or feeding ability when deep within sediments (Faulkes 2013).

1.7.3 Desiccation tolerance and habitat preference

Groundwater invertebrate species differ in their ability to tolerate desiccation and the habitat they prefer to live in. These traits influence their ability to survive drawdown events. Some species have a higher tolerance to drying or drought resistant forms or eggs (Dole-Olivier et al. 2000), as a result they can survive longer when stranded above the saturated zone and wait for the water to rise again. For example, some species of ostracods have high resistance to desiccation (Dole-Olivier et al. 2000), however, long periods of dry conditions will lead to mortality. Stumpp & Hose (2013) found that syncarids were more tolerant to drying than copepods. At 6% saturation 87% of syncarids were still alive, however at 6% saturation only 17% of the Wellington copepods were still alive. Examining habitat preference, studies have shown that species with a preference for larger sediments will be favoured by drawdown, as they can then move more easily downwards with a declining water table (Carmignani & Roy 2017). Most groundwater invertebrates prefer sediments that are larger than their body size (Korbel, Stephenson & Hose unpublished), thereby giving smaller animals more options. Copepods, for example, did not prefer either sand or gravel as they can fit comfortably in both.

1.7.4 Movement strategy

Species have different movement strategies in response to disturbance including human induced declines in water levels, for example, see Hoch et al. (2015). Some species react with random movement, in which direction is not altered in response to declining water levels, while others directed movement, in which direction is altered in response to declining water levels (Hoch et al. 2015). Not much is known about this for invertebrates living on or in substrate, however, studies show that many have directed movement and move downwards with the water table (Poznanska et al. 2015). Drawdown will favour animals with directed movement as they are more likely to find the water table as opposed to animals with random movement. To follow the water table, it may be necessary to move long distances vertically. As well as being challenged by the physical requirements and oxygen levels animals may be challenged by barriers leading to wrongful changes in direction. Poznanska et al. (2015) found that snails found it difficult to follow a straight course to the water after drawdown when the substrate was rough. Supporting this Palmer, Bely & Berg (1992) observed that copepods didn't travel in straight lines, but rather zigzag throughout

Table 3 – Shows groundwater invertebrate traits believed to favour drawdown. Modified from Bonada, Doledec & Statzner (2007).

Trait	Rationale	Reference
Body size	Animals with a small body size are better able to migrate downwards through the pore spaces with drawdown	(Bonada, Doledec & Statzner 2007)
Mobility	Fast animals can migrate downwards faster to avoid desiccation	(White et al. 2011)
Burrowing capacity	Animals better able to burrow favoured with drawdown	(Dole-Olivier et al. 2000)
Desiccation Tolerance	Animals with drought resistant forms favoured with drawdown	(Dole-Olivier et al. 2000)
Habitat preference	Preference for large sediments allows animals to move downwards with drawdown	(Dole-Olivier et al. 2000)
Movement Strategy	Directed, active migration better as opposed to random movement	(Hoch et al. 2015)

1.8 The role of drawdown rate

The ability of invertebrates to migrate downwards is impacted by the rate of drawdown. Drawdown rate affects the survival of animals, with faster rates leading to stranding if the animals cannot keep up with the decline (Carmignani & Roy 2017). A study by Poznanska et al. (2015) with *P. corneus*, an aquatic snail, compared horizontal migration with gradual water declines (8 days) and fast declines (immediate). Faster rates led to more animals being stranded as they were unable to follow the water level down but choose to move into their shells and wait on the substrate. While seeking refuge may be a valid strategy to enhance survival, long periods of drying would be expected to lead to mortality.

Some species would be more able to follow a declining water table due to their mobility (see above). A study by Palmer, Bely & Berg (1992) measured the speed of several stream invertebrates including copepods by measuring them between two points with a stopwatch. It was found that copepods had a range of 0.09-1.6 cm/min while Oligochaetes had ranges between 0.47-1.3 cm/min. However, while speed is an important factor in an animal's ability to reach a lowered water level their physical ability to travel long distances in a straight line is also important.

Chapter 2 - Aims and hypotheses

This study aims to examine the effect of drawdown on the movement of groundwater invertebrates and how rate and sediment size affect movement. It is not known if groundwater invertebrates move downwards with a declining water table or if they become stranded within the sediments of the unsaturated zone. If they become stranded they are prone to desiccation. Several different hydrological conditions will be examined including the effect of sediment characteristics, species traits and rates of drawdown on invertebrate stranding. Three different sized sediments (large gravel, small gravel and sand), two species of invertebrates (copepods and syncarids) and three drawdown rates (fast, medium and slow) will be examined. The study will determine the proportion of invertebrates stranded under the different hydrological conditions to determine vulnerabilities to drawdown to assist in their future management.

Manipulation experiments with columns simulating drawdown will be used to test the following hypotheses:

1) that coarse sediments and slower rates best facilitate the downward movement of invertebrates;
2) that syncarids and copepods will differ in their downwards movement with drawdown;
3) that coarse sediment will best facilitate the downwards movement of dead copepods.

Chapter 3 - Methods

3.1 Overview

Drawdown experiments were conducted in sediment filled columns in the laboratory using field collected animals. Experiments were performed in the dark at 17 ^{0}C to reflect the environmental conditions at the time of invertebrate collection. Columns were set up to simulate the effects of drawdown under different hydrological conditions (See Fig 3.1), see below for further details. Three different sediment sizes (coarse gravel (2-4 mm), fine gravel (1-2 mm) and sand (0.5-1 mm)), two different species of invertebrates (harpacticoid copepods, and syncarids) and three drawdown rates (400, 40, 4 cm/d) were investigated (See table 3.1). After drawdown columns were frozen and separated into cross sections. Invertebrates were stained and counted for each cross section to determine those stranded within the unsaturated zone.

Fig 3.1 Sediment filled columns were held suspended to a wooden board for the drawdown experiments

Table 3.1 Experimental design: invertebrate species in different hydrological conditions. The following table shows the different invertebrate species tested for the hydrological conditions below.

	Coarse gravel (2-4 mm)	**Fine gravel (1-2 mm)**	**Sand (0.5-1 mm)**
Fast 400 cm/d	Harpacticoid copepods Syncarids *(Dead Copepods)	Harpacticoid copepods *	Harpacticoid copepods *
Medium 40cm/d	Harpacticoid copepods Syncarids *	Harpacticoid copepods *	Harpacticoid copepods *
Slow 4cm/d	Harpacticoid copepods Syncarids *	Harpacticoid copepods *	Harpacticoid copepods *
No drawdown (control) **	Harpacticoid copepods Syncarids	Harpacticoid copepods	Harpacticoid copepods

* Dead copepods were added to each column with drawdown.

** A control was used for each rate (fast, medium and slow).

3.2. Preparation of columns

3.2.1 Preparation of bottles

Plastic bottles with a 64 mm base were obtained from Plasmo located in Malaga (WA). These were rinsed in water and a ring was cut out the bottom leaving a 3 mm edge. A stencil of a ruler was attached to enable easy measurements.

3.2.2 Preparation of sediments

Sediments were obtained from BC sands landscaping suppliers in Taren Point NSW and various aquarium retailers in Sydney NSW. Sediments were sieved into three different sizes - coarse gravel (2-4 mm), fine gravel (1-2 mm) and sand (0.5-1 mm) (See Fig. 3.2). Sediments were washed in alcohol then rinsed with water and finally autoclaved at 121 ^{0}C for 20 min to remove impurities and fungus.

Fig. 3.2 Sediments sizes right to left 2-4 mm, 1-2 mm and 0.5-1 mm.

3.2.3 Preparation of filters

A scintered plastic filter (Porous plate 1, 100-160 µm, ROBU, Germany) was placed before the bottle neck to keep invertebrates and sediments in the columns. The filters were held in place with silicone and a food grade rubber o-ring was placed outside the filter disc to form a seal when the disc inside the bottle.

3.2.4 Preparation of tubing

Wash bottle lids with the ends cut off were used to direct water out of the columns. These were attached to the thread of the bottle neck and in turn had aquarium hose attached. This hose was attached to a 60 ml syringe held on a retort stand when filling the column with water and to a dripper when drawing the water level down.

3.3 Test organisms

The test organisms used in this study were copepods, Harpacticoida (Ameiridae) and syncarids (Parabathynellidae). These organisms are commonly found in alluvial aquifers (Humphreys 2006) and are easily kept in the laboratory. These animals are all considered to be true groundwater invertebrates based on their physical characteristics such as enhanced sensory appendages and lack of eyes and pigmentation (Humphreys 2006). These organisms have different traits (see Table 3) and could be used to examine the effect of body size, mobility, burrowing capacity and habitat preference on the incidence of stranding with drawdown. Syncarids are much larger in size and have a more elongated body shape compared to copepods, on average syncarids are around 2500 µm in length compared to copepods at around 500 µm in length. These organisms also move differently with syncarids being very effective crawlers, while copepods were observed to swim more often. Syncarids are thought to be more able burrowers compared to copepods and prefer to reside in larger sediments because of their larger size compared to copepods.

Harpacticoid copepods have been described using the CO1 gene (Genbank accession numbers KF361325, KF361326 and KF361332),(see Hose et al. (2016))
Syncarids have also been described using the CO1 gene (Genbank accession numbers KF361321 – KF361324), (see Asmyhr et al. (2014)).

3.4 Collection methods

Copepods were collected from the Kulnura/Mangrove Mountain aquifer near Somersby NSW, Australia, which is located approximately 70 km southwest of Newcastle (33^{0} 22’ S, 151^{0} 18’ E). Invertebrates were collected from bores 75041/1 and 75041/2 on the 15th Nov 2017, 5th January 2018 and 16th March 2018.

Invertebrates were collected by pumping from the aquifer using a motorised inertia pump. Approximately 90 L was pumped from each bore and filtered onsite using a 63 µm sieve to collect and concentrate the invertebrates. Invertebrates were placed in 1 L plastic containers full of water from the bore and these were placed into an insulated cooler for transport back to the laboratory. Ice was used to keep the invertebrates at similar temperatures to their original environment. Additional water (between 10 L – 20 L) was collected from each bore for use in future laboratory tests. On arrival at the laboratory the invertebrates were placed in a temperature-controlled room at 17 ^{0}C to mimic the conditions at the time of collection.

Syncarids were collected from bore WRS05 located near the Macquarie River at Wellington (32^{0} 34’ S 148^{0} 59’ E). This bore is part of the University of NSW hydrological research station. The syncarids were collected on the 4th February using the same methods as above.

3.5 Filling of columns and calculation of drawdown

Drawdown tests were performed in a temperature-controlled room at 17^0 C. Columns were stabilised on a frame using bolts and wingnuts. The hosing and syringes were attached to each bottle. Filtered water (from the same bore where the invertebrates were collected) was then added to the column from the syringe (to avoid creating air bubbles) alternately with sediment which was placed in from the top. Columns were filled with sediment to the 60 mm mark followed by water to 70 mm (See Fig. 3.3 for diagram of the setting up process). Copepods and syncarids were then placed in separate columns from the top (20 copepods in each column for the large sediment and 10 copepods in each column for the medium and small sediments, 2 syncarids were placed in each column). Due to the low numbers of invertebrates it was decided that lower numbers of copepods should be used for the medium and small columns. Due to the logistical limitations of this pilot study, the tests with the syncarids were limited to larger sediment sizes (due to the latter being easier to handle than smaller sediments). For the experiments using live invertebrates, three column replicates were used for each sediment type and drawdown rate (see table 3.1). For information on the treatment of control columns and experiments with dead copepods see sections 3.6 and 3.7). Water was drawn down slowly to the 50 mm mark to encourage the invertebrates to move downwards to avoid being crushed by sediment to be added later. The column was then filled to the 140 mm mark with sediment and water. The amount of water and sediment added to each column was calculated.

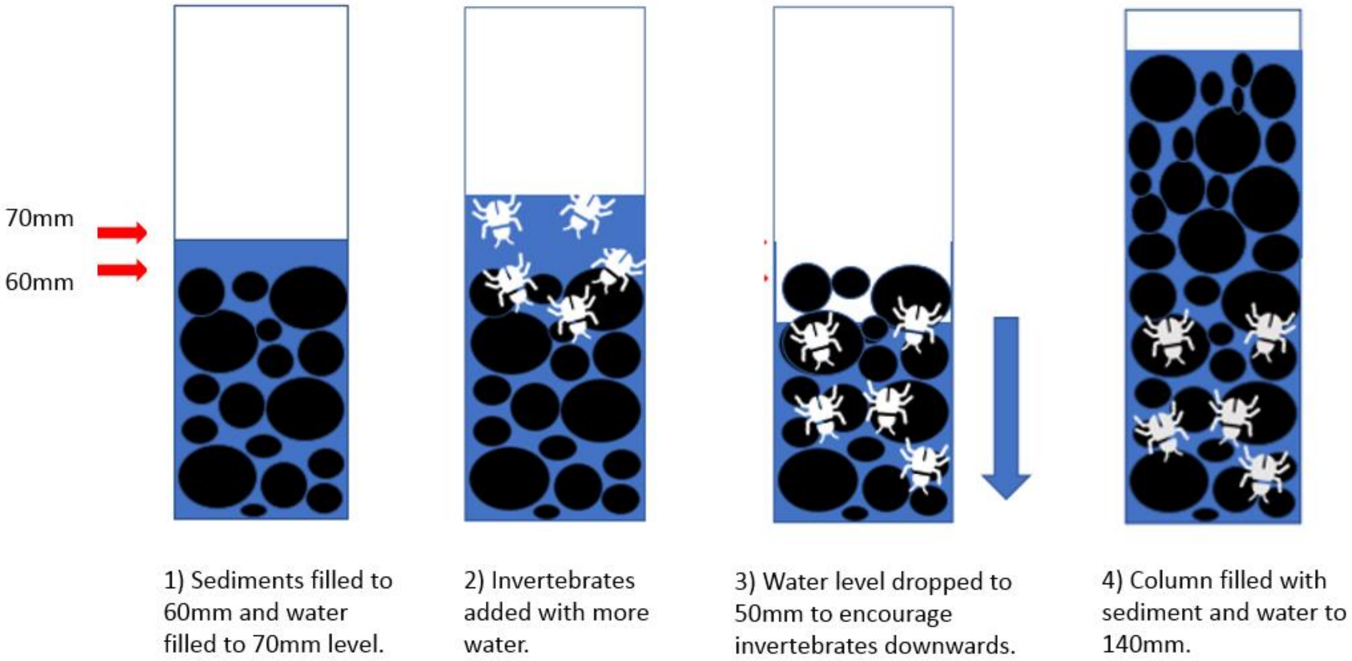


Fig. 3.3 Diagram of process for setting up columns

The columns were then left for 48 hours to give the invertebrates time to disperse evenly throughout the columns. After 48 hours drawdown was performed.

3.6 Control columns

Control columns were set up the same as drawdown columns in section 3.5. Three control columns were set up for each sediment size and assigned to a fast, medium or slow drawdown rate (20 copepods were placed in each column for the large sediment size and 10 copepods were placed in each column for the medium and small sediment size, 2 syncarids were placed in each column). During the experiment however, these columns were not drawn down, but were frozen at the same time as the corresponding drawdown columns. They were then treated the same as the drawdown columns (see section 3.9 and onwards). The control columns were separated into the same number of cross sections as the corresponding drawdown columns were (those columns corresponding with the same sediment size and invertebrates used (see table 4.1).

3.7 Dead copepod experiments

Drawdown tests with dead copepods were conducted to examine if the downwards movement of invertebrates was a function of invertebrate action (active) or was related to the downwards movement of the water (passive). It also enabled us to compare indirectly the movement travelled for alive and dead invertebrates. Dead copepods were added to the top of columns containing large, medium and fine sediment. For each sediment size, fast, medium and slow rates of drawdown were conducted. Ten dead copepods were added to each column (Each sediment size had 30 invertebrates in total). The frozen columns were separated into 1 cm cross sections with tap water (see section 3.11) starting from the top of the column until all 10 invertebrates were found (see table 4.1 for experiment replication).

3.8 Calculation of drawdown rates

Drawdown rates (expressed in terms of volume/time) were calculated by using the volume of water in each column and the specified drawdown rate (expressed as distance/time). The aim is to empty the column halfway for each treatment. The required drawdown time was calculated in conjunction with the drawdown volume rate to achieve a drawdown of half of the height of the sediment. The drawdown rates were 400, 40 and 4 cm/d, fast, medium and slow respectively. The upper limit was chosen from the literature to represent a value found in the real world (see table 2). The lower values were then chosen on a log scale to contrast this.

3.9 Column drawdown

To commence drawdown, the syringes were removed and replaced with roller clamps to control the rate of drawdown. A stopwatch was used to monitor the rate of drawdown over time. It was necessary to continually check and alter the drip rate as it tended to slow down as the water level was lowered and the pressure decreased. The drawdown times taken for the fast, medium and slow rates were 21 minutes, 4 hours and 18 minutes and 42 hours respectively,

3.10 Freezing of columns

After drawdown, columns were immediately frozen by immersion in liquid nitrogen. The columns were then placed in a freezer at – 20 ^{0}C until analysed.

3.11 Sediment analysis

Frozen columns were separated using tap water to selectively thaw the desired number of cross-sections (see table 4.1). These cross sections were collected using a sieve and rinsed to separate the invertebrates. Invertebrates were stained with rose bengal and counted under the microscope for each cross-section.

3.12 Statistical analysis

3.12.1 The effect of sediment size and drawdown rate on the movement of copepods

The proportion of copepods above the drawdown level was compared among sediment types and drawdown rates using a generalised linear model with a binomial error structure to permit analysis of the proportion data. The drawdown rates and sediment types were fixed factors. This analysis was done using R version 3.3.1 (R Core development Team 2018). A one-way ANOVA was used to determine if the proportion of syncarids above the drawdown level was significantly different when comparing rates (fast, medium, slow and control) across the large sediment size. The one-way ANOVA was conducted in minitab v 17 (minitab Inc, USA). The significance level (α) was 0.05.

3.12.2 The difference of the stranded proportion from zero

One-sample t tests were performed to see if the proportion of copepods and syncarids above the drawdown levels for each rate and sediment combination were significantly different from zero. This was conducted using Minitab v 17 (Minitab Inc, USA). The significance level (α) was 0.05.

3.12.3 Proportion of copepods versus syncarids stranded in large sediment

A Fisher's exact test was performed to compare the proportion of copepods and syncarids above the drawdown line in large sediment for columns that underwent drawdown (not controls). This test was conducted using the social science statistics calculator (www.socscistatistics.com/tests/fisher/default2.aspx). The significance level (α) was 0.05.

3.12.4 Distance travelled by dead copepods

The distance travelled by dead copepods in the sediment types and drawdown rates was compared using a generalised linear model as above. Drawdown rates and sediment types were fixed factors.

3.12.5 Power analysis

The generalised linear model used to determine the effect of sediment size and drawdown rate on the incident of copepod stranding was underpowered. According to the analysis in R sediment size had power of 0.15, drawdown rate had a power of 0.4. These values are substantially lower than 0.8 the desired level of power.

The one-way ANOVA used to assess impact of drawdown rate on the stranding of syncarids in the large sediment was also found to be underpowered with a value of 0.11, calculated in minitab V 17 (minitab Inc USA). To achieve a difference of 20 % between the drawdown rate means a sample size of 12 would have been required to have enough power.

The power of the 1 sample t tests was calculated in minitab V 17 (Minitab Inc USA). Most t-tests had insufficient power with values around 0.1.

The power of fisher's exact test, used to determine the proportion of syncarids versus copepods stranded in the large sediment, was performed in G Power 3.1.9.4. The power of this test was calculated at 0.05.

The generalised linear model used to determine the effect of sediment size and drawdown rate on the distance travelled by dead copepods had a power value of 0.69 for the sediment size and 0.91 for the drawdown rate.

3.12.6 Invertebrate Recovery

The percentage of copepods recovered in the large sediment after freezing and sediment analysis was 70 %, the percentage recovered in the medium and small sediments was 91 % and 88 % respectively. The percentage of syncarids recovered in the large sediment was 88 % and the total percentage of dead copepods recovered was 89 % across all sediment types.

Chapter 4 – Results

4.1 Overview

Although the author planned to use syncarids in the medium and small sediment, due to logistical problems and low numbers of invertebrates we had columns in the following conditions (see table 4).

Table 4. Experiment replication

The following table shows the experiment replication for all conditions.

Invertebrate	No. of invertebrates in each column	Column saturation	Sediment size	No. of columns	No. of cross sections (each column
copepod	20	drawdown	large	3*	2 (7 cms)
	20	control	large	3*	2 (1 cms)
	10	drawdown	medium	3*	14 (1cms)
	10	control	medium	3*	14 (1 cms)
	10	drawdown	small	3*	14 (1 cms)
	10	control	small	3*	14 (1cms)
syncarid	2	drawdown	large	3*	4 (3.5 cms)
	2	control	large	3*	4 (3.5 cms)
dead copepod	10	drawdown	large	3*	14 (1 cms)
	10	drawdown	medium	3*	14 (1 cms)
	10	drawdown	small	3*	14 (1 cms)

* 1 column for each rate (fast, medium and slow).

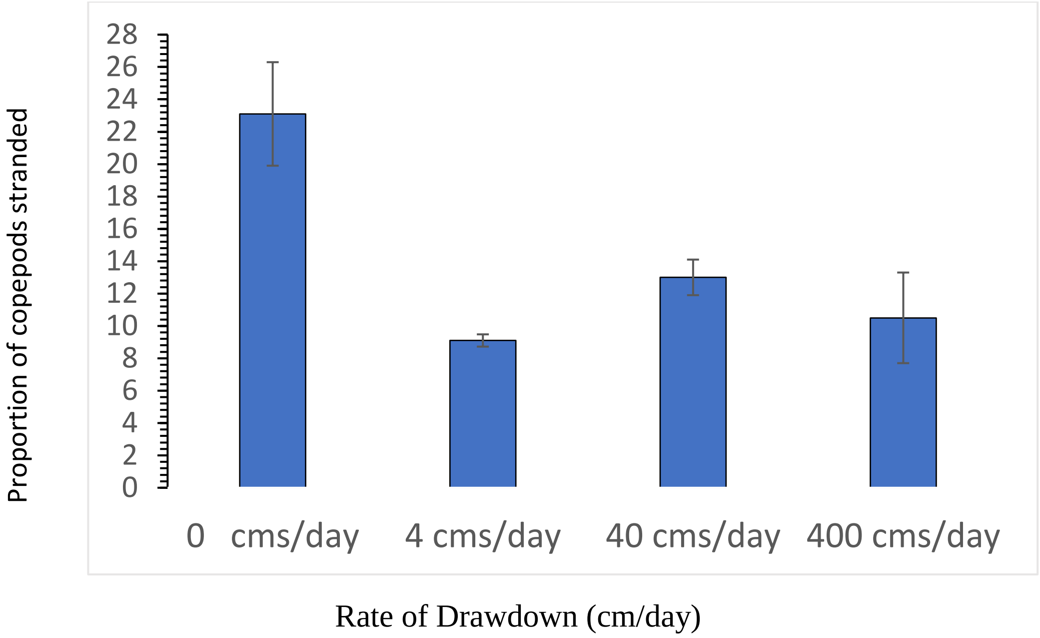
The proportion of copepods above the drawdown level following drawdown was not significant for the different rates and sediment sizes. However, although not statistically significant invertebrates seemed to move downwards with drawdown given that the saturated control columns had greater numbers of invertebrates in the top half of the columns compared to drawdown columns. The proportion of copepods above the drawdown level in the large sediment, slow rate and small sediment, control combinations were significantly different from zero, all others were not significant. The proportion of syncarids above the drawdown level was not found to be significant for all rates. The distance travelled by dead copepods was not found to be significant across sediment types or drawdown rates and only very small distances were achieved. Most statistical analyses were found to have insufficient power and further studies are needed with higher numbers of invertebrates. The GLM examining the distance travelled by dead copepods, however, had enough power for drawdown rate although, drawdown rate was not found to have a significant effect on the distance travelled.

4.2 The effect of sediment size and drawdown rate on the movement of copepods and syncarids.

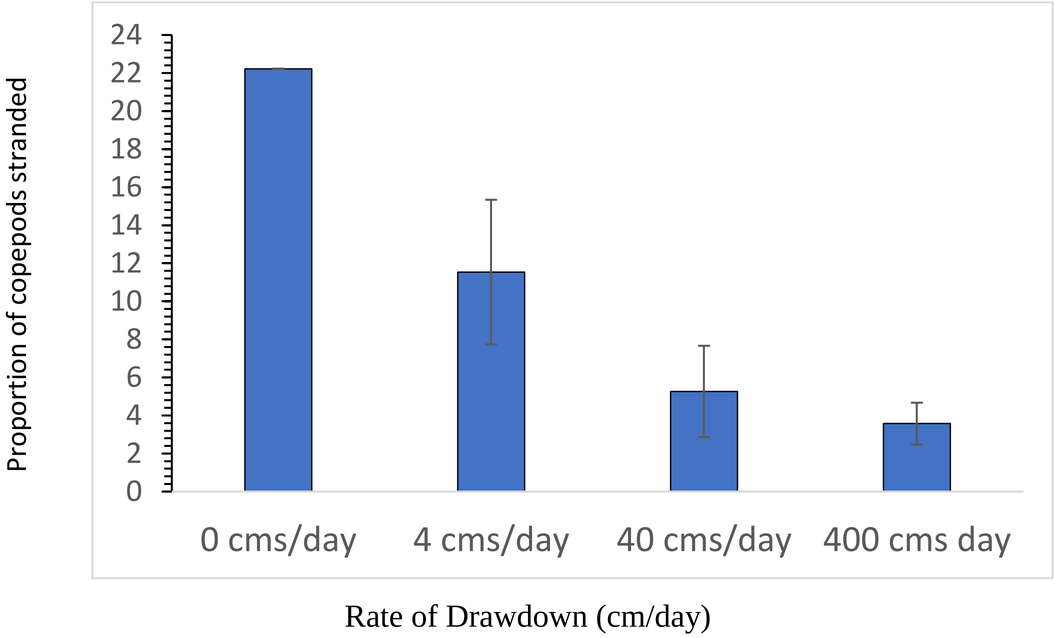
4.2.1 Copepods

The proportion of copepods stranded was not found to be significantly different across the different drawdown rates (fast, medium, slow and control) (z = 0.652, p = 0.514) or sediment sizes (large, medium and small) (z = 0.471, p = 0.638) (See Fig 4.1 graphs a), b), c) below for the proportion of copepods stranded in large, medium and small sediment sizes respectively) and the analysis had insufficient power. However, some trends were noted. More copepods were found above the drawdown line in the large and medium control columns compared to drawdown columns. While not the highest value in the small sediments the control columns were around the same value as the large and medium columns. In the control columns, large, medium and small sediment had 23 %, 22 % and 18 % of the copepods above the drawdown line respectively. In the drawdown columns, the highest rate of stranding was found with the combination of small sediment and fast rate (23 %), and overall small sediment seemed to have higher rate of stranding (at 13.9 %, compared with 11 % for large sediment and 7 % for medium sediment, though the differences were not statistically significant). Overall, the incidence of stranding in drawdown columns was 11 % for copepods across all rates and sediment types. Grouping the samples by drawdown rate alone, there was no association between drawdown rate and incidence of stranding – the risk of stranding was 12 %, 9 % and 11 % for fast, medium and slow drawdown rate respectively.

A


Proportion of copepods stranded
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0 cms/day
4 cms/day
40 cms/day
400 cms/day
Rate of Drawdown (cm/day)

B


Proportion of copepods stranded
24
22
20
18
16
14
12
10
8
6
4
2
0
0 cms/day
4 cms/day
40 cms/day
400 cms day
Rate of Drawdown (cm/day)

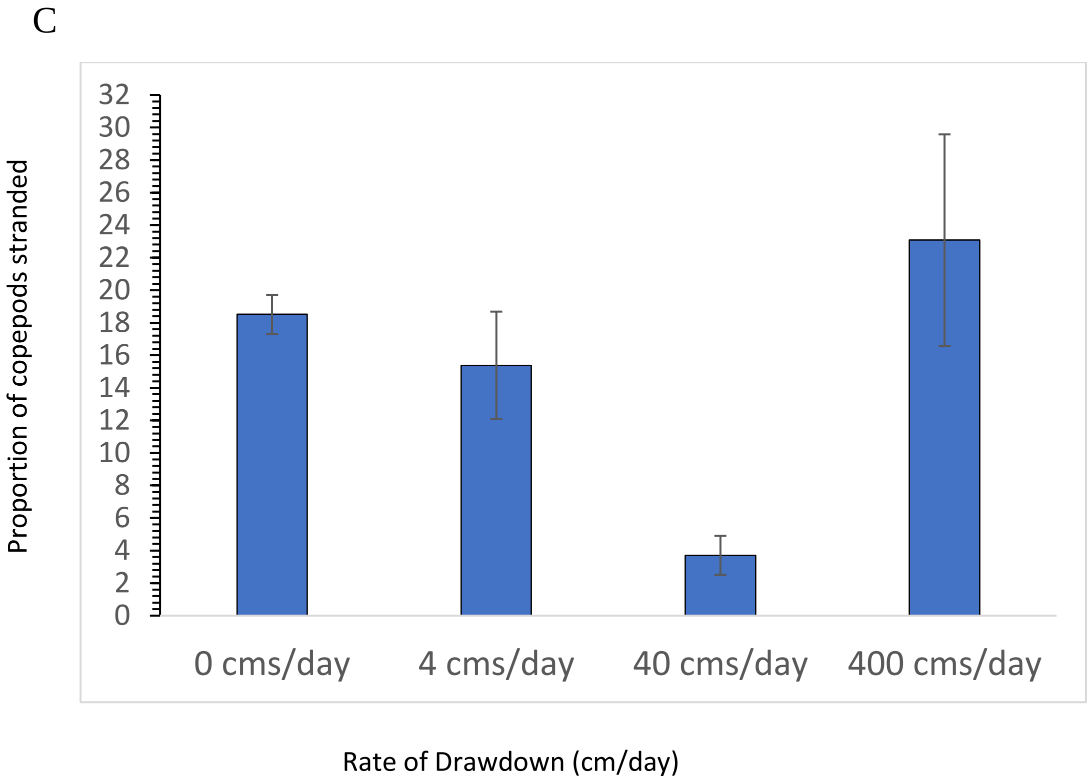
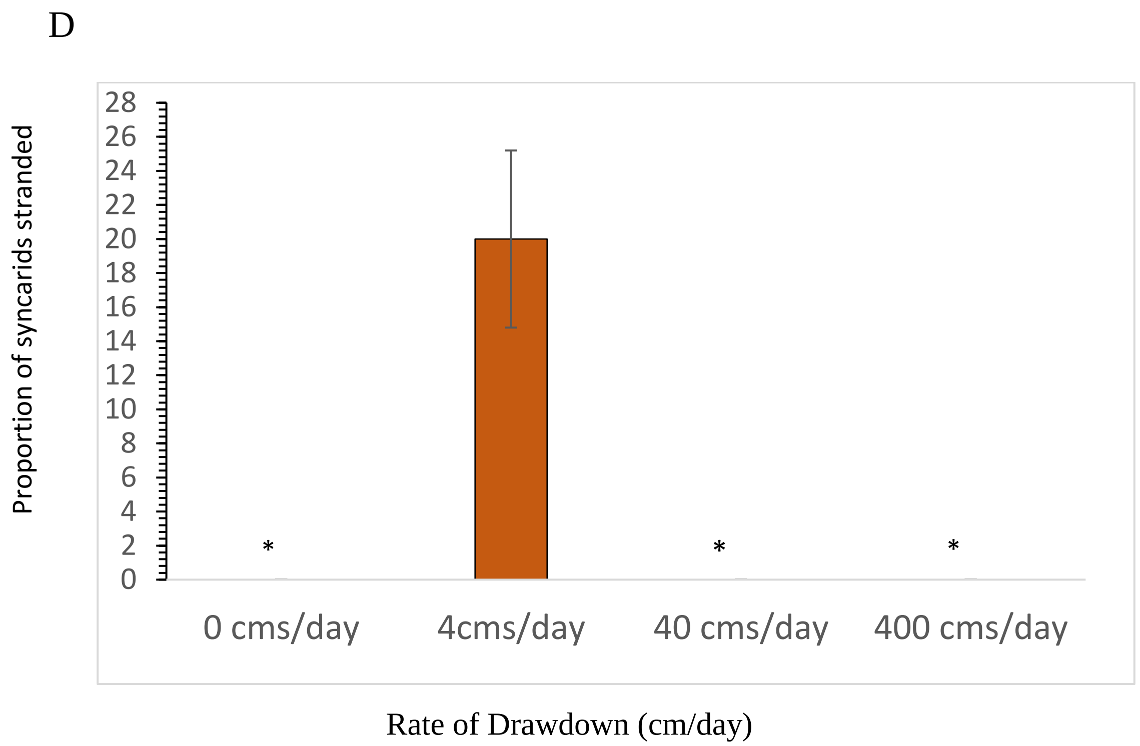


Fig 4.1. Mean (±standard error) proportion of groundwater copepods recorded above the mid column (drawdown) level following drawdown in A. coarse (2-4 mm), B. medium (1-2 mm) and C. fine (0.5-1 mm) sediments. Zero drawdown rate represents control columns. n= 3.

4.2.2 Syncarids

The proportion of syncarids above the drawdown level was not significantly different among the drawdown rates (fast, medium, slow and control) within the large sediment (F = 1.00, p = 0.44). (See Fig. 4.2 graph d) below) and the analysis was found to have insufficient power. Overall the incidence of stranding for syncarids was 5 % across all drawdown rates.



* No syncarids were found in the top half of these columns.

Fig 4.2. Mean proportion (± Standard deviation) of syncarids above the drawdown water level following drawdown with the different rates. n = 3. The graph shows that only the 4 cm/day drawdown treatment had syncarids above the drawdown water level following drawdown. All other drawdown treatments and controls had all syncarids below the drawdown water level following drawdown, as a result these treatments have no data on the graph above. Only five syncarids were found in the 4 cm/day treatment following analysis of the sediments instead of the possible six.

4.2.3 Difference of the stranded proportion from zero

The proportion of copepods above the drawdown level in the large sediment, slow rate combination was significantly different from zero (T = 6.31, p = 0.02), the proportion of copepods above the drawdown level for the small control was also found to be significantly different from zero (T = 5.00, p = 0.038). All other combinations for copepods were not significant and had insufficient power. The proportion of syncarids above the drawdown level was not significantly different from zero (p > 0.05) for any of the drawdown rates and had insufficient power.

4.3 Proportion of copepods versus syncarids stranded in large sediment

There was no significant difference when comparing the proportions of copepods and syncarids stranded in large sediment (not including control columns) (fisher's test, p = 0.69, df = 1) and the analysis had insufficient power. The incidence of copepods being stranded in large sediment was 11 % compared to 5 % of syncarids being stranded in large sediment (not including controls).

4.4 Distance Travelled by Dead Copepods

Sediment size and drawdown rate had no significant effect on the distance travelled by dead copepods (F = 2.36, p = 0.210 and F = 0.61, p = 0.587 respectively) (see Fig 4.3 graph e) below) and the analysis had insufficient power for the sediment size. On average dead copepods travelled less than 1 cm from the surface for different sediment types and drawdown rates. Smaller sediment sizes tended to restrict the downward movement of copepods more. While not significant, when all rates were combined the average distance travelled by copepods in large, medium and small sediment sizes was 0.73, 0.62 and 0.14 cm respectively. The power analysis suggests that there was sufficient power for the drawdown rate and it may be assumed that there is likely no difference in this case.

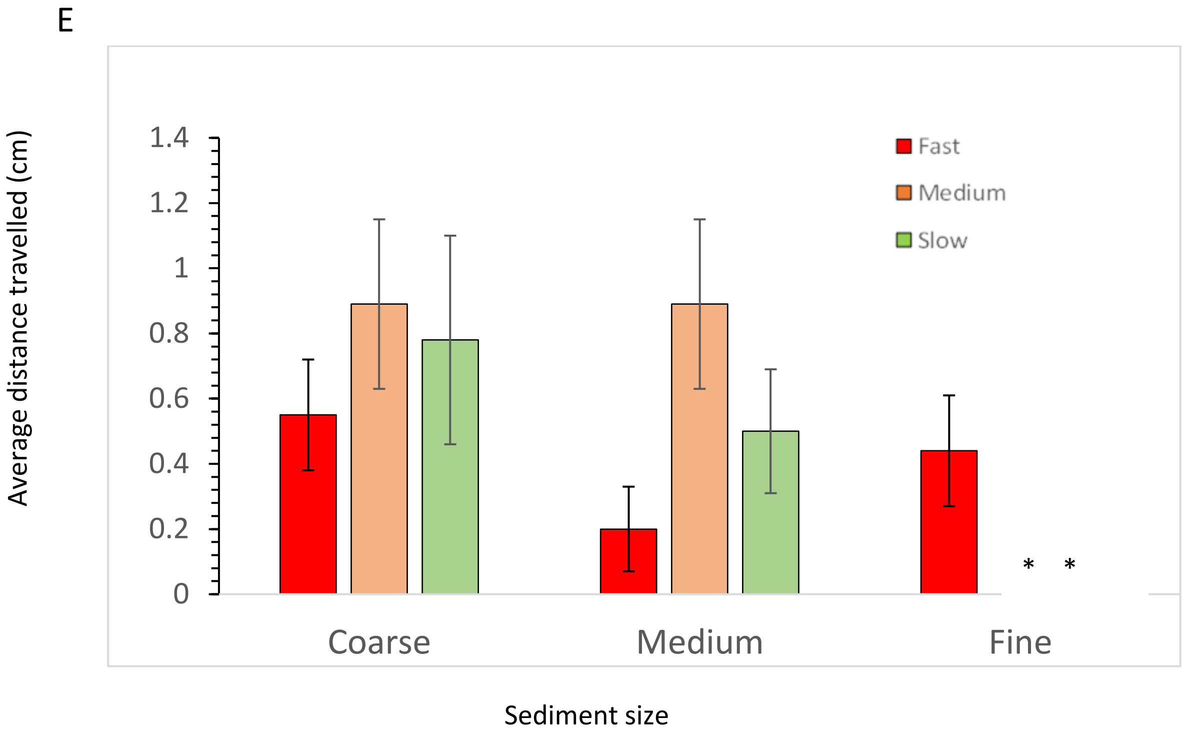


Fig 4.3. Mean distance travelled by dead copepods for each sediment and rate combination. * Copepods in small sediment, medium and slow rate combinations travelled less than 1 cm and could not be included in the graph.

4. 5 Comments on results

The study found few significant results; however, no consistent patterns were observed between the proportion stranded and the sediment size and rate of drawdown. This may be due to several factors including limited invertebrate recovery, problems with freezing and separation of the cross sections, lack of statistical power and the inconsistencies in methods used meant that useful comparisons could not be made. Invertebrate recovery following drawdown and freezing of columns was 70 % for the copepods in large sediment, for the medium and small sediment the recovery rate was 91 % and 88 %. For the dead copepods the recovery rate was 89 % over all sediment sizes. It was difficult to locate all the animals, it may be that some invertebrates degraded during the freezing process or separation of the sediment cross sections and could not be found. It was also very labour intensive to sort through the large amount of sediment and more time may have been needed to locate the missing invertebrates. The missing invertebrates may have an influence on the results obtained for the copepod studies. For example, if most of the missing animals had been in the top half of the columns following drawdown then the proportion stranded would be much higher than what was found here. A greater precision on the effects of drawdown on stranding could be achieved with a higher recovery rate of invertebrates.

The results here could also be affected by the method used to separate the sediment cross sections. Tap water was used and may have dislodged some invertebrates into different sections so the results are not as precise. The author suspects that animals are more likely to be dislodged downwards towards the bottom of the columns when using water to separate the cross sections.

The failure to find any meaningful significant results here may be due to a lack of statistical power. Here we had low numbers of invertebrates. A power analysis was not performed before the experimental design or sampling. Performing a power analysis beforehand would have committed the author to putting more effort into sampling. Logistics also limited my ability to collect larger numbers of groundwater invertebrates for this study. The post hoc power analyses that were undertaken show that most of the statistical analyses were underpowered due to low numbers of invertebrates meaning that there may have been a significant effect between the treatments which was not seen. However, the GLM used to examine the effect of sediment size and drawdown rate on the distance travelled by dead copepods had sufficient power for drawdown rate, however, drawdown rate was not found to be significant in this instance. There were observed trends suggesting that: animals move downwards with drawdown (saturated columns had higher numbers of invertebrates in the top half following drawdown compared to drawdown columns); smaller sediment sizes and faster rate treatments lead to higher rates of stranding with copepods and finally dead animals travel further in larger sediment sizes.

The inconsistency in methods used due to time constraints and lack of invertebrates meant that useful data comparisons between groups could not be performed. This reduced the usefulness of the statistical analyses. For example, the columns housing the copepod experiments using large sediment were only cut into two cross sections this made it impossible to gather depth distribution data for all three sediment types as only the medium and small sediment sizes were cut into fourteen 1 cm cross sections, likewise the columns used for the syncarid experiments were cut into four 3.5 cm cross sections. Also due to time constraints and low numbers of invertebrates syncarids were only used in the large sediment experiments. This data could then not be compared with the other sediment sizes. It was also not possible to compare the distance travelled by copepods and their cadavers in the different experiment treatments because the starting position for the live copepods was not known (they were left to acclimatise in the columns for 48 hours after being placed in the middle).

Chapter 5 – Discussion

5.1. Overview

This study looks at the impact of drawdown, specifically how the sediment size, invertebrate traits and rate of drawdown impact invertebrate stranding. Here we found that the proportion of copepods and syncarids stranded in the sediment above the water level after drawdown was not significant for drawdown rate or sediment size. However, while the difference was not statistically significant, organisms in the drawdown columns seem to have moved downwards compared with control columns. The proportion of copepods above the drawdown level in the large sediment, slow rate combination and small sediment, control was significantly different from zero, all other combinations were not significant. The distance travelled by dead copepods, as a surrogate of passive movement was not significant across sediment type or drawdown rate.

5.2 Limitations of study

1)Most of the statistical analysis were underpowered for this study due to low numbers of invertebrates (see section 4.5).

2) The inconsistency in methods used due to time constraints and lack of invertebrates meant that useful data comparisons between groups could not be performed. This reduced the usefulness of the statistical analyses performed (see section 4.5).

3) The failure here to find significant results may be affected by the attrition of samples which contributed to reducing the sample size even further. Some invertebrates that were placed in the columns were not found during analysis of the sediment (see section 4.5).

4) The results here could also be affected by the method used to separate the cross sections. Tap water was used and may have dislodged some invertebrates into different sections so the results are not as precise (see section 4.5). Stumpp & Hose (2013) also had difficulty separating the columns without creating bias.

5) The water level drawdown in these experiments was 7 cms. This cannot be extrapolated over larger distances because it is not known how far groundwater invertebrates can travel and over what speed. Studies examining the rate of drawdown over longer distances would be useful. For example, here syncarids were found at the bottom of the control and treatment columns

suggesting that they are good burrowers and prefer to live at lower depths at least in our laboratory conditions. Stumpp & Hose (2013) also reported syncarids at the bottom of columns. While it is evident that they are efficient burrowers it is not known how much further they could burrow if there was a larger water level drop. They may be safe at 7 cms but vulnerable to larger drawdown distances.

6) The sediments used in the columns were natural but too uniform to be found in the field. Studies using a more heterogenous sediment could help to gain more realistic results. The size, shape, packing and texture of the sediment will influence the ability of invertebrates to move through it. It is difficult to say what effect the uniform sediments used here would have on the movement of invertebrates. Increasing heterogeneity of sediment size often decreases the sediment pore size (Fraser 1935) while increasing heterogeneity of sediment shape acts to increase the pore size (Gayraud & Philippe 2003). The uniform texture would also influence invertebrate movement here the sediment texture was hard gravel and invertebrates would mostly relie on navigating through the pore matrix, although in the smaller sediment sizes animals would need to dislodge sediment. Invertebrates also adapt to the environmental conditions around them (Stumpp & Hose 2013) including the sediment type. If the invertebrates are adapted to a different type than we are using in our experiment, then our results will not be accurate.

5.3 Implications of this study

While our results here were largely inconclusive due to insufficient power some trends noted in this study support other studies examining the effect of drawdown on invertebrates. Results suggest that while animals move downwards with drawdown some animals are stranded above the water level, smaller sediment sizes and faster rates tend to restrict the downwards movement of animals, copepods are more vulnerable to drawdown events compared to syncarids and the small distances travelled by dead copepods suggests that active movement is required by invertebrates to avoid becoming stranded with water level declines.

Examining the effect of drawdown on groundwater invertebrates is a relatively new field of study and while there are some studies there is not a great deal of literature available for even the basic factors involved in groundwater level decline and its effects. Few studies exist because of the difficulty accessing the groundwater environment, the low numbers of invertebrates and the fact that groundwater invertebrates are small and thereby more difficult to work with compared to macroinvertebrates. However, increasingly factors affecting the biodiversity of our environment are becoming more important due to increasing anthropogenic pressures. This applies to the groundwater environment as well due to increased water consumption and issues such as climate change.

Although not statistically significant here, we found a trend suggesting that copepods move downwards with drawdown. This is consistent with other studies. (Korbel, Stephenson & Hose unpublished, Stubbington et al. 2011). Although not significant, a drawdown study by Stumpp & Hose (2013) using cyclopoid copepods suggested that the cyclopoid copepods could move downwards through ‘substitute sediment’ if they needed too. When examining the incidence of stranding with drawdown between control columns and treatment columns with drawdown they found that saturated control columns had less stranding when compared to treatments with drawdown. The authors speculate that this may be because the invertebrates may have progressively moved downwards more easily in the saturated columns compared to the unsaturated columns because a spoon was used to separate the sediment cross sections while the invertebrates were still alive. In a similar vein Korbel, Stephenson & Hose (unpublished) found that harpacticoid copepods and syncarids can borrow into sediments when faced with desiccation if pore space allows.

In our drawdown column experiments using copepods we found that the smallest sediment size and fastest rate combination had the highest rate of stranding (although our analysis was underpowered), Other studies suggest that smaller sediment sizes may be associated with higher rates of stranding as invertebrates are excluded from small pore spaces (Hose et al. 2017, Korbel, Stephenson & Hose unpublished). In a mesocosm experiment with water level decline Korbel, Stephenson & Hose (unpublished) examined the proportion of copepods that could burrow 20 mm into clay (<0.07 in diameter) and gravel (2 – 4 mm in diameter) and found a difference of 30 - 80 % respectively. A drawdown study by Vadher et al. (2017) using stream macroinvertebrates and artificial sediments also found that larger pore spaces were associated with greater burrowing depth through sediment compared to smaller pore spaces.

The drawdown rates used in this study had no significant effect on the proportion of copepods or syncarids stranded (although the fastest rate, smallest sediment size had the had the highest rate of stranding). This was unexpected as the rates in this study were spread over a large range and expected to cause stranding at least in the fastest rate. In a series of column drawdown experiments using groundwater invertebrates Stumpp & Hose (2013) also found that drawdown rate had no significant effect on the proportion of copepods stranded above the water level following drawdown. This study however, had a much narrower range for the rates used (2.6 m/d fast rate and 1 m/d slow rate) and it may not have been possible to detect a difference between the rates with the size of sediment used in the experiment. A study by Korbel, Stephenson & Hose (unpublished) found that Harpacticoid copepods could quite easily burrow into sediments with a smaller diameter (of 400 µm) than used in this study (500 µm was the smallest diameter used in this study) and the Stump and Hose drawdown experiments (1.55 – 1.85 mm). This may have enable the copepods to move easily downwards with the drawdown rates used. This is also supported by the fact that the copepods in this study were around 500 µm in length (the same size as the smallest sediment diameter) and much narrower and could therefore move between the sediment grains. However, this study only concluded that the harpacticoid copepods travelled more than 20 mm and did not give a precise distance, so it is difficult to speculate how far the invertebrates might have burrowed. Further supporting the hypothesis that the copepods might have been able to move downwards in our experiment conditions, Palmer, Bely & Berg (1992) found that pond copepods can travel at 1.6 cm/ min through water, at 0.3 cm / min, our fastest rate of water movement was much lower in this experiment, however, the copepods were required to move around sediment. The study by Stumpp & Hose (2013) did find a significant difference in the proportion of syncarids stranded with the rate of drawdown with more found in the top of the drawdown columns compared to the saturated control columns, however, the authors speculated that the syncarids were moving downwards in the saturated columns as the sediment was being removed from the column for analysis. Our failure to find a result here with syncarids may be due to low statistical power.

There was no statistically significance difference found here between the proportion of copepods stranded compared to the proportion of syncarids. Here it is only possible to compare the large sediment treatments as syncarids were only placed in the large sediment. However, a trend was noted. Larger numbers of copepods were stranded above the water level compared to the syncarids following drawdown. This is consistent with other studies that suggest that different species have different responses to drawdown. Stumpp & Hose (2013) found that syncarids were able to move downwards (either because of drawdown or during the sediment separation phase), by comparison the copepods were not as able to do this (although there were slightly less copepods in the top half of the saturated columns compared to the drawdown columns suggesting some ability to move downwards ahead of the sediment analysis). Vadher et al. (2017) found that species type had a significant influence on the chance of becoming stranded with drawdown. More here about species traits and why

Syncarids may be more adept at dealing with drawdown compared to copepods as a consequence of their mode of locomotion (being strong crawlers compared to copepods), higher tolerance to drying and their preference for lower depths in the sediments when compared to copepods (Hose et al. 2017). It is also possible to speculate that syncarids have a better movement strategy when dealing with water level declines. By observing them under the microscope it is evident that that syncarids tend to move more in straight lines when compared to copepods. A study by Palmer, Bely & Berg (1992) also supports this and notes that copepods tend to zigzag throughout the environment when they move. This is likely to lead to higher levels of stranding with drawdown events. Stumpp & Hose (2013) also noted that different species tended to be found at different depths in the saturated columns suggesting a depth distribution preference (although the authors also note that the syncarids and copepods may have moved downwards during the analysis of the experiment). Syncarids tend to move toward the bottom of the columns while some species of copepod were found near the water table at the surface (Stumpp & Hose 2013). This suggests that these species of copepods will be more affected by and vulnerable to drawdown compared to syncarids.

Dead copepods were tested among different sediment sizes and drawdown rates. Overall, there was very little movement of the cadavers even within the large sediment size, with copepods being restricted to less than 10 mm of movement. In comparison, a study by Korbel, Stephenson & Hose (unpublished) found that 80 % of live copepods could travel more than 20 mm in the large sediment size used here. (2 – 4 mm) and 75 % could burrow more than 20 mm into sand (diameter 400 μm). A study by Vadher et al. (2017) directly compared the distance travelled between alive animals and their cadavers and also found that live animals travelled much further though sediment These studies, as well as evidence suggesting that live animals can travel further in even smaller sediment sizes than the ones used here support the idea that active movement is involved in taking refuge from drawdown.

The results obtained here were unexpected given that the drawdown rates and sediment sizes used here were chosen to find a point where stranding of invertebrates would occur. We deliberately chose large ranges for the drawdown rate and sediment size based on the available literature. The drawdown rates were representative of real field data and occur with groundwater drawdown in the field. The sediment size also differed three-fold over the three sediment types and while the sediment treatments lacked the heterogeneity of sediment in the field, they were naturally occurring

Overall, drawdown leads to some invertebrates becoming stranded in the unsaturated zone, with some environmental conditions more likely to lead to stranding and some species being more vulnerable to stranding. It is known that drawdown can have detrimental effects on groundwater invertebrate populations by causing mortality (Stumpp & Hose 2013), shifts in community composition (White et al. 2011) and changes to ecosystem function (Boulton et al. 2008). With increasing pressures on groundwater resources further studies are needed to determine which groundwater invertebrates are vulnerable to drawdown and the environmental conditions under which stranding is likely to occur to protect the biodiversity of groundwater invertebrates in the future.

5.4 Recommendations for future study

1) This study could have been improved by conducting it on a larger scale to increase the sample size. Although this would also require more sampling effort and perhaps the use of different sampling sites to collect more invertebrates from the field. It may have also been possible to increase the density of invertebrates in each column. Here 10 invertebrates were used in each column, however, increasing this to 16 – 20 invertebrates per column (also required greater sampling effort and perhaps different sampling sites) would have increased the power of the analysis with little effort required elsewhere. Care must be taken not to increase the density too much so that invertebrate interactions will impact on the result of the study. Another option to increase power of the study would be to simplify the experiment, instead of using three sediment sizes and three drawdown rates these numbers could have been reduced.

2) Improving the consistency in the methods across the different treatments would enable more useful and powerful statistical analysis to get more meaningful results and conclusions for this study. The large sediment columns using copepods should have been separated into 14 cross sections to allow comparisons with the medium and small sediment sizes. The syncarids

experiments should have been conducted in the medium and small sediment sizes to allow for comparisons.

3) Improving the recovery of invertebrates following drawdown could provide more accuracy to the experiment. Using smaller columns would mean there was less sediment to sort through. Tagging the invertebrates with a dye or substance so all that was needed was to scan the column to find them would make recovery easy though I'm not sure which substance to use or how to scan the columns,.

4) Using tap water dislodges invertebrates and could lead to inaccuracies in assigning which section the organism came from. Using a cutting tool to separate the columns may help to avoid invertebrates being pushed into the sections further down the column. While a cutting tool may also lead to sample attrition, there could be less chance of moving invertebrates into different sections.

5) The limitation of not being able to extrapolate more information from 7 cms of drawdown could be improved by using longer columns or conducting studies out in the field.

6) The limitation of our sediments being too uniform could be improved by using a more heterogeneous mixture of sediment sizes that are more representative of the field. It may also be possible to collect sediment from bores for use in these column experiments. Drawdown studies out in the field could also offer a more representative sediment matrix.

Chapter 6 - Conclusion

Groundwater is under increasing pressure worldwide. Drawdown can impact on groundwater ecosystem functioning by causing changes to the community structure. Here we confirmed that stranding does occur with drawdown, despite what appears to be some capacity for invertebrates to follow a declining water table. Further research is needed to clarify the specific characteristics of the organism and drawdown conditions that might impact on invertebrates survival in a drawdown situation.

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