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DEVELOPING THE GROUNDWATER HEALTH INDEX (GHI) AS AN INDUSTRY-WIDE MONITORING TOOL.

Benchmarking and assessing groundwater ecosystems within cotton growing regions of the Murray Darling basin

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Executive summary

A well-managed supply of clean, usable groundwater is vital for the continued success and growth of the Australian Cotton Industry. The ongoing provision of clean groundwater depends on healthy groundwater ecosystems containing microbes and invertebrates (stygofauna). These biota improve water quality and aid water flow. Without the sustainable management of groundwater, it is likely that the health of these ecosystems will decline, with the consequence that growers may be faced with increased costs for groundwater extraction, water treatment and/or declining cotton yields.

In 2006, the cotton industry, through the Cotton Catchment Communities (CCC) CRC, became the first industry in Australia to recognise the importance of groundwater ecosystem health for the supply of sustainable groundwater resources by funding the development of a groundwater ecosystem monitoring program. The CCC CRC began a world first program to develop a toolbox of biological and chemical indicators to measure and monitor groundwater ecosystem health.

Further funding from the CRDC, through the project reported here (2014-2018), enabled the initial toolbox to be refined through additional sampling and research. From this work, the weighted Groundwater Health Index (wGHI; Korbel & Hose 2017) was developed. The new, refined method allows groundwater health to be assessed and monitored using a combination of biological and water chemistry variables. Since the publication of this method, the authors were invited to present the framework to the European Union Groundwater Working Group (April 2017) as a potential framework for adoption in the Groundwater Framework Directive and NSW DPI have been in discussions about including the approach in their monitoring for groundwater dependent ecosystems within NSW.

The project has succeeded in providing

- Documentation of the extent and condition of subterranean GDEs in 4 subcatchments of the MDB
- Provision of baseline data on groundwater biodiversity
- Enhancing knowledge of GW ecosystems
- Improve groundwater ecosystem sampling methodologies
- Development of GHI to be used on farms with potential incorporation into MyBMP

The outcomes from this work are an improved groundwater management program, shifting from the current approach of managing water quality and quantity to an approach that considers groundwater biodiversity and the maintenance of ecosystem function and services. It will allow for future assessment of potential groundwater health decline due to competing industries. The results of this report will be implemented through the cotton industry My BMP program, promoting ecological sustainability and natural resource stewardship, while concurrently improving the capacity for the future management of this resource.

Report

Developing the groundwater health index (GHI) as an industry-wide monitoring tool.

June 2018

Introduction

Groundwater is an important resource for agriculture throughout the world. Around 900km^3 of groundwater is used for agriculture each year. In Australia, over 80% of cotton is grown under irrigation, derived from a combination of sub-surface and surface sources. Although the importance of groundwater to agriculture is clear, groundwater also supports numerous ecosystems and provides valuable ecosystem services. Thus the sustainable management of groundwater resources includes the management of these ecosystems.

Groundwater Dependent Ecosystems (GDE) are ecosystems that rely upon groundwater for their existence. The mapping, monitoring and protection of Groundwater Dependent Ecosystem has come to the forefront of both Federal and State governmental policies, with GDEs incorporated in the National Water Initiative (2004), with many Water Management Plans requiring monitoring and protection of these ecosystems. There are several types of GDEs that can be grouped into three broad categories:

- Terrestrial GDEs
- Surface-aquatic expressed GDEs (eg rivers, wetlands)
- Subterranean GDEs

Within groundwater there consists a diverse and complex ecosystem consisting of microbes and invertebrates which provide ecosystem services of water purification and maintaining groundwater flow. These ecosystems are commonly know as *subterranean GDEs* or *groundwater ecosystems* and are 100% reliant on groundwater for their existence as the ecosystem is contained within groundwater itself.

The Cotton Industry recognises the importance of groundwater ecosystems for productivity and the need for benchmarks from which to assess current conditions, future change and potential impacts from other industries. The need to understand and sustainably manage these ecosystems and groundwater health, is of importance not only due to their biodiversity values but due to their functional importance and the ecosystem services they provide, such as maintaining aquifer flow, water quality and contributions to river flow and health

GROUNDWATER ECOSYSTEMS

Within aquifers there exists highly specialised, diverse and complex ecosystems consisting (typically) of small invertebrates (known as stygofauna), protozoan and microorganisms. These animals have adapted to the harsh conditions posed by subterranean environments with low levels of oxygen and nutrients, as well as the absence of light. Photosynthesis is absent in the dark subterranean environment, so the ecosystem is dependent on external sources of nutrients and oxygen from the surface.

A diverse group of Archaea, Bacteria and Fungi (known generically as microbes) inhabit groundwater environment. Through biogeochemical processes, these microbes are fundamental to the cycles of chemicals including sulfur, nitrogen, carbon and iron. These organisms are well known for their ability to degrade pesticides, organochlorides and nitrates within groundwater, with their importance in groundwater remediation recognised globally. Our current studies in NSW and QLD catchments have highlighted the presence of microorganisms with the capacity to degrade nitrates and reduce methane, hydrocarbons, iron and sulfur within groundwater. These microbes have the important ecosystem service of maintaining groundwater quality in agricultural areas.

Due to the lack of photosynthesis, microbes provide the foundation of the food web in the groundwater environment, with protozoans and large invertebrates feeding on the naturally occurring microbial biofilms. There appears to be a symbiotic relationship between microbe, protozoans and invertebrates within the groundwater, with the larger organisms providing organic material (through faecal matter and death) which microbes consume, but also it is thought that grazing activity of larger invertebrates promotes microbial growth within the groundwater. Thus both parts of the foodweb rely on each other for survival.

Most of the larger animals found in groundwater are highly evolved, obligate groundwater dwelling invertebrates that are not found in surface environments. They are predominantly crustaceans such as copepods, amphipods, isopods and syncarids (Fig 1), however also include species of oligochaetes (worms), mites and single-celled protozoans.

Figure 1: (clockwise from top left) Amphipod, syncarid, syncarid, mite, oligochaete, copepoda, mite, copepoda with visible eggs, typical sample diversity. Note Rose Bengal stain (pink) was used in some samples to enhance identification. Photos taken by K Korbel.

Groundwater ecosystems and their biological components are particularly important for maintaining the **quality and quantity of groundwater** available for agricultural and potable use. It is the interactions between microbes, invertebrates, water chemistry and the physical environment that enable the ecosystem to provide these crucial service. The need to understand and sustainably manage these ecosystems and indeed groundwater health is of importance not only due to their biodiversity values, but the ecosystem services they provide.

Groundwater health is the ability of an ecosystem to sustain its ecological function whilst maintaining ecosystem services. A previous CCC CRC project established an innovative framework to measure groundwater health (Korbel & Hose 2011). This framework was developed in the Gwydir catchment, and allowed a broad comparison of groundwater health between sites. The tool was instrumental in allowing the monitoring of groundwater health and providing baseline information.

CRDC: At the forefront of groundwater ecosystem research.

The cotton industry recognised the importance of groundwater as a resource and the role that groundwater ecosystem play in maintaining ecosystem services. To increase the understanding of this resource, and to allow the sustainable management into the future, the CRDC funded project **MQ1501.** The aims of this four-year project were to:

- Document the extent and condition of subterranean GDEs in four MDB sub-catchments
- Enhance the scientific knowledge of groundwater ecosystem and their interactions with farms and surface environments
- Further develop the GHI into a cost-effective tool to monitor groundwater health which can be used to monitor the environmental performance of cotton and other industries.

These ambitious aims have promoted the cotton industry as a leader in environmental management, and have seen it become the world's first industry to establish groundwater biodiversity and health baselines.

The broad objectives of the project were to:

- Document the current baseline groundwater biodiversity of the MDB catchment,
- Improve groundwater ecosystem sampling methodologies
- Improve the understanding of GDEs and their functions
- Improved understanding of the impacts of irrigation and cotton growing on GDEs
- Develop the GHI into a tool for monitoring groundwater health for use on farms.

This document will report on the above objectives and describe the knowledge gained through the project.

Project Study Area

The program has collated data from a total of 147 sites in the Murray Darling River catchment from the Gwydir catchment, Namoi R catchment, Macquarie catchment and the Condamine catchment (Fig 2). Catchments have all been sampled at least twice, with some of the more comprehensively studied sites in the Gwydir catchment sampled up to seven times. This research has now resulted in the one of the most comprehensive database of subterranean GDEs within Australia.

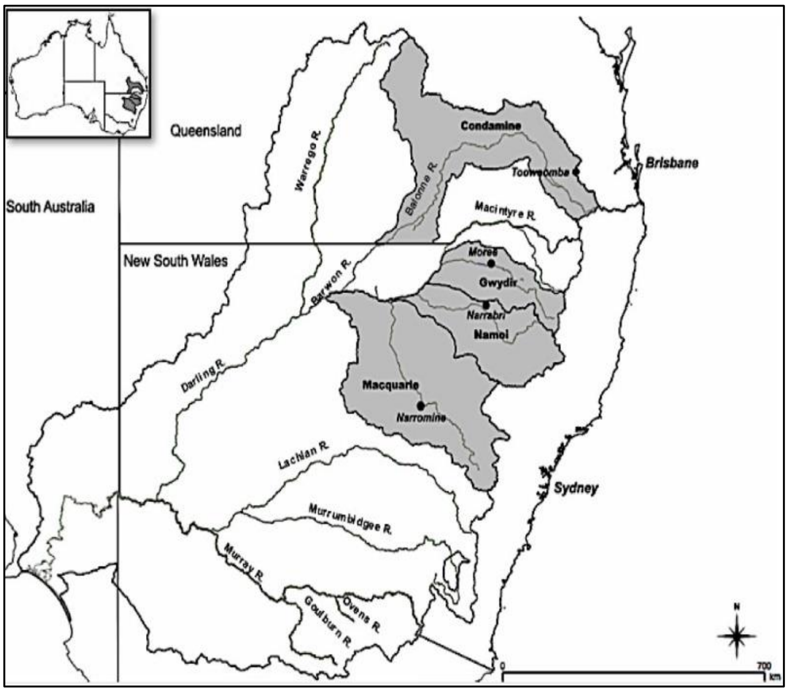

Figure 2: Map of study sites

The program was extended to incorporate molecular techniques, which extract DNA from water samples to characterise microbial (prokaryotic) (using 16S gene) and larger organism (eukaryotic) communities including stygofauna (using 18S). The number of sites that have this additional data totals 50, making this the single largest known study of eDNA in groundwater in the Murray Darling Basin.
Sampling within these catchments has been undertaken in the shallow alluvial aquifers (<40m deep). The majority of the bores samples were PVC pipes owned wither by the NSW or QLD governments. The bores sampled at Maules Creek (Namoi catchment) and at Wellington (Macquarie catchment) were accessed through collaborations with UNSW and were established under the NCRIS project.

GWYDIR AND NAMOI CATCHMENTS

The Gwydir and Namoi River aquifers have similar geology and geological histories. Both catchments have weathered pre-Quaternary kaolinised profiles, resulting in kaolinitic clays in the west of the catchments (Young *et al.* 2002). Throughout the Quaternary period, alluvium was widely deposited, and the transition from wet to dry climate during the Neogene and Quaternary periods has influenced sediment structure throughout the region, as noted in the distribution of sands/gravels and clays (Kelly *et al.* 2012).

The shallow aquifers of both catchments are dominated by surface interactions, characterised by good hydraulic connectivity between sand and gravel substrates in most regions (Carr & Kelly 2010). Groundwater levels are highly influenced by flow in the Gwydir and Namoi Rivers and flood events (Barrett 2009; Carr and Kelly 2010), with significant recharge occurring after major rain events (Barrett 2009; Carr and Kelly 2010).

MACQUARIE RIVER CATCHMENT

The Macquarie River catchment begins south of Dubbo and extends to the Darling River. The area sampled in this study concentrates on the semi-arid alluvial floodplain near Narromine. Upstream of Narromine the basement is Triassic, with downstream being Jurassic-Cretacous. The alluvial aquifers consist of an upper unconfined aquifer, consisting of mainly sand and gravels with the occurrence of confining clay bands (Giambastiani & Kelly 2010). Coarser grained sediments are located near the rivers. Alluvial aquifers in this region are locally hydraulically connected to the river, however the extent of connectivity between the alluvial aquifers and the underlying rock aquifers is poorly understood (Giambastiani & Kelly 2010).

Abstraction of groundwater in the Macquarie catchment began in 1967, and was associated with irrigated cotton and other crops. Ten sites in the lower Macquarie River, located 300 km south of the Gwydir catchment, were sampled around the township of Narromine (32°13'S, 148°14'E, population 6,854). Samples were taken from the shallow (10-35m depth) aquifers where the geology was very similar to the other three catchments.

A number of sites near Wellington in the upper catchment, were also sampled. These samples were included in addition to the sites funded under MQ1501**.** These sites were included in this report as they help to classify the biotic communities within the MDB and provide a representation of sites located away from cotton growing activity.

CONDAMINE RIVER CATCHMENT

The Condamine catchment is located approximately 180 km to the north of the Gwydir catchment, near Toowoomba (27°33'S, 151°93'E, population 161,970) in southern QLD. The study area is characterised by three dominant hydrogeological units, the sedimentary basement aquifer, the basalt aquifers and the alluvial aquifers. This study concentrates on the Quaternary-formed, sand, gravel and clay based alluvial aquifers of the catchment. There is high hydraulic connectivity between the alluvial aquifer and the Condamine River and tributaries, and inter-connections between all the aquifers within the catchment (Dafny 2014).

Fourteen sites were sampled in the shallow (<40m) alluvial aquifers of the Condamine catchment between Warwick and Dalby, west to south west of Toowoomba.

LANDUSE IN THE STUDY AREAS

Agriculture is the main industry in all four catchments, with irrigated cotton being the largest financial contributor to the Namoi and Gwydir catchments (ACG, 2010), and a dominant landuse in the Condamine (MDBA, 2015) and lower Macquarie catchment. Other landuse includes grazing and both dryland and irrigated cropping (e.g. sorghum, lucerne, mung beans) and, consequently, all catchments have been extensively modified for agriculture. Groundwater is widely used for irrigation and stock in all four catchments, with irrigation activities undertaken for over 50 years (Carr and Kelly, 2010; Giambastiani and Kelly, 2010; Kelly et al., 2012). Declining groundwater levels and pesticide contamination have been linked to irrigation in the Gwydir and Naomi catchments (Barrett, 2009; Carr and Kelly, 2010; Muschal and Cooper, 1998) and groundwater extraction in the Condamine and Macquarie catchment are believed to have contributed to declining groundwater water levels (MDBA, 2012, 2015).

The following sections of this report are divided into the specific objectives of MQ1501 project. They provided the results from the past 4 years of research.

1. Biodiversity study in the catchment.

EUKARYOTIC ORGANISMS (IE ANIMALS, PROTOZOANS AND FUNGI)

The aquifers of the Murray Darling Basin support a significant biodiversity of eukaryotic communities. Using a combination of traditional 'collect and count' methods and metagenomics methods, sampling has revealed a relatively high diversity of taxa. Over 77 eukaryote OTUs have been identified at the order level (where possible) or class as a result of the sampling from this project. Several of these OTUs appear to be species new to science. Results for each of the 4 study catchments are provided in Appendix A.

Numerous protozoans were detected using 18S DNA sequencing. Protozoans, particularly those from the class Spirotrichea (sub-class Hypotrichia) significantly contributed to the eukaryotic community in all aquifers. Centramoebida (Amoebozoan), Eugregarunorida (Protozoan), water mites and fungi were relatively abundant within groundwater, indicating a complex community of micro, meio- and macro fauna within aquifers. Within groundwater, it is believed that protozoans have the function of stimulating bacterial metabolism through secreted nutrients and by grazing on bacterial cells. It has also been suggested that the grazing of bacteria by protozoans prevents clogging of interstitial spaces in the aquifer matrix and contribute to the cycling of nitrogen and carbon within groundwaters.

The presence of fungi in groundwater was detected through DNA analysis, including many of the fungal genera reported from aquifers in other parts of eastern Australia. The fungi Kickxellomycotina was the most abundant eukaryote, but little is known of the ecological significance of this group of organisms. Blastocladiales were high in abundance, and members of this taxon are known to contribute greatly to the foodchain and are associated with decomposition of dead crustaceans or are parasites of nematodes, crustaceans, and tardigrades. Fungi from the order Pezizomycotina (including the class Dothimdeomycetes) have been detected in all catchments, and likely play a significant role in nutrient cycling and biofilm production.

There was high diversity of stygofauna within the study catchments, including Nematoda, Syncarid, Amphipoda, Oligochaetes, Copepoda, Platyhelminthes, Annelida, Tardigrades, Gastropoda and Ostracoda. Particular 'hotspots' of biodiversity noted within the Namoi and Gwydir catchments. These catchments had relatively higher abundances and diversity than the other two catchments. Comprehensive lists of biota are presented in Appendix A. eDNA analysis revealed taxa new to sciences, however these taxa remain to be formally identified and named.

Studies in the Gwydir and Namoi catchments have reported significant differences in stygofaunal assemblages, however total richness and abundances of stygofauna between the catchments are similar. The results suggest that the functional role of biota between catchments are similar, for example there were differences in the species of syncarids and amphipods between catchments, but no differences at higher taxonomic levels, and total biomass was similar between catchments. The similarity in composition, size and abundance of stygofauna between catchments is likely evidence that the biota are providing similar ecosystem service and functions between catchments (Korbel et al 2013). The differences in stygofaunal assemblages even at family level, may reflect the limited distribution of some taxa and evolutionary divergence of species.

PROKARYOTIC ORGANISMS (MICROBES)

Diverse communities of groundwater Archaea and Bacteria were revealed in the MDB through DNA analysis. The study of the Condamine, Gwydir and Namoi catchments indicated 7 Archaea and 88 Bacteria orders, with six bacteria and one archaea only being identified to the domain level, indicating species new to science. The results from this study are summarised in Figure 3, which indicates the typical composition of archaea and bacteria within the catchments. Full results of the microbial biodiversity will be provided to the CRDC in electronic form due to the amount of data associated with DNA sequencing.

The prokaryotes identified in this study perform a range of metabolic functions, which suggests that biogeochemical cycling of carbon (e.g. the methanogen, *Methanomassiliicoccales*), sulfur (e.g. sulfur reducing bacteria from the order *Desulfobacterales*) and nitrogen (e.g. the ammonia oxidizing archaea *Nitrososphaerales*) is occurring within groundwaters. As well, there were taxa capable of aerobic and anaerobic processes. Interestingly, we recorded a number prokaryotic organisms that could only be classified as either bacteria or Archaea, with no confidence in lower–level taxonomic detail, and many taxa for which a functional role is unknown. Additionally, several unidentified species of oganohalide respiring bacteria (ORB) eg Dehaloccoides, Dehalobacter, Desulfitobacter, as well as sulfate reducing microorganisms (SRM) eg Desulfotomaculumm, Desolfobacter and choroflexi species have been identified in the groundwater samples. Such new species may be considered valuable in the bioremediation of organohalide and crude oil polluted groundwaters (eg Sherry et al 2013).

Aquifers contained high proportions of prokaryotes that have the potential to fix nitrogen, including *Nitrosopumilales, Nitrososphaerales*, and *Nitrospirales*, with members of these orders recognised as important sink for nitrogen in freshwater environments. The high proportion of these taxa in the prokaryotic community suggests that some aquifer ecosystem are providing a predominantly N-fixing environment. In other catchments several commonly identified denitrifiers were prominent, including *Sphingomodales* and *Comamonadaceae* (Order: *Burkolderiales*). The presence of these taxa may suggest that aquifers may be enriched in nitrate, or previous enrichment of nitrates has occurred, however further studies are required.

Pathogenic organisms, particularly *Xanthomonadales* (Family: *Xanthomonadaceae*) and *Pseudomondales*, were detected in cotton growing regions. *Xanthomondales* is known to cause significant losses to cotton crops and *Pseudomondales* a known component of biofertilisers, although members of this order are also common soil denitrifiers with no plant pathogenicity. Additionally *Burkholderiales* (e.g. *Comamonadaceae*), which were most abundant, are known biocontrol agents of pathogens on cotton crops. The greater abundance of these organisms, as well as *Xanthomondales* and *Pseudomondales*, at cotton sites suggests that either pathogens may be entering groundwater from terrestrial source, however further studies would be required to determine the source.

Further studies of the Gwydir, Condamine and Macquarie catchment indicate that the Gwydir and Condamine catchments have very similar microbial communities, whereas the Macquarie catchment appears to have different relative abundances of various microbial communities (Figure 4), most notable higher abundances of Hydrogenophilales and lower abundances of Psuedomondales.

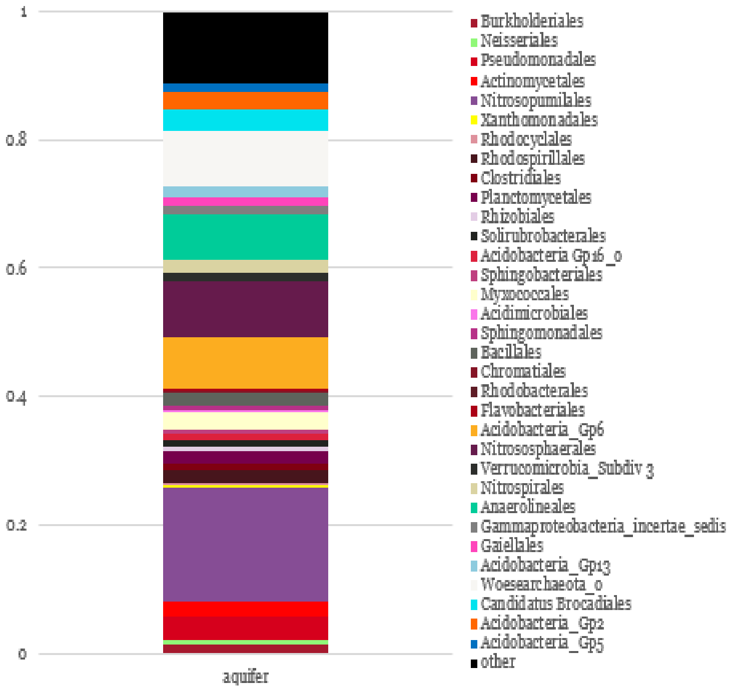

Figure 3: Typical prokaryotic communities within MDB

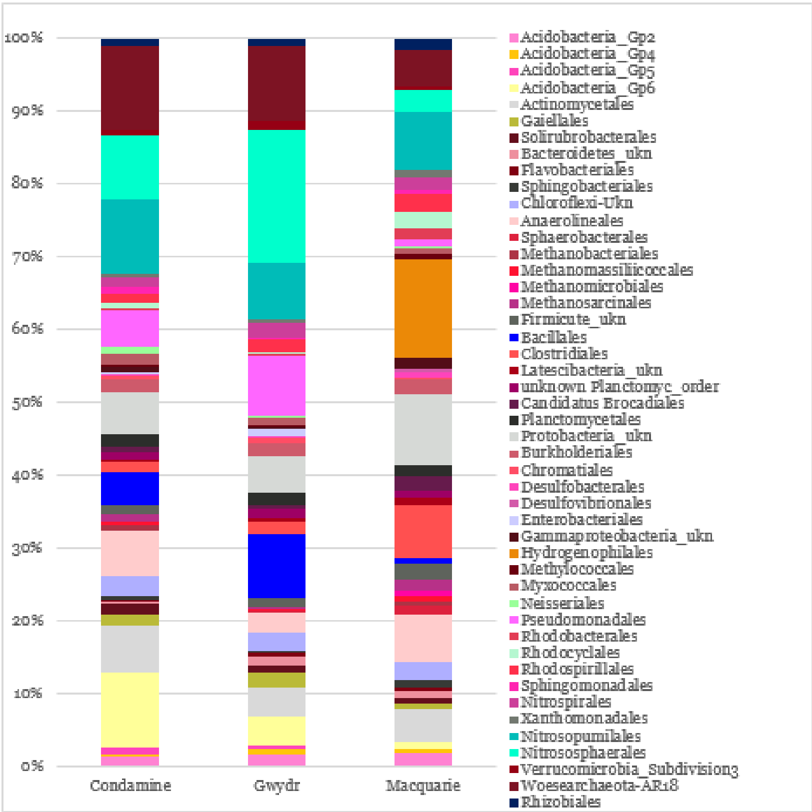

Figure 4: Microbial communities within catchments of the MDB

2. Impacts of irrigation on groundwater microbial communities

A number of papers, have indicated the impact of irrigation and cotton growing activities on groundwater biota, describing differences in biotic community diversity and abundances under differing agricultural landscapes. This current project (MQ1501) further developed these investigations to look at functional role of biota and differences in community profiles.

When comparing irrigated to non-irrigated areas on a functional level, significant differences between the microbial communities particularly in the nitrogen cycling functions are evident. Dissolved inorganic nitrogen (DIN), in the form of nitrates, nitrites and ammonium, in groundwater arises mainly through leaching of fertilisers from agricultural systems and can be enhanced through irrigation. Thus, we suggest that the increased abundance of nitrifying microbes at irrigated sites may reflect the long term impacts of the application of nitrogen based fertilisers on groundwater biota. Following fertiliser application, the microbial community may actively convert the ammonia to nitrates, which may cause elevated nitrate levels in the groundwater, however additional testing is required to confirm this suggested mechanism.

Our studies have indicated

1. groundwater microbial communities change in response to the surface landuses,
2. higher relative abundances of denitrifying bacteria and lower ammonia oxidation microbes in non- irrigated compared to irrigated agricultural.

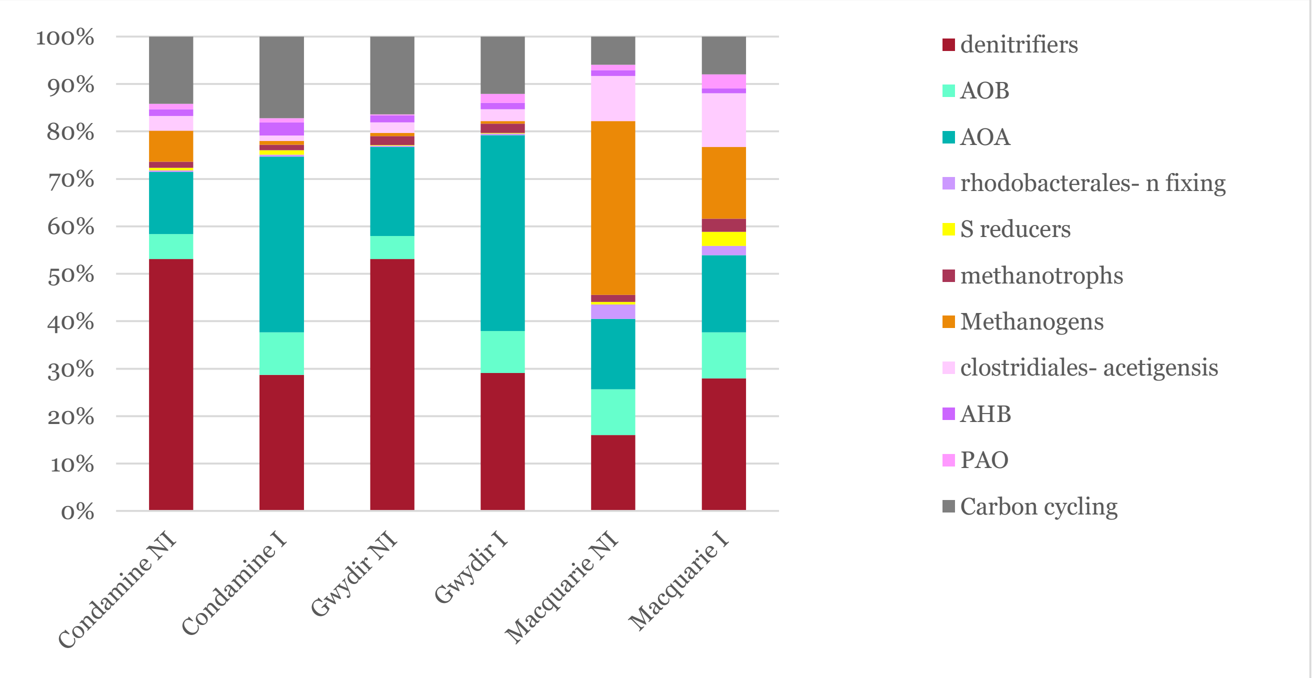

Figure 5: Comparison of microbial community functions within non-irrigated (NI) and irrigated (I) sites within catchments of the MDB (Korbel & Hose unpubl.)

3. Improving knowledge of groundwater biota

STYGOFAUNA HABITAT AND SEDIMENT PREFERENCES

Research from this study indicates the importance of environmental variables in the distribution of groundwater biota. This is important research as it means that human impacts on biota can be distinguished from natural variation. Our research has concentrated on the impacts of sediment size and groundwater drawdown on groundwater fauna.

There were increases in average stygofaunal richness and abundance with course sediments in both the Namoi and Gwydir catchments (Figure 6). Larger animals were not present in fine sediments such as clay or fine sands, with smaller taxa (copepoda) also increasing in abundance with coarser sediment types (Figure 7).

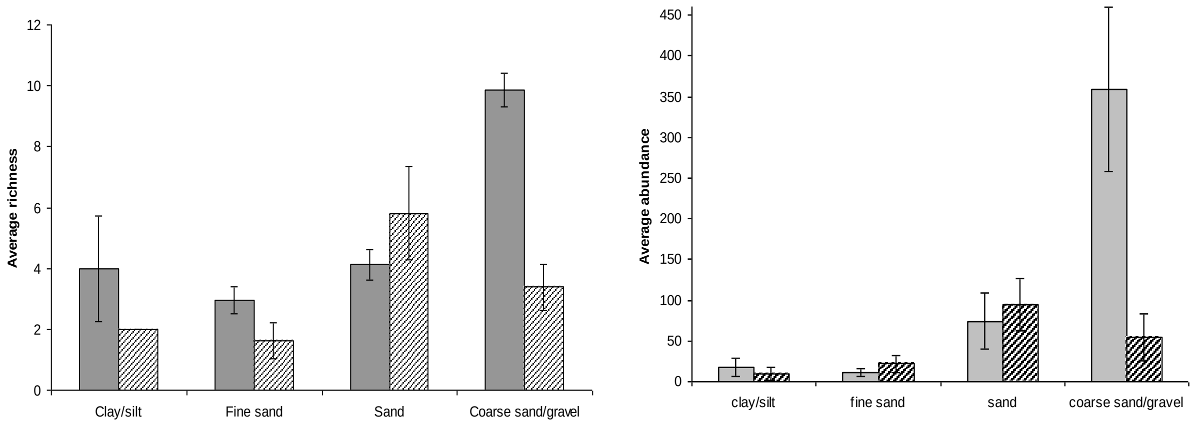

Figure 6: Mean (+ Std Dev) of stygofaunal average total richness and abundance with soil type in the Gwydir (shaded) and Namoi (striped) alluvial aquifers. (from Korbel et al 2013)

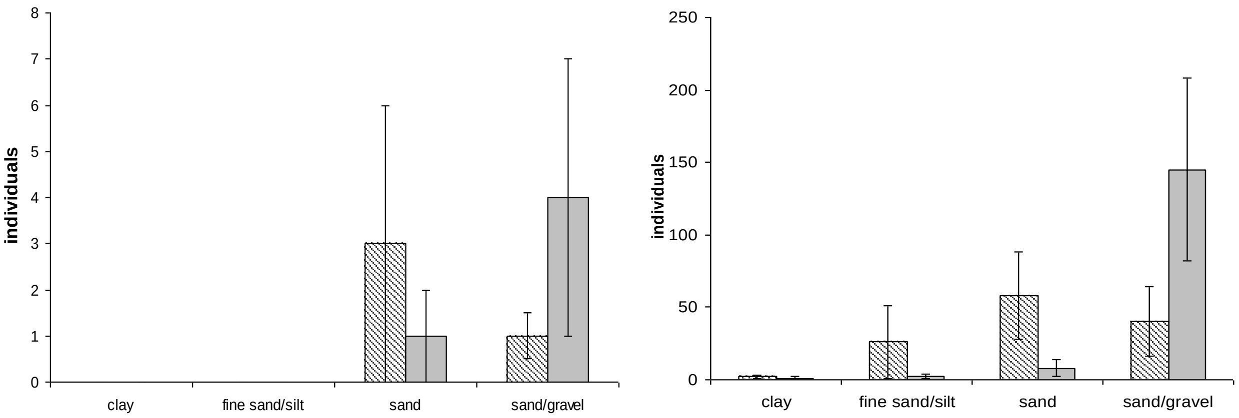

Figure 7: Abundance of large taxa (syncarid and amphipoda) and small taxa (copepoda) relative to sediment size in the Gwydir (shaded) and Namoi (striped) alluvial aquifers.

Further investigations compared the preferences of groundwater Harpacticoid and Cyclopoid (Copepods), Amphipods and Syncarids for sediment types (Figure 8). Results indicated that both the harpacticoids and cyclopoids were able to use fine clay sediments however showed a preference for larger substrates. Whereas larger amphipods and syncarids preferred larger gravels and sands, with many unable to burrow into in the clay sediments. This study demonstrates the general inability of some stygobiotic macroinvertebrates to use clay sediments and overall differences in sediment use among the small (copepods) and large (amphipods, syncarids) stygofauna. These findings assist the interpretation of field studies, particularly the apparent 'hit and miss' detection of stygofauna.

The differences in mobility of stygofauna will impact their ability to survive groundwater drawdown, thus making less mobile species more vulnerable to such impacts. This knowledge is critical to the conservation and protection of subterranean fauna, which are under pressure globally due to declining groundwater levels caused by over-exploitation in industries such as mining and irrigation.

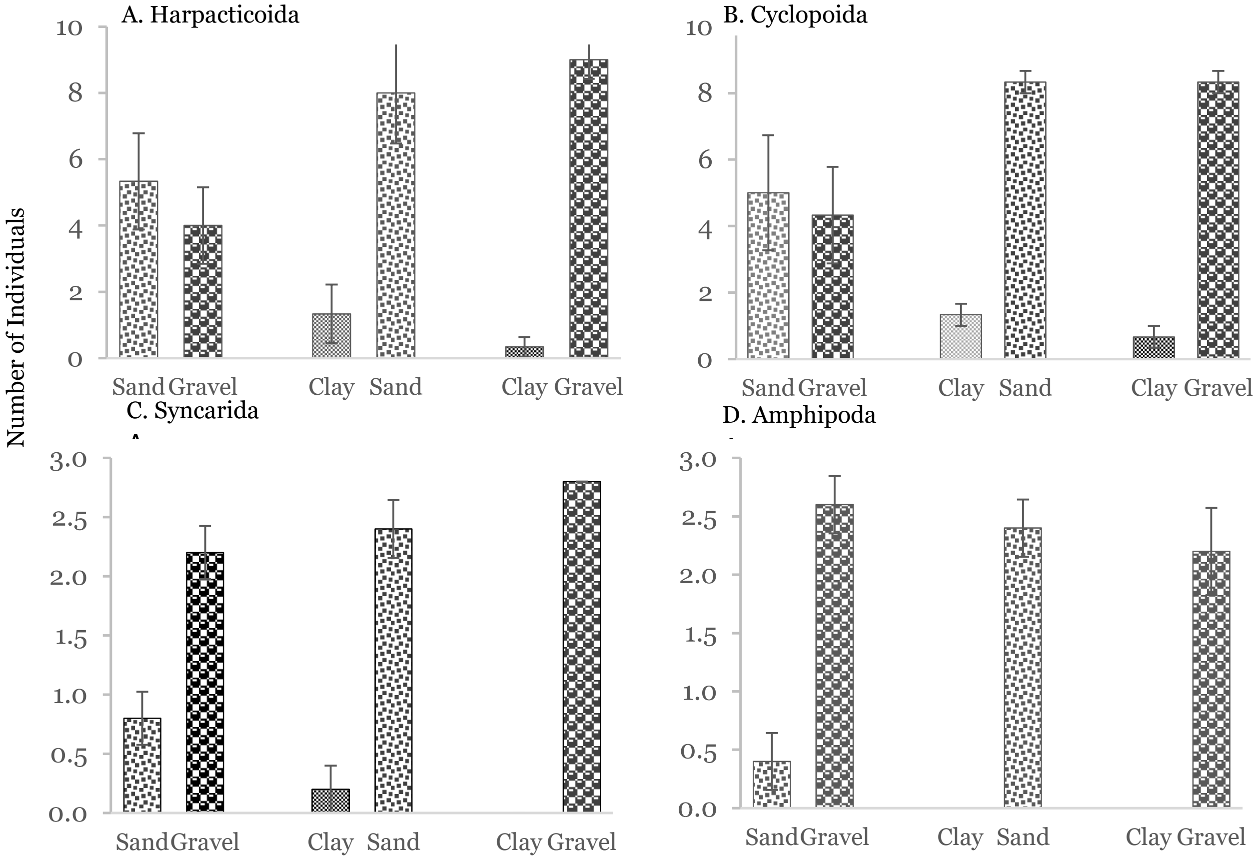

Figure 8: Mean (+ SE) number of animals living in sediment type when two choices of substrate given (Korbel & Hose under review).

4. Improving sampling methods

TRADITIONAL OR METAGENOMICS ANALYSIS?

Research compared the traditional 'collect and count' methods to metagenomics methods with results indicating differences in fauna detection levels between sampling methods. DNA analysis identified more meio-fauna such as Tardigrades, Platyhelminthes and protozoans, which can be cryptic and difficult to process and identify under a microscope. Whereas net/pump sampling located syncarids and amphipods which were not detected in metagenomic analysis. This indicates that both methods in combination, may be necessary to detect all stygobitic fauna (Table 1). The inability to detect stygofauna in some metagenomics samples highlights the need for further refinement of the techniques used for the DNA analysis.

Table 1: Sampling methods for detection of groundwater biota

Component	Traditional collection alone	eDNA alone	Traditional + eDNA
Stygofauna abundance	No	No	yes
Stygofauna richness	No	No	yes
Microbial abundance	No	Yes	No
Microbial richness	No	yes	no

PURGING OR NOT PURGING WELLS?

Through this project it was indicated that bores must be purged before sampling for microbial communities because the bore environment is artificial and not reflective of the conditions in the wider aquifer. This research has definitively answered an important question in groundwater ecology, and has implications for sampling methodologies and groundwater health assessments (Korbel et al 2017).

Stygofauna abundance and richness is also dependent on groundwater sampling methods. Bores provide an artificial environment enriched in nutrients, leading to the greater abundances of animals in bores compared to the surrounding aquifer. For example, amphipods were only found within bores and not within aquifer waters (collected after purging the well) (Korbel et al 2017). Biased assessments of abundance based on bore only sampling may lead to inaccurate conclusions about aquifer condition and productivity. Similarly, differences in the relative abundance of taxa in wells and aquifers means that proportion-based metrics used for health assessment, such as the proportion of crustaceans to oligochaetes (such as the wGHI), will also be affected by the sampling approach.

The need to purge wells for biological sampling is dependent on the objectives of the study and the biota being analysed. The two most common objectives of sampling biota in groundwater are

1) to assess biodiversity (often required by legislation prior to commencement of development activities) in which the desired outcome is a comprehensive list of the taxa present,

2) to investigate some aspect of groundwater ecology, which requires analysis of the composition, function, health or other attribute of the aquifer. E.g. the groundwater health index

Table 2: Best practice collection and analysis methods for stygofauna and groundwater microbes

Component	Purge bore	Non-purged	Purged + non purged	Traditional collection	eDNA	Traditional + eDNA
Stygofauna abundance	YES	NO	NO	NO	NO	YES
Stygofauna richness	YES	YES	YES	NO	NO	YES
Microbial abundance	YES	NO	NO	NO	YES	NO
Microbial richness	YES	NO	NO	NO	YES	NO

Based on the findings of this study, we suggest that groundwater biological sampling should consider separating the collection of water into pre- and post-purge samples for stygofauna analysis, with all microbial (prokaryotic and eukaryotic) samples collected after purging (Table 2). The volume of water required to purge the well will depend on well depth and diameter, and we suggest that at least three well volumes be removed (see Baskaran et al 2009), and that this water be processed separately as a 'well' sample. A second volume of water no less than 100L could then be collected as a separate 'aquifer' sample. This method will results in two different data sets depending on what analysis is to be completed. Community analysis of stygofauna (e.g. abundance or proportions of species) should be measured using the 'aquifer' sample without the purged 'well' sample, thus eliminating the high relative abundances and compositional differences between well and aquifer samples. Whereas richness or species lists should be compiled using stygofauna by compiling both 'well' and 'aquifer' samples to provide the best estimate of stygofauna richness at the site.

5. Improving the Groundwater health index (GHI)

BACKGROUND

Previous research defined a healthy groundwater ecosystem in terms of its function, organisation (structure) and the ecosystem services provided. This definition underpinned the development of a monitoring and evaluation tool called the Groundwater Health Index (Korbel & Hose 2011). This method incorporated a number of biological indicators as well as human induced stressors to give an overall holistic indication of health and enabled the monitoring of groundwater health and human impacts over time.

Our research has shown clearly that biota respond to both human impacts and environmental variables, meaning that factors influencing the natural distribution of biota needed to be considered when undertaking monitoring and assessment of groundwater health. The major component of the CRDC funded research into groundwater health was to improve the original GHI. As a result, we have incorporated four crucial environmental variables into the GHI assessment regime, thus allowing for the discrimination between natural biotic variation and human induced changes to biotic communities. Additionally, the new weighted Groundwater Health Index (GHI) applies a weighted score to discriminate three levels of health by comparing various biotic and abiotic indices to predetermined reference conditions.

APPLICATION OF THE WEIGHTED GHI IN THE MURRAY DARLING BASIN

The wGHI framework (Korbel & Hose 2017) allows for two levels (or *Tiers*) of assessment based primarily on detail of investigation required and associated cost. Tier 1 is a generic assessment, with tier 2 being a catchment based assessment, with locally determined thresholds and taking into account natural variation of groundwater biota due to factors such as sediment type, oxygen levels, presence of trees and dissolve organic carbon concentrations. The framework was extensively trialled in four catchments of the MDB between 2014 and 2017, with the results of these studies presented in Table 3.

Tier 1 assessment proved to be an adequate tool for assessing groundwater ecosystem health allowing the discrimination of 'impacted' from 'non-impacted' sites. However, due to the use of generic indicators, it does not provide any mechanisms to distinguish between levels of health, or to incorporate factors that may influence biotic distribution and thus perceived 'health' of the site. Thus, it is recommended that sites that fail Tier 1 assessments should undergo the more comprehensive Tier 2 assessment.

Our results indicated the degree of impact of sites within four catchments of the MDB as measured by tier 2 assessment (Table 3). The catchments have been sampled with varying sample effort, with the Gwydir sampled on four occasions and the other catchments on two occasions. This has an impact on the confidence of the results with the most reliable results gathered from the Gwydir catchment. The general trend is that grazing areas contain sites which reflect either similar to reference condition or mildly impacted groundwater health. Irrigated cropping sites (including cotton) had impaired groundwater health. The Macquarie catchment showed the most significant impact due to irrigated cropping with 4/5 irrigated sites having poor ecosystem health. The other catchments containing around 60-70% of the sites in irrigated areas that displayed major deviations from reference conditions. Full results of the wGHI and scores for each catchment can be seen in Korbel et al (2017).

Our results demonstrate that not only does Tier 2 assessment allow managers to classify health of sites into one of three categories, it also indicates the areas which have impaired health and should be prioritised for further monitoring and assessment. The wGHI Tier 2 assessment, incorporating environmental variables and weighing system, provided a more comprehensive assessment of health than the GHI Tier 2 assessment.

The wGHI framework provides a tool that can be selected dependent on budget, is sensitive to relevant environmental issues, is easy to apply and readily understandable by the community, all of which are necessary components for environmental monitoring tools. It is possible to choose different subsets of indicators and methods for tier 2 assessment, depending on the situation or resources available, in this way the wGHI provides a useful tool to meet groundwater monitoring requirements. By incorporating these factors and remaining flexible to evolve as knowledge of groundwater ecosystem expands, the framework provides environmental managers a much needed, cost-effective tool with which to monitor groundwater health over time and space and is proven to have scientific rigour.

Table 3: Tier 2 assessment of groundwater health in 4 catchments using the WGHI
Health classification: Green = similar to reference health, Orange = slight deviation from reference health, Red= major deviation from reference health. * indicates no stygofauna present at site during sampling events. Sites were sampled varying numbers, Gwydir catchment n=2-4, Namoi n=1-2, Condamine catchment n=2, Macquarie catchment n=1-2

Gwydir catchment

Site	Landuse	wGHI score
G1	Irrigated crop	5
G2	Irrigated crop	4
G3	Irrigated crop	3
G4	Irrigated crop	5
G5	Irrigated crop	4
G6	Irrigated crop	5
G7	Irrigated crop	4
G8	Irrigated crop	5
G9	Irrigated crop	3
G10	Irrigated crop	2
G11	Irrigated crop	3
G12	Irrigated crop	5
G14	Mixed non-irr.	2
G16	Mixed non-irr	3
G21	Grazing	2
G22	Grazing	1
G24^	Irrigated crop^	5^
G25^	Grazing^	1^

Namoi catchment

Site	Landuse	wGHI score
N1	Irrigated crop	1
N2	Irrigated crop	4*
N3	Irrigated crop	4
N4	Irrigated crop	2
N5	Irrigated crop	3
N6	Irrigated crop	4
N7	Irrigated crop	5*
N8	Irrigated crop	4
N9	Grazing	2
N10	Grazing	2*
N11	Grazing	1
N12	Grazing	2*
N13	Grazing	1
N14	Grazing	4
N15	Grazing	3

Condamine catchment

Site	Landuse	wGHI score
C1	Grazing	2
C2	Mixed non-irr	2
C3	Mixed non-irr	5
C4	mixed crop	1
C5	Grazing	0
C6	Mixed non-irr	2
C7	Irr. crop	4
C8	Irr. crop	4
C9	Irr. crop	5
C10	Irr. crop	2*
C11	Irr. crop	3*
C12	Mixed non-irr	0*
C13	Irr. crop	3
C14	Grazing	0
C15	Grazing	0

Macquarie catchment

Site	Landuse	wGHI score
M1	Grazing	1
M2	Grazing	2
M3	Mixed non-irr	1*
M4	Irr. Crop	2
M5	Grazing	1
M6	Irr.crop	5*
M7	Grazing	2*
M8	Irr.Crop	5
M9	Irr.crop	5*
M10	Irr crop	5

FARMER GHI TRIALS

1. Development of sampling protocols

Due to the success of the wGHI framework, the CRDC commissioned a study which adapted the framework methods to enable farmers to complete groundwater health sampling using production bores on their farms, and send to scientific specialists for analysis. The ultimate goal of this was to adapt the methods to incorporate the water quality monitoring requirements of My BMP and be suitable for inclusion in the cotton myBMP guidelines. This would allow for sustainable management and monitoring of groundwater resources. This ambitious goal would see the cotton industry pioneer the monitoring and assessment of groundwater health.

Initially, a method was trialed by farmers using a specially designed kit that provided farmers with the tools needed to undertake an assessment of health. Farms were visited in mid-2016 to assess availability and practicality of using bores and establish trial properties. The existing GHI methods (Korbel & Hose 2017) were then modified taking into account costs of each test, and a suitable range of 'indicator' measurements were established. Kits and sampling protocols were established in conjunction with Geoff Hunter (CottonINfo) and Stacey Vogel (CRDC) and tested with farmers between October 2016 and Feb 2017 to coincide with the irrigation season.

Figure 9: Farmer trial near Wee Waa (photo: Stacey Vogel CottonInfo)

The first round of sampling identified several practical issues with the sampling methods not being appropriate for on-farm sampling. Additionally, the timing of sampling and rainfall proved to be an issue. These results were not unexpected, as with all new monitoring tools trials and subsequent modifications are required to provide a suitable end product. As a result of the identified issues, the project team developed new techniques to monitor microbial activity, one of the key requirements of the GHI.

In late August 2017, the project team revisited farms to trial the new methods and discuss sampling issues that had arisen in the initial trial. The GHI monitoring kit was updated with the new equipment, which included specially designed nets to allow sampling directly from production pump outlets.

2. Methods and results

A crucial part of the GHI is the tiered assessment process. Tier 1 benchmarks can be used to indicate whether sites are showing any ecosystem stress, whereas Tier 2 assessment can be used to give a rank of good, moderate or poor ecosystem health. The Tier 1 benchmarks used for the trials are presented in Table 4. These benchmarks were chosen from a variety of suitable health indicators previously established as generic to all groundwater ecosystems (Korbel & Hose 2011). The benchmarks have proven to be generic and have been used with success in a variety of different aquifers, including overseas.

Table 4: Tier 1 indicators (from Korbel & Hose 2011;2017)

Indicator Type	Indices	Tier 1 'pass' threshold
Functional	Dissolved Organic Carbon	< 4 mg/L
Organisational	Total abundance of crustaceans	>50%
	Total abundance of oligochaetes	<10%
	Stygoxenes	absent
Stressor	Pesticides*	Absent (detection at 2ppb)
	Nitrate-N	<2 mg/L

*atrazine and diuron test strips (available through Department of Agricultural and Environmental Chemistry, University of Sydney, Australia)

The sampling methodology adopted for the trials allowed for the more detailed Tier 2 assessments, with the overall Tier 2 health indicator classification worked out by averaging the score for each sampling events (Table 5). It should be noted that sampling events per farm varied due to unforeseen circumstances such as pump maintenance and rainfall events. All farms were sampled a minimum of 2 times and a maximum of 4 times.

Figure 10: On-farm sampling, November 2017

The Tier 2 benchmarks used were established in the Gwydir catchment, however past research has indicated that these benchmarks are also applicable for use in the Naomi catchment (Korbel & Hose 2017). Overall average health score of 0-0.5 indicated good health, 0.5-3 moderate health and an overall score of 3+ displayed poor groundwater health. As it was not possible to gather information on total organic matter or dissolved oxygen in these trials, the weighting systems that accounts for natural variation in stygofaunal population, was not implemented in this trial. This could be considered for implementation in future trials.

The Tier 1 testing indicated that all sites showed some level of deviation from reference-like conditions (Table 6), thus all sites were classified as impaired by this level of assessment. Tier 2 results indicated a range of groundwater health within the farms, from good health to poor health.

Table 5: Additional Tier 2 indicators (from Korbel & Hose 2017)

Indicator Type	Indices	Tier 1 'pass' threshold
Functional	Dissolved Organic Carbon	< 4 mg/L
	Microbial activity (when available)	Sig. Diff ($p<0.05$) to controls
Organisational	Stygofauna count (abundance)	12 ± 3
	% crustacean	>50%
	%oligochaetes	0-25%
	Stygoxenes	absent
	Stygofauna richness	1-8
Stressor	Pesticides	Absent (detection level 2ppb)
	Nitrate-N	<2 mg/L
	Turbidity	<2 (on scale provided)

*Environmental conditions (turbidity, presence of trees, sediment type) were recorded and analysed as part of tier 2 assessment (see Korbel & Hose 2017)

Table 6: **Results of Tier 1 and Tier 2 farm GHI trials**

Farm	GHI **Tier 1** classification	GHI Tier 2 average score	GHI **Tier 2** classification
1-	impaired	2	Moderate health
2-	impaired	1.6	Moderate health
3-	impaired	4	Poor health
4-	impaired	1.5	Moderate health
5-	impaired	0.5	Good health

Whilst many farms in the trails returned results of moderate health, this is to be expected as the groundwater in these sites is impacted by pumping and addition of nutrients. One farm returned a good health score, despite the pressure placed on this farm by irrigation and agricultural practices. Further sampling of the farms throughout the annual cropping cycle would better inform the application of the method.

Atrazine and nitrate levels were a concern on a number of properties, and these indicators contributed to the degraded health classification at these farms. Atrazine is a widely used herbicide used in a variety of crops in Australia. Atrazine is a mobile chemical which is known to contaminate aquatic ecosystems. Within Australia, groundwater samples often contain detectable levels, but at levels above 2ppb, (the detection limit of the sampling methods provided) this can indicate point-source pollution or historical improper usage/handling of pesticides. The concern with our results, is that the testing provided only indicates if levels are above 2ppb, it does not measure the exact quantity of atrazine, therefore it is unknown how high the levels actually are. Groundwater pesticide testing is expensive, and for the purpose of the GHI, the exceedance value of 2ppb is sufficient to indicate a potential stress to groundwater health.

Another surprising result was the diversity of groundwater biota both at the start of the irrigation season in October, but also at the end of irrigation in February. The highest abundance recorded was 146 individuals, with species richness varying between 0-5. Whilst these numbers do not appear high when compared to surface ecosystems, within groundwater the diversity and number of individuals was relatively high. Interestingly, there were three families of syncarids identified from the samples, with copepods and ostracods making up the remaining crustaceans. Other species found included worms, mites, protozoans and rotifers (Figure 11).

Figure 11: Stygofauna located in trial, copepod, syncarid, mite (top to bottom).

3. Implementation plan for MyBMP applications

The monitoring program presented here is a world-wide first trial of a method to measure groundwater health. It has incorporated the water quality monitoring requirements of MyBMP with monitoring groundwater biota to add a level of detail such that the overall 'health' of groundwater on individual farms can be assessed, and compared to predetermined health benchmarks. This monitoring will allow the tracking of groundwater health over time, providing holistic monitoring of groundwater and allowing the early detection of potential issues rather than the 'snap shot' view of the environment that water chemistry testing provides.

IMPLEMENTATION PLAN

The farmer trials indicated that the GHI could be incorporated into the myBMP framework, integrating water chemistry and biological monitoring of groundwater to give a holistic view of the overall groundwater health by implementing a long-term monitoring program.

From the trials it was evident that where baseline assessments have been conducted and the general biota of the aquifers is known, once a year sampling would be sufficient to monitor the long-term health of a site within catchments. At this stage this includes sites within alluvial aquifers of the Condamine, Gwydir and Namoi catchment. The Macquarie catchment can use the existing baselines, however caution must be used as this catchment has not been sampled as often as other catchments thus the threshold measurements may not be as accurate. In other catchments, it is advised that baseline sampling over a minimum of 4 events be conducted throughout the catchment by groundwater ecology experts to establish the GHI tier 2 assessment baseline data.

The smooth implementation of the GHI requires a number of key personnel to manage the sampling process. This includes a scientific officer (who will process samples eg Macquarie University) and a kit maintenance officer in each catchment. The key issues surrounding the implementation of the program will include:

1. ***Kit maintenance and management officer***
   It is suggested that the monitoring kits be located in central officers within each catchment. The kits need to be managed by these offices, with the kit maintenance officer given the tasks of
   i. contacting the scientific officer to restock kits when required
   ii. ensuring all farmers have been made aware of hazardous materials and correct handling procedures
   iii. calibrating equipment before each sample event
   iv. printing and allocating unique codes and labels to each farm/sample
   v. develop and manage an on-line or app booking system for the kits
   vi. develop and manage online booking system for courier
   vii. handle samples (including freezing) once farmer returns to the office

2. ***Chain of custody procedures***

It is essential that a correct procedure is established to maintain a chain of custody from the farm to the scientific officer. Suggestions for this include:

i. Provision of unique barcodes and labels for each run
ii. A spreadsheet in the form of email for the farmers to enter in data related to water quality, irrigation activity, nitrate application and date/time of sampling
iii. The samples will be stored at a relevant CottonInfo office and the kit maintenance officer will be responsible for creating the chain of custody form, booking the courier and contacting the scientific officer.
iv. Scientific officer will log samples and store appropriately until they are processed.

3. ***Farmer responsibilities***

Each farmer utilising the kit will be responsible for the kit and its contents once it is loaned to them. They also will be responsible for any chemicals given and need to ensure they have read the appropriate material data safety material on each chemical and understand the safe storage and handling of these chemicals.

It is recommended that the farmers

i. Sample at the beginning of the irrigation season
ii. If the bore is not in use, allow the bore to run for 30sec to clear any remaining iron/sediment before sampling
iii. Follow the sampling protocols and storage requirements of samples (Appendix B) and label all samples with unique code.
iv. Fill in the details required on the field sheet or on the online spreadsheet.
v. Email the field sheet to the scientific officer once sampling is complete
vi. Return the kit and samples to the central office

4. ***Scientific officer***

The scientific officer will have the responsibility of receiving samples via the courier, processing the samples for microbial activity and stygofauna and reporting on the results annually. The scientific officer needs to

i. Ensure labels are allocated to each farm with unique code
ii. Provide material for restocking kits
iii. Receive and store samples on delivery
iv. Process samples for microbial activity and identify stygofauna
v. Report on the health of site using the GHI framework
vi. Be available as a contact for both farmers and kit maintenance officer as required.

5. ***Indicative costs:***

The indicative costs of sample processing are provided based on a minimum of 20 samples for processing, and excluding transport costs. This cost also excludes the initial cost of kits and restocking kits with water testing equipment, the initial kits are costed at approximately $500. It is estimated that the costs for water quality and biotic analysis per sample would be as follows:

ITEM	cost
Sample jar and chemicals	$10
Dipstick (USyd)	$10
Microbe and stygofauna analysis	$30
Reporting	$30
Total (per sample)	$80

PROJECT OUTPUTS

Dissemination of knowledge to the community has occurred through presentations at Cotton Industry conferences, national and international groundwater conferences, workshops, scientific publications and informal lectures. The research team has also conducted several meetings with farmers on implementation of the GHI methodology as well as speaking on ABC Rural Radio on the participation of the cotton industry and famers in trialling groundwater health monitoring programs. There have also been a number of short interviews and presentation of findings in CottonInfo publications. The detailed list of outputs is found in Appendix C.

CONCLUSIONS

Groundwater is essential in sustaining many rural communities and industries, including cotton. By gaining knowledge on groundwater health and establishing a monitoring program, this project is encouraging the stewardship and sustainable management of the resource, thus aiding the entire rural community through the benefits of clean and well managed groundwater.

The main aims of this project were to benchmark groundwater health within the four specified cotton growing catchments of the MDB. This was completed, with stygofauna and microbial diversity and abundances provided in this report, providing the first comprehensive database of groundwater biota and documenting the current condition of groundwater ecosystem health for the region. By defining the values of natural ecosystems, the CRDC has furthered the global understanding of the biodiversity values of groundwater ecosystems.

In addition, the GHI was trialled at 5 sites, within 4 farms in the Namoi catchment. This tool was refined and developed for use as cost-effective method to record and monitor groundwater chemistry and biota as a yearly monitoring tool for measuring groundwater health.

By implementing a long-term monitoring program using the GHI, the cotton industry can

- evaluate long-term impacts and identify degrading ecosystem health over time
- detect any impacts due to competing industries
- measure improvements in health due to improved environmental performance

The project has improved scientific knowledge of groundwater ecosystems and their responses to environmental change. This research has highlighted the industry as a leader in managing biodiversity both practically through groundwater management, and through the establishment of a groundwater biodiversity database. The project has provided baseline data on groundwater health throughout the catchments to allow for the monitoring of future impacts on this resource and for the management of groundwater for the sustainable future of the industry.

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Appendix A

List of stygofauna (presence/absence) recorded at sites within all sampled catchments (2006-2016)

	Copepoda			Syncardia								Ostracoda	Amphipoda			Isopoda	Acari	Oligochaete	Nematoda	Tardigrades	Platyhelminthes	Rotifera	Mollusca	Brachiopoda	Gastroticha	Annelida		
	Cyclopoida	Harpacticoida	unknown	unidentified	anaspidaceae	family A	Notobathynellida	Psammaspidadae	Parabathynellida	Bathynellidae	chilibathynelid	unidentified	paramelitidae	melitidae	neoniphargidae	janiridae sp	unidentified	unidentified	unidentified	unidentified	unidentified	unidentified	Bivalvia sp	unidentified	unidentified	unidentified	Sample size (n)	DNA sampled
P indicated species present in pump sampling; X indicates sample taken * indicates species present in DNA analysis																												
Gwydir catchment																												
30159	P	P				P	P										P	P									2	
30391	P	P				P	P			P		P	P				P	P									4	
30398		P					P										P	P									1	
30436	P	P										P					P	P									4	
30444	P	P							P								P	P	*		*	P					2	X
30456	P	P					P			P		P	P				P	P									3	
30457	P	P							P	P							P		P			P					3	
30458	P	P				P			P	P		P	P				P	P	P								6	
30459		P							P			P					P	P	P								3	
30460	P	P					P			P							P	P									3	
30461																	P	P									7	
30462		P															P	P	P								3	
36017		P					P			P	P	*	P				P	P	P*		*		*			*	4	X
36035	P	P					P		P			P	P				P	P	P								5	
36048	P												P				P										3	
36049	P						P			P																	5	
36050	P	P					P		P	P			P				P	P	P		*	P					7	X
36051	P	*					P		P	P		P					P	P	P		*	P			*	*	6	X

36052	P	P					P			P	P	P	P				P	P	*		*				*	*	7	X
36110	P	P							P	P			P				P	P	P			P					6	
36112		P							P	P								P				P					5	
36114	P	P					P			P		P					P	P				P					3	
36146		P					P			P								P									2	
36159	P											*					*	P	P		*	*	*		*	*	1	X
36205																											1	
36206																											1	
NAMOI CATCHMENT																												
30132	P	P		*								*					P	P	*		*				*		1	X
30134		P								P		*	P	P			P	*	*						*		1	X
30232	P			*						P		*	P				*	*	P		*	P	*		*		1	X
30236	P	P	P									*					*	*	*			*			*		1	X
30446	P									P									P								1	
30447	P								P	P		P					P	P	P		*	*					3	
36094	P	P		*					P								P	P	P		P	*			*		2	X
36093	P																P					P					2	
36096	P	P							P								P	P				*					2	
bh7.2			P					P	P	P		P	P		P		P	P			P	P					2	
BH9	P								P			P			P		P				P						2	X
BH10.2	P								P	P		P					P		P								2	
BH11.2	P		P						P	P		P					P	P	P		P						2	
BH18.4	P		P						P								P		P								2	
BH19.1			P						P	P							P	P									2	
BH19.2									P	P							P	P	P			P					2	X
BH20.4																	P	P	P			P					2	
BH20.2																	P	P	P			P			*		2	x
BH21.2																											2	
BH18.2										P		P	P			P	P		P								2	
BH12.4				*								P					P		P			*	*		*	*	2	X

BH8												P	P		P		P	P	P		P						2	X
EC21									P							P		P	P		*				*		2	X
BV1.1																	P										2	
BV1.2																	P										2	
BV15																		P	P			P					2	
21482	P																P	P									2	
25338		P								P							P	P									2	
25339																	P										2	
25332																	P										1	
25333																	P										2	
25334	P									P							P	P									2	
25419	P	P								P		P					P	P				P					2	
30071																											1	
25040		P															P										2	
25341																											2	
25010	P	P										P					P					P					2	
30281																											2	
25330	P	P															P										2	
30170	P	P					P			P		P	P				P	P				P					2	
25329	P									P							P					p					2	
CONDAMINE CATCHMENT																												
42230831			*									*					*	*	*		*	*	*	*	*	*	2	X
42230957	*	P	*						P								*		*		*					*	2	X
42230963		*	*						P			*					*	P	P		*	*				*	2	X
42231065																	P										1	
42231179	P											P						P									2	
42231230			*									*					P	*	*		*	*				*	1	X
42231334	P	P															P	P	P		*	P			*	*	2	X
42231339				*								*					*		*		*	*					1	X

42231363		P			P				P								P		P			P					2	
42231398		P			P				P								P	P	*		*					*	2	X
42231404		P	*	*	P				P	P		*					P	P	*		*	*	*		*	*	2	X
42231413	P									P							P	P									2	
42231414			*									*							P	P	*	P				*	2	X
42231416	P		*									*					P		*		*	*				*	1	X
42231432				*													*	*	P		*	*				*	2	X
42231469												P															2	
42231492										P								P									2	
42231530	P	P		*					P	P		P					*	P	P		*	P			*	*	2	X
MACQUARIE CATCHMENT																												
36519		P							P	P		P					P	P	P		*	P					2	
36726	*	*										*					*	*	*		*	*			*		1	X
36729	*	*										*					*	*	*		*	*		*	*	*	1	X
36971	*											*					*	*	*		*			*	*	*	1	X
96009	P	P							P	P		*					P	P			*	P				*	2	X
96046	*											*						*	*		*		*		*	*	2	X
96047	P	P							P	P		*					P	P	*		*	*	*		*	*	2	X
96048	*	*							P			*						*	*		*	P	*			*	2	X
96077	*			*								*						*	*		*	*	*			*	1	X
801050		P		*					P	P		P					*	P	P		*	P	*			*	2	
801052	*	*										*					*	*	*		*	*	*		*	*	1	X
801054												*					P	P	*		*	*	*	*	*	*	1	X
801058																		P								*	1	
801059	*	*										*					*	*	*		*	*	*	*	*		2	X
96008																		P	P							*	1	
96037	*											*					*	*	*			*			*	*	1	X
WELLINGTON RIVER SITES																												
1																											1	

2																											1	
3		P		P													P										1	
4	P	P		P			P										P										1	
5									P	P																	1	
7									P			P					P										1	
8																											1	
9																											1	
10																											1	
11																											1	
12		P							P									P									1	
13	P	P					P															P					1	
14		P							P								P					P					1	
15																											1	
16	P																	P	P								1	
16		P								P																	1	
17							P			P		P															1	
18																											1	
19										P																	1	
20																											1	
22	P	P								P								P									1	
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Appendix B: Groundwater Health farm sampling protocols

Groundwater Health Field Sampling Trial

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Groundwater Quality and Biological sampling.

Groundwater is a valuable resource for the cotton industry with many farms relying on groundwater resources for the growth of crops. Understandably, there is concern about the protection of the quantity and quality of this resources. If water is over-extracted and exceeds natural recharge rates, then the availability of this resource in the future will diminish. Equally important is the quality of water.

Current research indicates that within groundwater, there is a diverse and complex ecosystems consisting of both small animals (stygofauna) and microorganisms. These two components of the ecosystems are interlinked and depend on each other for survival. The importance of these biota for water quality should not be underestimated. Micro-organisms within groundwater have the capacity to significantly alter groundwater chemistry which can, in turn, impact on the quality of the extracted water for irrigation. Recent studied within NSW and QLD catchments have indicated several microorganisms have the capacity to degrade nitrates and reduce methane, iron and sulfur within groundwater, thus have very important consequence for water quality.

The monitoring program presented here has incorporated the requirements of MyBMP, such that these requirement will be completed once sampling has occurred. Additionally, the monitoring of groundwater biota adds a level of detail such that it will indicate the overall 'health' of your groundwater. This monitoring will allow the tracking of health over time, providing holistic monitoring of groundwater allowing the early detection of potential issues. Aspect included in monitoring:

1. **pH** : pH level in irrigation waters that is less than 4 can contribute to soil acidity and greater than 9 can contribute to alkaline soils. pH that is greater than 8.5 or less than 6 can affect spray mixes, result in precipitation of salts and/or cause corrosion & fouling (CottonInfo 2015).
2. **Electrical Conductivity** (EC): measures the concentration of salt in groundwater. The effect water salinity has on plants depends on soil type; salt tends to stay longer in the root zone and harm plants in slower draining soils. Groundwater is prone to higher salinity an should be monitored
3. **Nitrate**: Studies have found that nitrate contamination of Australian groundwaters is widespread. Nitrogen is used in agriculture mainly in fertiliser applications, excessive application of nitrogen cause nitrates to migrated below the soil zones and infiltrate the aquifer waters. Background concentrations of nitrate are less than 2mg/L (Bolger 1999). Increased nitrates impacts directly on groundwater usage and groundwater ecosystems, as well as eutrophication in surface waters.
4. **Temperature**: When studying groundwater biota, temperature is an indication of the habitat available to the biota.
5. **Standing water level**: Water level is routinely monitored to give an indication of the changes in water level due to extraction over time.
6. **Stygofauna**: Current knowledge indicates there are specific species of biota that will indicate the health of groundwater ecosystems. For example the proportion of crustaceans to worms in indicative of ecosystems health.
7. **Microbes:** Various aspects of microbial ecology and function can be studies from groundwater. The monitoring method we use provides a cheap method to analyse the overall microbial activity within groundwater and may be used to indicate human impacts on groundwater.
8. **Atrazine:** There has been known atrazine contamination in groundwaters of the Gwydir and Namoi catchments
9. **Hardness:** general hardness and carbonate hardness are measured to give an overall indication of water quality.
10. **Turbidity and sediment type**: This can impact the types of stygofauna that are able to live in the groundwater.

Sampling Kit Contents

Each kits contains the following:

- Tape measure with water 'plopper' attached
- Bailer (large plastic tube to be attached to fishing line)
- Fishing rod and attachments
- Marker pens to label each container
- Water quality tests
    - Hack^{tm} nitrate strips
    - API 5 in 1 strips
    - Electrical conductivity, pH and temperature multi-meter
    - Turbidity jar and disc
- Stygofauna sampling
    - Stygofauna net (to be attached to fishing line)
    - Plastic tubes
    - Ethanol (for preservation of animals)
    - Buckets to collect water
- Microbial analysis
    - Plastic vial
    - foil
- Marker pen
- Folder with pencil and field sheets
- Zip lock bags
- Envelopes for postage of cotton strips

NOTES:

Please rinse all containers and nets at the start of your sampling to ensure that all contaminants are removed. This can be done with tap water.
Keep the kit out of direct sunlight. Some of the test strips need to be stored below 10°

If additional equipment is required or you need help with sampling please contact :
Kathryn.korbel@mq.edu.au or Grant.Hose@mq.edu.au

Sampling schedule A- FOR BORES WITH ACCESS TO STANDING WATER- ie can insert measuring tape into bore

Order	Process: follow this order	Description
1.	**Site observations**	Fill in the site observation details on the field sheet. • Note anything unusual about the site/smell/colour of water • Note down the current landuse and fertiliser application/irrigational regime
2.	**Water level (if possible)**	1. Lower the tape down the bore hole (at speed) until you hear the weight enter the water with a 'plop'. 2. Record the depth from GROUND LEVEL to the water. (ie subtract the height of the bore from the overall measurement)
3.	**Stygofauna sampling**	1. Attach the blue-lid bottle to the stygofauna net then the net to the fishing line. 2. Lower the net into the bore to the bottom (if possible) and agitate the sediment at the bottom by 'shaking' the net. 3. Wind the net up slowly, leave to settle for 2 minutes. 4. Carefully discard the top 20-30ml of water from the plastic vial and add ethanol to the container, if ethanol runs out you can use methylated spirits 5. **Label on outside of container with marker pen**, and insert paper label written on in pencil. 6. Repeat this 3 times at each bore hole. 7. **Please seal all stygofauna bottles using the plastic film provided and drop into the CRDC in Narrabri**.
4. (a-h)	**Water quality testing**	1.Empty as much of the water from the bore as possible using the bailer attached to the fishing line (10 bails of water should be sufficient unless the bore is <30m deep) 2. Use the bailer to collect 500ml of water and fill plastic container provided. 3. Procedures for each water analysis are provided in the next section (4a-4g), record data on field sheet
5.	**Microbial activity**	After collecting the water and stygofauna sample, collect 20ml of water and place in plastic vial with chemicals. **BE CAREFUL NOT TO SPILL CHEMICALS- THEY ARE HAZARDOUS**, refer to MSDS provided. 2. Label with permanent marker 3. Place in esky, wrapped in foil, 4. Freeze as soon as possible **Please label & drop to CottonInfo for processing**..

Notes for field sheets.

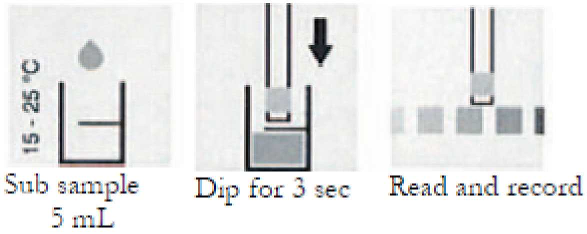
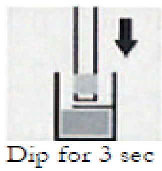
Please record data as accurately, the data along with any necessary information and/or explanations is vital to the success of the monitoring program. Please fill out the additional information on the second field sheet as an indication of the fertiliser application and irrigational practices currently in use. We would also like to know how long each sampling event took. This information will be used to further refine the sampling protocol.

Sampling schedule B- FOR MOST PRODUCTION BORES

Order	Process: follow this order	Description
1.	**Site observations**	Fill in the site observation details on the field sheet. • Note anything unusual about the site/smell/colour of water • Note down the current landuse and fertiliser application/irrigational regime
2.	**Water level**	1. If possible recorded the standing water level before pump starts
3.	**Stygofauna sampling**	1. Start pump on slowest speed (if already pumping water no need to wait) 2. Attach bottle to the sample net 3. Go to discharge point and place net under the flowing water 4. Let water pass through for approximately 3 minutes 5. Place the net in a bucket momentarily 6. Wash sides of the net down using the 'squeeze bottle' filled with bore water- this will detach any animals that are attached to the side of the net. 7. Make sure you wash sediment down as well- this can contain bugs. 8. The sample bottle needs to be half empty- gently pour water out of the net and then rinse with water into the bottle 9. Add ethanol **at least 50% volume** to the container (or if ethanol runs methylated spirits) 10. **Label on outside of container with marker pen**, and insert paper label written on in pencil. 11. Repeat this 2 times at each bore hole. 12. **Please seal all stygofauna bottles using the plastic film provided and drop into CRDC office**.
4. (a-h)	**Water quality testing**	1 After stygofauna sampling, collect water into 500mL jar provided. 2. Procedures for each water analysis are provided in the next section (4a-4g), record data on field sheet
5.	**Microbial activity**	1. After collecting the water and stygofauna sample, collect 20ml of water and place in plastic vial with chemicals. **BE CAREFUL NOT TO SPILL CHEMICALS- THEY ARE HAZARDOUS**, refer to MSDS provided. 2. Label with permanent marker 3. Place in esky, wrapped in foil, 4. Freeze as soon as possible **Please label & drop to CottonInfo for processing**.

Record notes on field sheets.

Water quality analysis.

Measurement		
4 (a-c). Temp (°C) pH EC (conductivity)	 temperature Acidity/alkalinity salinity (using Meter)	The bailer should be emptied into the plastic container provided and temperature taken ASAP. **DO NOT** leave the water in the container in the sun. Turn probe on and use mode/ent key to switch between measurements The pH will need to stabilise for a few minutes before the reading is taken. If pH meter stops working please take reading from API strip as instructed below
4d-e GH KH	 Total Hardness Carbonate hardness (using API pond 5 in 1 strips)	** **READ GH and KH immediately**** 
4f NO_3^-	 Nitrate (using Haack nitrate/nitrite strips)	 ** **Ensure you are reading the NITRATE colour code. Ranges 0-50ppm****
4g. Turbidity		Using disc provided, place in the bottom of container provided. Pour water into the container, leave for 10seconds Record the clarity of water on the scale (next page)
4h. Atrazine	Agrochemical dipstick (only if available in kit)	 Use pipete provided to pipette water into the small circle Wait until you see water moving up the line Leave for 3 minutes Record result – 2 lines= negative 1line=Positive (Note on the field sheet if error shows)

Turbidity protocol

Collect the sample in the 1L plastic beaker and place on the circle pattern (printed underside of test kit lid). After 10 seconds, observe the level of turbidity against the chart below. Record the score in the data sheet.

0	1	2	3	4	5
LESS					MORE
					

Procedure directly from:

Crossan, A. N (2007) On-Farm Water Quality Monitoring Program: Protocol and log book. The University of Sydney and Cotton Catchment Communities Cooperative Research Centre. Sydney.

Please record data as accurately on sheets provided (next page), the data along with any necessary information and/or explanations is vital to the success of the monitoring program. Please fill out the additional information on the second field sheet as an indication of the fertiliser application and irrigational practices currently in use. We would also like to know how long each sampling event took. This information will be used to further refine the sampling protocol.

FIELD SHEET FOR EACH SITE

Date/time: _______________ Location: ____________________.

Environmental attributes:
Notes:

Large trees (within 10m of bore)

1. Abundant 2. Scarce 3. None

Sediment 1. Clay 2. Sand 3. Other ....

Weather 1. Dry 2. Rain........ how much and duration

Number of stygofauna bottles collected: ....

Volume of syringed water for microbial tests:.....................................

Time frozen: ....

Measurements	
Water level:	EC:
Temp (°C):	pH:
Total Harness (GH):	Carbonate Harness (KH):
Nitrate (ppm):	Atrazine : (if C only indicates positive)
Turbidity (0-5):	Time required to sample?

Landuse practices:

1. **Application of fertilisers/pesticide.**

   Date of last application..........................

   Name of fertiliser/pesticide used..

   Application /ha...

1. **Irrigational activity**

   Date of last irrigation...

   Irrigation type (circle) groundwater surface water

   Application rate - estimate ML/hr (per ha) ....

2. Crop being grown ....

Other notes on landuse

Sampler name and signature:

Appendix C: Main outputs since beginning of MQ1501 project (2014-2018)

Output type	Description
Scientific papers	Menció, A, Korbel, K., & Hose G. (2014). River- aquifer interactions and their relationship to stygofauna assemblages. A case study of the Gwydir alluvial aquifer (NSW, Australia). Science of the Total Environment 479-480:292-305.
	Korbel K. & Hose, G (2015). Habitat, water quality, seasonality or site? Identifying environmental correlates of the distribution of groundwater biota. Freshwater Science 34:329-343.
	Korbel K., Chariton, A., Stephenson, S., Greenfield, P., Hose, G. (2017) Wells provide a distorted view of life in the aquifer: implications for sampling, monitoring and assessment of groundwater ecosystems, Scientific Reports DOI: 10.1038/srep40702
	Korbel K, Hose, G (2017) The Weighted Groundwater Health Index: Improving The Monitoring And Management Of Groundwater Resources. Ecological Indicators 75: 164-181
	Korbel & Hose (under review) Sediment Size Influences Habitat Selection And Use By Groundwater Macro And Meio Fauna, Aquatic Sciences
Masters completions	Little, J (2015) A comparative assessment of groundwater ecosystems under irrigated agricultural and pasture, MRes thesis, Department of Biological Sciences, Macquarie University.
	MacDonald, F (2017) Assessment of the salinity/stygofauna relationship and determinative elements of stygofauna habitat, Mres thesis, Department of Biological Sciences, Macquarie University.
Current student	DiCairano, Maria. PhD Thesis, commenced in January 2017
Conferences/ Presentations	Korbel (2015) Groundwater health and sustainability , CRDC conference Brisbane
	Korbel (2016) The importance of groundwater biota- understanding the structure and roles of groundwater communities, ESA, Freemantle, November 2016.
	Korbel & Hose (2016) Groundwater health and sustainability, RLM Forum, Brisbane 2016
	Hose & Korbel (2016) Assessing groundwater ecosystem health- where to next? Flinders University, Adelaide 2016
	Hose & Korbel (2016) What happens to groundwater ecosystems when you take out the groundwater? Ecological Society of Australia, Freemantle, Nov 2016
	Korbel & Hose (2017) Does agricultural practice impact on groundwater ecosystem functions, Australian Groundwater Conference, UNSW, July 2017.
	Hose & Korbel (2017) What happens to groundwater ecosystems when you take out the groundwater? Australian Groundwater Conference, UNSW, July 2017.
	Hose, Korbel & Chariton (2017) Metabarcoding for stygofauna assemblages. Society for Australian Systematic Biologists Conference, Adelaide, 2017
	Korbel (2017) Groundwater biota and water chemistry, Australian Cotton Research Conference, Canberra September 2017
	Hose & Korbel (2018) Assessment of groundwater ecosystem health: An Australian perspective, Meeting of the CIS Groundwater working group, Malta 2017.
Working groups	Korbel (2017) Representation as groundwater specialist at MAEGA workshop, Netherlands
	Korbel (2016) Namoi bioregional assessment and RIM workshops, CSIRO, Sydney
	Hose (2016-17) Namoi, Gloucester, Hunter, Sydney Bioregional Assessment and RIM workshops
Other forums	Korbel & Little (2015) New and rare species of stygofauna in our aquifers, CottonInfo Korbel (2017) Spotlight publication focusing on research issue.
	ABC rural- interview on groundwater health. November 2017

Bibliography

Barrett, K., 2009. Lower Gwydir Groundwater Source: Groundwater Management Area 004. Groundwater Status Report 2008. NSW Department of Water and Energy, Sydney

Baskaran, Feitz, S.A.J., Caritat, P., Plazinska, A., Brodie, R.S., Coram, J., Ransley, T.,2009. Groundwater Sampling and Analysis: A Field Guide. GeosciencesAustralia, Canberra.

Carr, J.R and B.F.J. Kelly 2010. Gwydir catchment groundwater hydrographs, UNSW, Sydney

Dafny, E., Silburn, D.M., 2014. The hydrogeology of the Condamine River Alluvial Aquifer, Australia: a critical assessment. *Hydrogeology Journal* 22, 705-727.

Giambastiani, B., Kelly, B 2010 Macquarie-Bogan Catchment groundwater hydrographs, Connected Water Initiative, NCGRT, UNSW, Sydney.

Kelly, B.F.J., Giambastiani, B.M.S., Larsen, J., Ralph, T., Timms, W., Baker, A., 2012. Paleoclimate impacts on aquifer architecture and hydraulic connectivity, 38th Symposium on Geology of the Sydney Basin, Hunter Valley

Korbel K. & Hose, G 2015. Habitat, water quality, seasonality or site? Identifying environmental correlates of the distribution of groundwater biota. *Freshwater Science* 34:329-343.

Korbel K., Chariton, A., Stephenson, S., Greenfield, P., Hose, G. 2017. Wells provide a distorted view of life in the aquifer: implications for sampling, monitoring and assessment of groundwater ecosystems, *Scientific Reports*

Korbel K, Hose, G. 2017. The Weighted Groundwater Health Index: Improving the Monitoring and Management Of Groundwater Resources. *Ecological Indicators*

Korbel, K. L., Lim, R. P. & Hose, G. C. 2013. An inter-catchment comparison of groundwater biota in the cotton-growing region of north-western New South Wales. *Crop and Pasture Science,* **64**, 1195-1208 .

Korbel, K. L., Hancock, P. J., Serov, P., Lim, R. P. & Hose, G. C. Groundwater ecosystems vary with land use across a mixed agricultural landscape. *Journal Environmental Quality* **42**, 380-390 (2013).

Murray Darling Basin Authority (MDBA), 2015. The Basin Catchments: Condamine-Balonne catchment snapshot, Commonwealth of Australia, http://www.mdba.gov.au/discover-basin/catchments/condamine-balonne.

Murray Darling Basin Authority (MDBA), 2012. Groundwater Sustainable Diversion Limit Resource Unit Summary Report Cards, Commonwealth of Australia, Canberra.

Muschal, M., Cooper, B., 1998. Regional level monitoring of pesticides and their behaviour in rivers in: Schofield, N., Edge, V. (Eds.), Minimising the impact of pesticides on the riverine environment: key findings from research with the cotton industry. Land and Water Resources Research and Development Corporation, Canberra.

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