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Water treatment to control influent water biosecurity risk on Australian prawn farms; Effectiveness and impacts on production ponds

David Mann, Paul Palmer, Stephen Wesche, Tom Gallagher

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Researcher Contact Details

Name: David Mann
Address: Bribie Island Research Centre
144 North St Woorim QLD
Phone: 07 3471 0923
Email: david.mann@daf.qld.gov.au

FRDC Contact Details

Address: 25 Geils Court
Deakin ACT 2600
Phone: 02 6285 0400
Fax: 02 6285 0499
Email: frdc@frdc.com.au
Web: www.frdc.com.au

In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

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An essential component of this project could not have been completed without the contribution of Gold Coast Marine Aquaculture. Their willingness to provide full access to the farm during operation, as well as staff assistance and equipment, ensured that information valuable to the whole industry, could be derived from their ground-breaking endeavours to continue production in the face of high biosecurity threat.

The rotating drum filter used in experiments, analogous to much larger units used commercially, was supplied by Reparator Pty Ltd. The filter was ideal for project needs and performed flawlessly.

David Mayer's (Department of Agriculture and Fisheries) biometry expertise was essential in extracting clear meaning out of a challenging data set. Thanks also to Julian Uribe-Palomino from the CSIRO for his expert training and ongoing support in identification of zooplankton.

Abbreviations

RDF – rotating drum filter
WSSV – white spot syndrome virus
WSD – white spot disease
BIRC – Bribie Island Research Centre
APFA – Australian Prawn Farmers Association
GCMA – Gold Coast Marine Aquaculture prawn farm

Executive Summary

The Australian prawn aquaculture industry is committed to a strategy of enhanced farm biosecurity to reduce the potential for production losses caused by disease agents. Evidence from prawn farming regions around the world indicates that source seawater is a significant route for pathogen transmission into the farm. Further, the highest risk mechanism for disease transmission is considered to be the organisms suspended in the water that without treatment of the influent water will be directly transferred into the farm. All prawn farms in Australia use seawater sourced from adjacent waterways to fill production ponds and exchange water throughout the production season.

In consultation with local and international experts, the Australian prawn aquaculture industry has identified that influent seawater filtration and chemical disinfection, either alone or combined, are key measures for enhancing biosecurity on prawn grow-out farms. These water treatment approaches can remove potential disease vectors present in the farm source water. Given the large investment required to implement seawater treatment systems, and the critical role it can have for farm biosecurity, prawn farms need information regarding practical design parameters, effectiveness of different treatment intensities and relative risk reductions of different options. This project was initiated to address the general lack of technical information on the application of water treatment systems and their impact on pond dynamics under Australian prawn farming conditions. It assessed the performance and impacts of mechanical filtration of influent seawater in an experimental tank-based system, and at a large operational farm growing black tiger prawns (*Penaeus monodon)* in Southeast Queensland. The technical details provided by this project directly informs the design and operation of farm biosecurity facilities, as well as the refinement of biosecurity operating standards for industry.

In the wake of the White Spot Disease (WSD) outbreak in Southeast Queensland in 2016 prawn farms that recommenced production in the white spot control zone (Biosecurity Queensland 2017) implemented greatly increased levels of influent water treatment. Project staff worked closely with the largest prawn farm on the Logan River, Gold Coast Marine Aquaculture (GCMA), which is positioned close to the mouth of the Logan River and Moreton Bay. The study was conducted during the first two production seasons (2018/19 and 2019/20) following a mandatory shut down (i.e. fallowing) of prawn farms within this zone in 2017. This was the first time rotating drum filters (RDFs) had been used to treat influent water on an Australian prawn farm. Throughout the two production seasons, the project monitored the plankton exclusion performance of the filter system and the downstream impacts on plankton populations in the on-farm seawater storage and distribution network, and production ponds. Upgrades to the RDFs, particularly the replacement of 80 µm mesh with 50 µm mesh between the production seasons, provided a valuable opportunity to compare filtration intensity in a commercial prawn farming environment. The plankton exclusion performance of 20, 40, 80 and 150 µm filter mesh sizes, was also examined in tank-based experiments using a scaled-down RDF analogous to that operating at the farm, and mesocosms to determine the viability of plankton remaining in filtered seawater.

A ranking of disease transmission risk associated with the organism groups identified in the farm influent seawater was used to assess the biosecurity benefits of fine filtration. This relative risk ranking was based on the organism's potential capacity to host, or otherwise vector, diseases of relevance to prawns, particularly White Spot Syndrome Virus (WSSV) responsible for WSD. The potential disease vector organism's life-history and population dynamics within farms was also considered in formulating this ranking. Penaeids and other decapods occurring within the local environment were considered the highest disease transmission risk, followed by small planktonic and epibenthic crustaceans, such as amphipods and copepods, and then non-crustacean groups including rotifers and molluscs. Phytoplankton were included in the ranking based on several reports of association with viral pathogens under particular circumstances but rated the lowest risk. It

should be noted that in the absence of detailed local data on actual levels of disease transmission by influent organisms and no realistic method for assessing it directly, the ranking was based on a combination of vector possibility, probability and potential magnitude.

From the perspective of organism transmission risk, the implementation of mechanical filtration of influent seawater at the GCMA Logan River prawn farm can be considered to have been highly effective in reducing their biosecurity risk. Juvenile and adult stages of the top three risk groups, penaeids, decapods and peracarid crustaceans, were effectively restricted from entry into the farm. The high density of decapod larvae and various other crustaceans in the filter backwash, many of which occur at very low abundance in seawater entering the farm, is a good indicator of this risk reduction effectiveness. Furthermore, the investment by GCMA in upgrading the original 80 µm mesh to 50 µm mesh, was similarly justified by a clearly enhanced level of exclusion of the highest risk organisms.

In the commercial-scale RDF system, mesh with a nominal rating of 50 µm permitted around 20% of crustacean nauplii that were present in the source seawater to pass through. In the small-scale experimental system, the 20 µm mesh excluded all crustaceans, including copepod eggs and nauplii, and the proportion of nauplii passing through 40, 80 and 150 µm mesh was approximately 3%, 10% and 50%, respectively. It was evident that the majority of nauplii passing through the 40 and 50 µm mesh were copepods, though this early life stage was not allocated to taxa. Therefore, this level of filtration allowed a founding population of zooplankton to enter. Copepods in particular are considered beneficial as an early live feed source for post-larvae, and for helping to stabilise pond blooms. Findings suggest that the lower abundance in filtered influent water retards growth of zooplankton populations within the prawn farming ponds. Filling ponds earlier at the start of the production cycle, to allow natural feed levels to amplify to desired levels before stocking post-larvae, may be a corrective management action.

The nominal mesh size rating was found to not be a reliable indication of the maximum body size of zooplankton that can pass through the mesh. In Season 2 at the farm, when a 50 µm filter mesh was in use, almost 30% of zooplankton in the RDF filtrate, predominantly copepods, had a minimum linear dimension in the 50-75 µm size class, and 23% were in the 75-125 µm size class. There were also individuals larger than this, though at very low abundance. The more controlled small-scale mesocosm experiments confirmed that organisms that are substantially larger than the mesh aperture can remain viable after passing through the filter mesh. It is considered that the flexible nature of the chitinous exoskeleton confers copepods and other zooplankton the capacity to reversibly compress and distort when forced through a small aperture.

Filter mesh construction is an important element affecting filter zooplankton exclusion performance. When the 80 µm stainless steel and polymer thread filters were both operating concurrently at the farm, a significantly greater abundance of zooplankton passed through the stainless-steel mesh. The different weave pattern of the two mesh types, twill weave for the stainless steel and plain weave for the polymer mesh, create very different aperture geometries which may explain the difference. The twill weave aperture is more complex and 3-dimensional when compared with the plain weave and has a maximum linear measurement well in excess of 80 µm. It is therefore recommended that farms installing filtration systems consider the characteristics of the mesh when designing a system to deliver a specific outcome.

This study showed that zooplankton of relevance to disease transmission risk are not completely excluded by filter mesh even as small as 20 µm. Rotifers and polychaetes were the most notable taxa that established populations in mesocosm tanks filled with 20 µm filtered seawater. RDFs with mesh finer than this is presently considered impractical for large scale farms due to the high restriction on flow rate this would confer. Therefore, to achieve more stringent zooplankton exclusion capacity, application of a chemical disinfectant may be the only viable option that presently exists. As there is no clear quantification of the disease transmission risk posed by various zooplankton groups, cost-

benefit assessment of this extra step would only be based on a potential or perceived risk under the local circumstances. A recent history of disease outbreaks in the region can be considered an indicator of elevated local risk that may warrant implementation of additional biosecurity measures.

There was no evidence that the retention of filtered seawater within the farm's distribution and storage network impacted copepod or other zooplankton populations, though high temporal variability of taxa abundance in farm inflow and a necessarily restricted sampling program may obscure fine-scale changes. In contrast, seawater retention on the farm led to an overall increase in phytoplankton abundance. In terms of the composition of phytoplankton blooms, the relative proportions of the main groups that were identified, including diatoms, dinoflagellates, and cyanobacteria, did not significantly change with location in the seawater network. The taxonomic composition of the bacterioplankton at different areas of the seawater distribution network had consistent differences but there was no indication of adverse changes. Taxonomic diversity, considered an indicator of healthy aquatic systems, was retained, and there was no evidence for the proliferation of bacterial groups (e.g. *Vibrio*) that are known to include pathogens.

Even with all influent water filtered by a 50 µm RDF, high biosecurity risk species still occurred within the farm waters. Throughout the two production seasons studied, juvenile and adult glass shrimp (*Acetes sibogae;* ~ 15 to 30 mm) were observed within the water distribution system, as well as on the feeding trays within the production ponds. In production ponds, barnacles (cirripede crustaceans) established an extensive population within several weeks of filling. Additionally, at the end of Season 2, a small number of juvenile and adult banana prawns, (*Penaeus merguiensis)*, school prawns (*Metapenaeus macleayi*) and palaemonid shrimp (of the decapod family Palaemonidae) were identified in the main water distribution channel. The penaeids and other shrimp observed could not have passed through the filter mesh intact and a likely scenario is that they entered the farm as eggs or early larvae and subsequently developed within the farm. Though it is also possible that occasional breaches of the filters when small tears in the mesh occurred, may have provided opportunity for small numbers of these species to invade the farm. Barnacles, being sessile once transforming to the juvenile stage, could only have entered the farm as eggs or larvae. Decapod crustaceans, and particularly penaeid prawns, are considered the highest risk for disease transmission to the black tiger prawns farmed at GCMA, however the risk associated with eggs and nauplii of these species is uncertain. Barnacles are also significant in prawn farming operations for their capacity to foul aerators and increase operating and maintenance costs.

The critical demands on a prawn farm influent water treatment system and the variable operating conditions mean that consistently achieving a high level of biosecurity control is challenging. Under typical pond management regimens employed on Australian prawn farms, a high rate of seawater inflow over extended periods is considered necessary to supply the total daily volume demanded to maintain favourable production pond water quality. Also, supply waters regularly have high organic and inorganic particle turbidity, high microorganism load, and are highly variable in physico-chemical qualities, including temperature and salinity. The Logan River prawn farm monitored during this project has demonstrated filtration can be a practical biosecurity measure that can deliver the seawater requirement of a large farm, even when source water quality has deteriorated due to high rainfall and severe weather. Additionally, of importance to pond management, filtering seawater to a level that significantly reduces biosecurity risk does not greatly impact the presence of natural planktonic organisms that are essential to establishing a stable, healthy pond microbiome in production ponds. On the other hand, the presence of zooplankton in filtered seawater means that residual biosecurity risk remains, even when a filter mesh size as low as 40 µm nominal aperture is used. To achieve complete disease vector removal and provide an even higher level of biosecurity control in areas with high disease risk, chemical treatment may be necessary. This approach is likely to disrupt the essential microbial community and may require remedial measures in the farm water management strategy.

Based on project derived information, the following points related to RDF design and operation are considered important to achieving a high level of influent water biosecurity when implementing a single pass filtration system:

- A drum filter mesh size of 40 to 50 µm is a practical and effective level of filtration intensity. This mesh size is a balance between excluding all zooplankton, particularly crustaceans, and delivering sufficient water flow rate to meet peak farm demand, and provides a very high level of disease vector exclusion. 20 µm mesh size can potentially exclude all crustacean larvae but is concomitant with more fragile mesh and reduced flow rate.
- Stainless steel mesh of twill weave construction is robust and may be a preferred option in situations where mechanical damage to mesh is anticipated, but the organism exclusion performance of plain weave mesh with an equivalent nominal mesh size rating was superior in farm comparisons.
- Mesh constructed from polymer thread is susceptible to tearing which can be responsible for release of unfiltered water into the farm. Filter mesh should be inspected regularly and repaired.
- The capacity to secondarily treat filtered influent water, either by additional, finer mechanical filtration or chemical disinfection, would be advantageous. This would reduce the probability of accidental release of untreated water into the farm and in circumstances where disease transmission is considered high, would provide further reduction of biosecurity risk.
- A high rate of filter backwash frequency, or even continuous backwashing, will reduce the extent of unfiltered organisms flowing into the farm when mesh breaches occur.
- The RDF seals that prevent water by-passing the drum can also be a point of failure and route for unfiltered water reaching farm ponds. Ideally the RDF design has minimal likelihood of ineffective sealing events, but seals will also need to be inspected regularly.
- When filtering with a mesh aperture size of 40 to 50 µm typical zooplankton communities will still establish in production ponds; consisting largely of copepods, as well as rotifers and other invertebrate group larval forms.
- For farmers that consider natural live foods are critical to early stock performance, the timing of pond filling and post-larvae stocking may need to be reassessed as time taken to attain maximal copepod abundance in ponds may be extended when 40 to 50 µm mesh filtration is used.

Introduction

The prawn aquaculture industry is committed to a strategy of enhanced farm biosecurity to reduce the potential for production losses caused by disease agents. This was elevated in priority by the 2016/17 WSD outbreak in southern Queensland but is also driven by considerations that current farming practices are vulnerable to other disease agents that could emerge in the future. It is recognised by aquaculture disease experts and the industry that a critical pathway for disease incursion is via the externally sourced water (van Wyk et al. 2014; World Bank 2014; Esparza-Leal et al. 2009; Walker and Mohan 2009), typically pumped from an adjacent estuarine creek or river. Therefore, treatment of farm influent water to eliminate or reduce disease agent load, particularly that associated with live vectors of disease, such as crustaceans or fish, is seen as a fundamental course of action to address farm exposure to this risk.

In consultation with local and international technical experts, the Australian prawn aquaculture industry has identified that filtration and some type of chemical disinfection are practical biosecurity approaches for prawn grow-out farms. However, the level of information available on design criteria and effectiveness of proposed water treatment systems is lacking, particularly in relation to Australian farming conditions. Information derived from overseas experiences of farming prawns under threat from multiple diseases has been instructive, however due to significant differences in farming systems, the local industry sees a need for additional information that has direct relevance to Australian farms and practices.

A key component of the new biosecurity measures is the treatment of influent water derived from local environmental waters. These water sources are used to initially fill production ponds and subsequently to exchange water during the grow-out cycle. Upgrading influent water treatment requires a large investment by farms, prompting concerns regarding the low state of knowledge on the effectiveness of potential treatment options and their impact on pond dynamics under Australian farm conditions. Consequently, improved information for systems design parameters and relative risk reductions for different options under consideration are a high priority.

A national white spot biosecurity workshop held in October 2017 discussed industry priority R&D needs. The workshop was attended by representatives of the prawn farming industry, state and federal biosecurity agencies and the Fisheries Research and Development Corporation (FRDC). The current project directly addresses one of the immediate research priorities that was identified: influent water treatment options that would reduce the biosecurity risk posed by farm influent water (Crane et al., 2017). This work also contributes information on two other aspects of water management important to farm operation; 1) intake water as a source of fouling organisms that colonise solid structures within the water distribution system and production ponds, and 2) harmful phytoplankton that occasionally bloom in production ponds and compromise prawn health.

The project was designed to assess the performance and impacts of mechanical filtration as a means by which prawn farmers could lower the risk of disease agent transfer into farms by selective removal of disease hosts and other vectors naturally present in farm source water. It sought to provide technical detail for the effectiveness of water filtration options to control the risk of disease incursions and investigate impacts these water treatment options may have on production pond dynamics and productivity.

The technical detail provided by the project directly contributes to the design and operation of farm biosecurity facilities, as well as refinement of industry biosecurity operating standards. While the impetus for this project was a need for individual farms to increase their biosecurity measures to prevent white spot disease, the upgrade of influent-water treatment was anticipated to have additional potential benefits for farms, including the prevention of fouling organisms, such as barnacles that enter the farm as microscopic larvae and then colonise aeration equipment and other

hard surfaces. Fouling incurs significant yearly costs across the industry (>$1,000 per hectare per year; (Mann, 2013), requiring resources for cleaning and reducing equipment life span. A potential benefit of a high level of influent water treatment likely only achievable by chemical disinfection, is exclusion of harmful algal species that regularly reduce farm productivity (Mann 2017). These additional benefits, would contribute to lowering the cost of production and improving productivity, offering subsidiary value to farms implementing new water treatment systems for disease management.

The project sought to work closely with farms on the Logan River, as they were in the white-spot disease (WSD) control zone (Biosecurity Queensland 2017) and were implementing treatment of influent water in the 2018/19 production season. This was the first farm stocking following the post-WSD outbreak fallow period. One farm, Gold Coast Marine Aquaculture (GCMA) provided regular farm access and assistance to project staff so that rigorous scientific assessment of the performance and impacts of their newly installed commercial scale water treatment system could be undertaken. This was considered essential to achieving outcomes directly applicable to the wider industry, as well as enabling the project to provide ongoing feedback to GCMA on filtration system effectiveness.

Initially the project planned to also investigate the impact of ozonation, and so a bacterial biome component was included to provide information about how this form of chemical disinfection impacts the bacterioplankton. Although the opportunity to investigate ozone treatment did not eventuate, the bacterial biome work was retained with the objective of investigating the changes that naturally occur as the intake water progresses through the farm channels and reservoirs. This provides useful baseline data for the study of chemical disinfectants and microbiome management in the future.

Objectives

1. Provide the Australian prawn farming industry with robust, locally generated information on practical farm influent-water treatment methods to optimise biosecurity investment.
2. Assess the impact farm influent water filtration has on the plankton blooms in prawn production ponds.
3. Evaluate the capacity for influent water filtration over a range of mesh apertures to reduce the presumptive risk of WSSV transmission onto prawn farms via plankton vectors.
4. Assist epidemiological investigations in the event of another WSD outbreak impacting the region.

Methods

The investigation of prawn farm intake-water filtration proceeded in two parts;

Part A. Experimental comparisons of different filtration mesh sizes performed under controlled conditions; and

Part B. Assessment of a filtration system operating on a commercial prawn farm throughout a production cycle.

Both project parts used analysis of zooplankton and phytoplankton in natural seawater before and after treatment as the primary method for assessing filtration impact. Plankton assemblages were analysed by microscopy to identify and quantify taxa and measure sizes of various life-stages present. In Part 2, bacterioplankton were also assessed using DNA sequencing to quantify changes that occurred within the farm water supply system.

Part A. Impact of different filtration mesh sizes on plankton populations

Location

Filtration experiments were conducted in a hatchery facility located on a non-operating prawn farm located adjacent to Bullock Creek, close to Donnybrook in southern Queensland, Australia. The hatchery had a translucent membrane roof covering and shade cloth sides (Figure 1) and housed bulk water storage tanks, filtration equipment and an experimental array of smaller tanks.

Source water

Experimental seawater was sourced directly from the tidal section of Bullock Creek, which opens directly onto Pumicestone Passage (Figure 2). Seawater from the farm's intake point was pumped to temporary storage tanks (3 x 10 kL and 1 x 30 kL) located within the hatchery facility. The pump's intake had a large-object exclusion screen of around 1 cm aperture size to exclude large objects. The seawater therefore contained natural plankton and sediment loads that were analogous to the influent water sourced by commercial prawn farms in estuarine environments. Seawater was pumped to the facility over a period of several hours either side of the high tide. To ensure consistency of water quality and plankton assemblages across all temporary storage tanks, the influent water was diverted to each of three tanks on a regular rotation (e.g. every 5 minutes during filling). Seawater in the storage tanks was used the day after filling after being aerated overnight.

Figure 1. Hatchery facility on a prawn farm in which experiments were conducted.

Figure 2. Location of experimental facility and source of test seawater. Bottom photos: Bullock Creek near intake (left), and intake point (right).

Experimental design

The impact of selected filter mesh sizes on plankton assemblage was assessed, both immediately after treatment and after longer term cultivation of filtered seawater in 2 kL mesocosm tanks. In the context of this work, a mesocosm is defined as a contained environment that sustains and promotes growth of naturally occurring organisms. An experimental system consisting of an array of 21 mesocosm tanks equipped with aeration (two airstones per tank) was established. (Figure 3).

The mesocosm approach was used to assess the longer-term viability of planktonic organisms surviving passage through the filter and their capacity to reproduce and form a persistent population. Mesocosms of post-treatment seawater were maintained for two weeks as static cultures with no further addition of water. Plankton assessments of these mesocosms were undertaken at one- and two-weeks post-treatment, giving sufficient time for egg and early life-stages to appear in the zooplankton and be identifiable to taxa.

Figure 3. Experimental mesocosm array used for filtration experiments.

Two replicated experiments were conducted using this system, commencing 5/04/19 and 24/04/19, with each set of mesocosms running for 2 weeks. Both experiments comprised five filter treatments, each with four replicate mesocosms distributed among 20 mesocosm tanks in a row/column design. The experimental design provides for inclusion of potential influences of tank position in the statistical analyses comparing treatment effects. The two replicate experiments were conducted three weeks apart to broaden the range of water quality conditions and plankton composition/abundance characteristics that typically prevail in natural estuarine systems. A third experiment was attempted but the high impact of heavy rainfall on source seawater plankton composition led to it being abandoned.

Water filtration

The experimental filtration unit used was a small, automated rotating drum filter (RDF), made by Adriyatik (model AMDF-571) and supplied by Reparator Pty Ltd (Figure 4). This filter is analogous in design and operation to those employed on commercial prawn farms, including automated backwash and water level alarms, but with lower water flow capacity. It was well suited to these experiments because its flow rate capacity was appropriate for the scale of the experiments and

because the stainless steel filter screens of different mesh sizes could be readily exchanged. The nominal mesh sizes used for the experimental treatments were 20, 40, 80 and 150 µm. These mesh sizes were selected for assessment on the basis that:

- 20 µm mesh should exclude all metazoan life forms; all life stages, from egg onwards, of multicellular animals like copepods and many other micro- and macro-scopic organisms. This filter size effectively fulfils the role of a negative control for the experiments, though may not be technically feasible to implement on some farms.
- 40 µm mesh is expected to allow only the smallest size fraction of metazoans to pass through and have reasonable potential for application to commercial-scale filter systems.
- 80 µm mesh is the same size as that initially applied in the 2018/19 season at the GCMA farm to exclude the highest disease transmission risk organisms.
- 150 µm mesh is likely the largest mesh size a farm may consider using as a final filtration step for biosecurity purposes. Larger mesh sizes will have a diminished benefit for the infrastructure investment.

An unfiltered, raw seawater treatment was included in the design to serve as a positive control for assessing treatment effects.

Seawater in the storage tanks was vigorously mixed before and during pumping to the RDF to ensure suspended material and organisms were consistent during initiation of the experiment. The storage tanks were equipped with multiple air diffusers, and a large bubble diffuser powered by a spa blower was frequently swept across the bottom of the tank. Additionally, a strong lateral current within the tank was created regularly using a paddle.

Seawater was pumped from each storage tank to the RDF with a submersible pump, and after passage through the filter was collected in a small sump and pumped to the treatment tanks with another small submersible pump (Figure 4). Flow rate through the experimental filter was approximately 6000 L h^{-1}, well within the theoretical filter capacity stated by the manufacturer; up to 50,000 L h^{-1} for large filter mesh sizes. In comparison to the large rotating drum filter installed at the commercial prawn farm studied in this project, the experimental filter was operated at a lower water flow rate relative to the potential maximum flow of each unit as stated by the manufacturer.

Figure 4. Rotating drum filter used for filtration experiments. The side view in the left image shows the raw water supply entering on the right of the RDF at the centre of the drum and filtrate flows into a sump below filter level. The right image is looking down on the filter drum with stainless steel mesh in place.

Mesocosm experiments

The viability of planktonic organisms surviving passage through the filter was assessed by retaining filtered water in mesocosm tanks for two weeks. Each tank within the experimental array received 1,700 L of seawater. Four replicates of each filtration level (mesh sizes of 20, 40, 80 and 150 µm) and an unfiltered control were included in each experiment. A randomised row/column design of treatment and replicate position within the array was implemented to account for the potential impact of position, potentially arising due to small variations in light and temperature. To reduce potential for cross-treatment contamination, the RDF and water distribution system was thoroughly rinsed between treatment operations, and the order for mesocosm tank filling proceeded from the smallest mesh size to the largest and finishing with raw seawater. For the raw seawater treatment, the source water was passed through the rotating drum filter without a filter mesh in place.

To maintain appropriate conditions for plankton growth, all mesocosm tanks were equipped with two 50 mm fine-bubble airstones with moderate airflow rates. These ensured continuous water circulation and high (> 90%) dissolved oxygen levels (Figure 3). To stimulate plankton proliferation, a single low dose of inorganic fertilisers was added on the day tanks were filled, which was calculated to provide 1 ppm of nitrogen (N) and 0.1 ppm of phosphorous (P) (Table 1). Previous experience from pilot experiments (data not presented) had demonstrated that organic fertilisers, such as fish extract concentrate, promoted a very high level of particulate material that made plankton collection, identification, and enumeration problematic. Additionally, pilot experiments previously showed that these low levels of inorganic N and P were sufficient to quickly stimulate phytoplankton and zooplankton growth. Water quality parameters, temperature, pH, salinity, dissolved oxygen, and turbidity were monitored and recorded for all tanks on alternate days and water temperature in selected tanks was also continuously recorded with submersed loggers.

Table 1. Fertiliser regimen applied to mesocosm tanks to provide a nitrogen to phosphorous (N:P) ratio of 10:1.

	Monoammonium phosphate	**Urea**	**Potassium Nitrate**	**Total N and P**
Amount (g)	0.64	2.68	2.74	
Equiv. to N (g)	0.078	1.25	0.38	1.708
Equiv. to P (g)	0.171	0.0	0.0	0.171
				Ratio N:P 10.0

Plankton assessment

Zooplankton and phytoplankton samples were taken prior to water filtration, immediately after filtration on day 0, and at day 7 and 14 for all tanks of the mesocosm experiments.

Zooplankton in the untreated source seawater, and that remaining in the filtrate immediately after passage through the RDF was sampled by concentrating 200 L of seawater in a 20 µm filter sock. Filter residue (i.e. the particulate material retained by the collection filter) was drained of seawater and preserved in 70% ethanol for later analysis. For mesocosm sampling, each tank was manually mixed with a paddle for homogeneity before taking samples. On days 7 and 14, a 50 L sample was concentrated in a 20 µm filter sock and similarly preserved in ethanol.

Phytoplankton samples were taken at the same time as the zooplankton samples, by filling a 600 mL bottle directly from the mixed mesocosm tank and preserving it with Lugol's iodine to a weak tea colour (approx. 0.25% solution). Phytoplankton samples were subsequently stored in black containers in the laboratory until processed for analysis.

Zooplankton sample analysis

Prior to commencing sample processing, staff undertaking zooplankton identification and counting received extensive training and ongoing assistance from Julian Uribe-Palomino from the CSIRO plankton ecology group.

In the laboratory, zooplankton concentrates were filtered from the ethanol preservative using a 20 µm screen and transferred to a set volume of clean water. After thorough mixing, a 5 mL sample of the plankton suspension was taken using a Hensen-Stempel pipette and transferred to a Bogorov counting chamber. This sample was examined using a Nikon SMZ-18 zoom dissecting microscope to identify and count the taxa present. Copepods were identified to Order level (Calanoid, Cyclopoid, Harpacticoid, Poicilostomatatoid) and most other organisms were identified to the Phylum level. The counts of organisms in these subsamples were converted to numbers per litre in mesocosms, based on volumes of original samples taken and dilutions necessary to assess sample concentrates.

For samples with very high levels of particulates and flocculent material, where identification of organisms in samples was difficult (even with higher sample dilution), it was necessary to use a thin, flexible probe to sweep through samples in the counting chamber. The addition of a filtration step to remove extraneous material in the preparation of microscope samples was not effective as various organisms were the same size as the flocculent material.

Zooplankton body size measurement was undertaken for samples prepared similarly to the identification process, except 5 mL samples were transferred to a gridded petri dish for microscopic examination. Within transects, the body length, width and total length of all specimens were measured using camera specific software (Amscope).

Phytoplankton sample analysis

For algal taxa identification and enumeration, sample settlement was used to concentrate suspended cells to a volume suitable for microscope analysis. Sample bottles containing 600 mL of Lugol's preserved seawater samples were allowed to settle for over 24 hours and then the supernatant slowly siphoned using a narrow bore tube secured centrally above the bottom of the bottle, to leave a volume of 37 mL. The remaining volume was mixed by swirling the bottle and poured from the bottle into a small jar and the bottle rinsed thoroughly with a further 13 mL of seawater which was then added to the sample to attain a final sample volume of 50 mL. Depending on the algal density of this final volume, it was either used directly for microscopic analysis or in the case of low cell densities, this volume reduction process was repeated to provide a final sample volume of 10 mL before analysis. Original sample cell densities were calculated using each sample's volumetric treatment.

For phytoplankton cell identification and counting, 1 mL of a well-mixed suspension was transferred to a Sedgewick-Rafter slide and inspected at 100x and 400x magnification. In many cases, cells were identified to genus level, but for statistical analyses the main taxonomical or morphological groupings of diatoms, dinoflagellates, cyanobacteria and phytoflagellates (Table 2) were used. Algal counts were made for columns evenly spread across the entire slide area, with the column frequency, and therefore total number of columns counted, determined by the density of cells. Due to the often high load of suspended particulate matter in samples, small-celled species, particularly small chlorophytes and single celled cyanobacteria species, were not counted.

Table 2. Groupings of phytoplankton used for analyses.

Group	Sub-group
Diatoms	Pennate solitary
	Centric solitary
	Chain (# chains and cells)
Dinoflagellates	Thecate
	Athecate
Cyanobacteria	Oscillatoria
	Pseudanabaena
	Other (Small solitary cell species not included)
Phytoflagellates	All (Species under 10 µm not included)

Plankton data statistical analysis

Statistical analyses were conducted on the calculated plankton taxa abundance data derived from the microscope counts and volumetric dilutions. Abundance data were highly skewed so were first ln(x+1) transformed before being analysed using a general linear model (McCullagh and Nelder 1989) under the Normal distribution with an identity link, using GenStat (2018). Residual plots validated this model. The independent experimental unit was the mesocosm, with the successive weekly samples modelled as regression trends (curved, if significant). The fixed effects were pond-type and time, with their interaction being retained if significant. Significance was determined at the $P<0.05$ level. Adjusted means were estimated for the mesocosm treatments, and significant differences between these were detected using protected least significant difference (LSD) testing. The bias-correction (Kendall et al. 1983) was applied when back-transforming the ln-means to raw densities. Standard errors were similarly back-transformed, and these asymmetrical ranges about each mean were averaged as approximate overall standard errors.

Part B. Monitoring intake water filtration on a commercial prawn farm

Prawn farm monitoring

Gold Coast Marine Aquaculture (GCMA), the largest prawn farm on the Logan River, provided project staff with assisted access to the farm including the water intake and filtration system, water storage and distribution network, and production ponds. Project staff sampled selected water bodies within the farm over the production season. Initially the project planned to undertake sampling activities only during the 2018/19 production season, starting at stocking in September 2018 and finishing with harvest in April 2019. However, with FRDC project variation approval, sampling was extended to include a second production season, from October 2019 to March 2020. This project extension made it possible to similarly study the performance effects of significant modifications that GCMA made in 2019 to their filtration system, for added project outcomes. The most critical change made to the filtration system between Season 1 and Season 2 was the replacement of 80 µm mesh with 50 µm mesh.

At the commencement of the project another farm had planned to participate in the same way as GCMA, however this did not eventuate due to operational considerations of that farm. This reduced the scope of the project monitoring and data collection, particularly in relation to ozone treatment of filtered water. This change in project activities allowed extra (weekly) sampling at GCMA for the first 10 weeks of the Season 1 (from 25/09/18 to 27/11/18) to improve the resolution of data over this time. From then to the end of February in Season 1, sampling was undertaken on a fortnightly basis. The change in project activities also allowed the extension of activities to include extension of farm system monitoring activities to a second season, though at a lower sampling level.

Prior to commencement of farm project sampling visits, a biosecurity standard operating procedure (SOP) was drafted and approved by the project participants, GCMA, BIRC and Biosecurity Queensland (Appendix C). This protocol was followed for the entire period of the project. GCMA provided use of a small laboratory and equipment storage facility as well as use of an all-terrain vehicle (ATV) to move around the farm.

Farm seawater treatment and supply system

In response to the threat of white spot syndrome virus (WSSV) presence in farm source water in the lower reaches of the Logan River, GCMA installed a filtration system in 2018 to restrict the transfer of potential disease vectors into the farm. For both production seasons monitored (2018/19 and 2019/20), all seawater entering the farm was passed through large-capacity rotating drum filters (RDF) operating in parallel (Figure 5). Seawater was pumped from the Logan River into a small receiving pond, before passing to a filter supply pond via underground pipe (locations 1 and 2 respectively in Figure 6). Seawater then was gravity fed through the RDFs before entering the extensive storage and distribution network within the farm. This included two reservoirs and several kilometres of supply channels. Seawater had variable residence time in the supply channels before entering production ponds, depending on the stage of the production cycle and location of ponds within the farm.

Initially there were two identical RDFs in operation, and later in the first season a third was commissioned. The filter mesh of the RDFs in Season 1 was constructed of woven stainless steel thread and was rated at a nominal aperture size of 80 µm. This original (Season 1) filter system operated at a flow capacity of up to 5 ML h^{-1}, and used timed, regular backwash cycles to remove accumulated residue lodged on the mesh. The mesh of all filters was reduced to 50 µm for Season 2, using a Hex Filter™ mesh of polymer construction. Backwash from the filters was directed to an adjacent collection pond where it was settled and disinfected prior to release.

Figure 5. Rotating drum filters in original configuration as used in Season 1. Note the small pond, in the background (sampling location #1) receiving raw water pumped from the Logan River, and filtered water flowing into the farm distribution channel in the foreground.

Farm plankton sampling and water quality measurement

On each sampling occasion, seawater and concentrated plankton samples were taken from the farm intake, prior to filtration, immediately after the filters, and at strategic points across the farm as detailed in Figure 6 and Table 3. The primary sample points were selected to provide sufficiently detailed coverage of significant zones within the farm. The sampling program was designed to quantify the impact of filtration on the plankton naturally present in the source water and identify changes as the water passed through the farm distribution and storage network to the production ponds. At most sampling points, multiple sample types were taken to provide for assessment of the phytoplankton, zooplankton and bacterioplankton communities.

Water quality parameters, temperature, pH and salinity were recorded at all sampling locations using hand held TPS meters. Turbidity was also estimated at each point using a turbidity column, which actually provides a value of water clarity that is negatively correlated with turbidity. The turbidity column used had a maximum readable value of 85 cm, equivalent to approximately 4.8 NTU (nephelometric turbidity units). Because of the inverse exponential relationship between turbidity column readings and actual turbidity, there is very little optical difference in readings above 80 cm. Also, turbidity column values over 80 cm have very low NTU values; 100 and 120 cm column readings are 3.8 and 2.9 NTU respectively.

Figure 6. Layout of the GCMA farm indicating the locations of sampling points (1 to 12) and water supply flow (Blue arrows). Note that some farm features have changed since this aerial photograph was taken. S1 and S2 denote production ponds sampled in Season 1 and Season 2 respectively. Farm 1 and Farm 2, as they are generally referred to, indicate old and new sections of the same farm. See Table 3 for description of sampling points.

Table 3. Summary of primary sampling points for GCMA. See Figure 6 for location of sample locations within the farm layout.

Location ref.	Name	Description	Sampling type
1	Intake pond	Raw water inflow direct from Logan River supply pumps	Zooplankton Phytoplankton
2	Pre-filter pond	Drum filters supply reservoir	Bacteria Water quality
3	Post-filter	Water within the filter compartment outside the filters	Zooplankton Phytoplankton
4	Filter backwash	Backwash water pipe discharge point	Filter residue WSSV test
5	New filter, Season 1	Additional filter commencing operation in January 2019	Zooplankton
6	Rachael Reservoir (R1) outlet	At pipe entry into distribution channel from the reservoir	Water quality Zooplankton Phytoplankton Bacteria
7	End of Conrod Straight channel	At transfer pumps that supply Farm 2	Glass shrimp
8	Big Hole Reservoir (R2) outlet	Concrete channel at outflow from Reservoir 2	Glass shrimp
9	Farm 2 channel end	End of southern water distribution channel adjacent to pond inlet	Water quality Zooplankton Phytoplankton Bacteria
10	Production pond A (front of farm)	Sampling at feed tray jetties in 3 locations	Water quality Zooplankton Phytoplankton
11	Production pond B (mid-farm)	Sampling at feed tray jetties in 3 locations. Sampled pond varied between Season 1 and Season 2	Water quality Zooplankton Phytoplankton
12	Production pond C (back of farm)	Sampling at feed tray jetties in 3 locations. Sampled pond varied between Season 1 and Season 2	Water quality Zooplankton Phytoplankton

Plankton sampling

Phytoplankton samples were collected in 600 mL PET bottles by either directly filling with the bottle held 5 to 10 cm below the water surface or, where water access was difficult, from a bucket with a rope attached that was filled by casting it into the water flow. Samples were preserved with Lugol's iodine to a weak tea colour (approx. 0.25% solution) and stored in the dark in a black container in the BIRC laboratory.

Zooplankton samples were taken at the points 1, 3 and 5 (Figure 6) by collecting 200 L of water by 20 L bucket and gently pouring it through a 40 µm mesh net partially submersed in an additional 20 L bucket. The residue retained in the net was transferred to a small 20 µm sieve. The drained material was washed from the sieve into a 70 mL sample jar with 80% ethanol. Elsewhere in the farm, zooplankton was collected using an 80 µm-plankton tow net (30 cm diameter, 90 cm long) attached to a 3.5 to 3.9 m pole. Feed tray jetties in production ponds and monk outlets on distribution channels provided a position for the operator to apply an oblique tow which directed the net through the water column from near the bottom to the surface as it passed through a circular arc of 180°. At sampling locations where the operator was positioned on the bank of a channel, the tow net was obliquely towed through a 130° arc. The volume of water passing through the tow net, was calculated from the length of the total tow. Additionally, where the sampled water was flowing, an estimate of flow direction and velocity at the sampling point was included in calculation of the filtered volume. Zooplankton collected in the tow net was washed down into the collection jar using clean water supplied from a manually pressurised hand-pump spray, dewatered in a small 20 µm sieve, and preserved in 70% ethanol in a 70 mL sample jar.

Backwash from the farm's drum filters, containing a concentrate of plankton and particulate material, was collected with a 20 L bucket at the backwash discharge point. Collection started immediately after the pulse of backwash flow was noted at the pipe discharge so as to capture a representative sample of material being retained by the screens. The 20 L sample was dewatered using a 40 µm mesh hand net and apportioned into duplicate samples that were preserved in 70% ethanol. One sample was used for plankton identification and one for WSSV testing.

Bacterioplankton sampling

A straightforward sampling protocol was developed at the BIRC laboratory to monitor the suspended bacterial biome in the farm water supply system. Preliminary tests using this protocol consistently delivered sufficient bacterial DNA for PCR amplification and product sequencing. The protocol was designed to simplify the in-field sample collection process that was carried out under sometimes challenging weather conditions. The steps of the protocol were as follows:

1. Collect samples from the body of water using disposable 200 mL plastic cups, attached to a pole for extended reach if necessary. Submerse the cup with the open end down and then once at approximately 10-20 cm depth rotate it to fill. Submerse the cup in areas of moderate surface water turbulence and avoid any material floating at the surface.
2. Pour sample through an 11 µm microscreen filter (50 mm diameter PVC construction) into another clean disposable plastic cup to remove large particulate material. After each use clean the microscreen filter with freshwater then thoroughly rinse with 70% ethanol.
3. Without delay, use a 50 mL syringe to draw 20 mL of the sample from around the middle of the cup's volume.
4. Add the 20 mL subsample of seawater to a 50 mL Falcon tube containing 30 mL of absolute ethanol.

5. At each sampling location take three replicate samples. Collect the samples in approximately the same area 1 to 3 minutes apart, effectively sampling a new ‘plug’ of seawater as it flows past the sampling point.
6. At each sampling location collect a single field negative control sample. This sample is reverse osmosis water, in an uncontaminated container, poured into a new sampling cup and transferred into the 50 mL Falcon tube in the same manner as for the farm seawater samples.
7. Immediately put the Falcon tube samples in a zip-lock bag and place on ice.
8. Transfer the samples to a -20 °C freezer within 20 hours for storage until processing. Processing to be carried out within 3 weeks of sample collection.

On each sampling day, bacteriological samples were collected from three locations:

- pre-filter at the exit of the first reservoir
- just prior to first point of entry into a production pond, and
- at the end of the water distribution network (sampling locations 2, 6 and 9, respectively, Figure 6).

Glass shrimp collection

Glass shrimp (also referred to as jelly shrimp; *Acetes sibogae*) juveniles and adults were observed in aggregations at different sampling locations, though were not collected using standardised volumetric methods designed for smaller, planktonic species. Glass shrimp were previously known to aggregate at the end of supply channels and corners of the storage reservoirs at GCMA. When collection opportunities were identified, twenty or more individuals were collected using a hand net and preserved in 70% ethanol for WSSV testing.

Plankton sample analysis

Phytoplankton and zooplankton samples collected from the farm water supply system were processed and analysed following the same protocols as those used for the filter mesh size experiments described in Part A. Filter residue samples however, were not quantitatively assessed due to the imprecise way samples could be obtained, so were assessed in a more qualitative manner. This involved identifying the taxonomic groups present and determining their relative, rather than absolute, abundance. The filter residue results formed a reliable record of the larger species that were present in the raw seawater at only very low abundance. This group included species that were not considered regular inhabitants of the plankton, such as benthic amphipods, which otherwise would have entered the farm in the intake water to colonise the storage and distribution system substrates.

Except for barnacle nauplii, all nauplii stage crustaceans were grouped together as it is difficult to assign this early life stage to specific taxa. Barnacle nauplii were able to be classified separately, because they have a characteristic morphology readily differentiated from that of copepods and other crustaceans.

WSSV testing

At approximately fortnightly intervals, one of the duplicate filter residue samples collected from the backwash water was submitted to the Queensland Biosecurity Laboratory (BSL) for WSSV testing. Initial laboratory analyses determined that the high load of vegetative and algal matter present in the sample inhibited the PCR process, so a sedimentation step was added to the laboratory protocol. This involved strongly agitating the by hand and allowing to settle before taking a sub-sample from the second observable subsurface layer that formed for DNA extraction and PCR. Microscopic observation of the layers that formed after mixing and settling confirmed that the uppermost layer contained a very high content of flocculent organic material and debris of apparent vegetal origin. The second layer contained a high proportion of crustacean species, copepod adults, amphipods and other micro-crustaceans, and had a much lower content of vegetal material compared with unprocessed samples.

Preserved filter residue and other zooplankton samples were retained for use in epidemiological investigations if a WSD outbreak occurred in the region during the project. In Season 2 a WSSV outbreak was identified on other prawn farms in the region on 12 April, the very end of the production season when harvesting at GCMA was well advanced and water intake had greatly reduced. This project's last sample collection was prior to the outbreak, on 5 March, when farm inflow was low and monitored ponds had been harvested. The filter residue samples throughout the production cycle, as well as extra plankton samples taken from the farm water distribution network leading up to March, were submitted for WSSV testing to establish the WSSV status of the farm supply water prior to the WSSV outbreak.

Bacterial biome profiling

The process of bacterial profiling of the seawater samples involved DNA extraction at the BIRC laboratory, submission of DNA samples to the Australian Genome Research Facility (AGRF) laboratory for 16S region amplification, and sequencing using the Illumina MiSeq platform. Raw sequence data received from this laboratory was processed with bioinformatics software (Quantitative Insights into Microbial Ecology (QIIME 2) version 2019.1) to derive a list of phylogenetic groups present in each sample. Results were used to describe the bacterioplankton communities and identify patterns in community profiles that established at three key water supply and distribution system locations within the farm, 1, 6 and 9 as shown in Figure 6.

A full description of methods used in this molecular bacterial biome work and results obtained at each step of the bacterial profiling process are provided in Appendix D.

Statistical analyses of plankton counts

Plankton count data from farm samples were highly skewed, as they were for counts of the experimental samples (Part A) so were similarly ln(x+1)-transformed then analysed using a general linear model (McCullagh and Nelder 1989) under the Normal distribution with an identity link, using GenStat (2018). The independent experimental unit was the sample location, with the successive weekly samples modelled as regression trends (curved, if significant). The fixed effects were location (Table 3) and time, with their interaction being retained if significant. Significance was determined at the $P<0.05$ level. Adjusted means were estimated for the location, and significant differences between these were obtained using protected LSD (least significant difference) testing. The bias-correction (Kendall et al. 1983) was applied when back-transforming the ln-means to counts. Standard errors were similarly back-transformed, and these asymmetrical ranges about each mean were averaged as approximate overall standard errors.

Results and Discussion

Part A. Impact of different levels of filtration on plankton

Filter mesh geometry

The filter meshes installed on the experimental RDF were all from the same manufacturer and constructed of stainless steel, but the weave design was quite different among the filter sizes trialled (Table 4). Microscopic examination revealed that the three weave designs present a different aperture geometry to the flow of water through the filter (Figure 7). Aperture shape and dimensions will likely influence the relative performance of the filter mesh, i.e. the maximum size of organisms passing through relative to the nominal mesh size. It may also affect the viability of organisms that pass through it since the 3-dimensional weave mesh may cause a high degree of contortion of the organisms resulting in injury.

Table 4. Characteristics of the filter mesh of different nominal aperture ratings used in experiments.

Nominal mesh rating (µm)	Mesh weave	Aperture description	Aperture dimensions*	
			Longest (µm)	Shortest (µm)
20	twill dutch	3-dimensional; tri-angular	80	40
40	dutch	3-dimensional; tri-angular	170	95
80	dutch	3-dimensional; tri-angular	200	125
150	plain	2-dimensional; square	170	170

** Measurements were made with a microscope and are only an approximate guide due to the 3-dimensional nature of the weave's apertures and the relatively large diameter of the weft and warp threads. For solid, spheroid particles the effective aperture will be smaller than these figures indicate.*

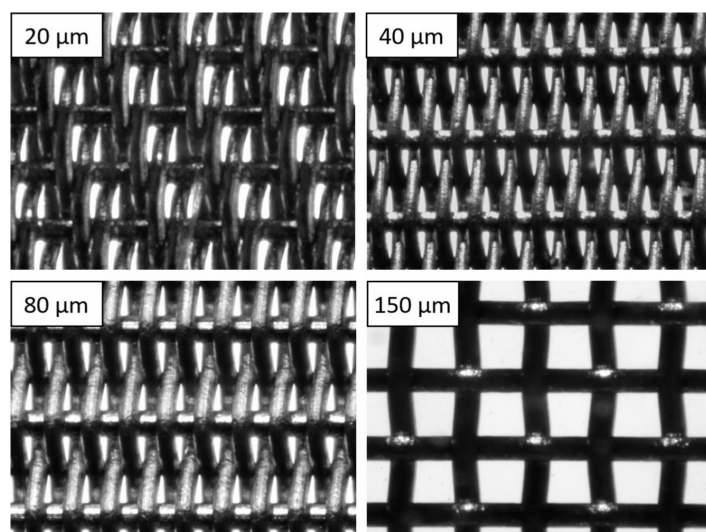

Figure 7. Microscope images of the filter mesh installed on the rotating drum filter used for the experiments. Labels are the manufacturer's nominal size rating. Weave patterns are 20µm - twill dutch, 40 and 80µm - dutch, 150µm - plain. Note that magnification varies among the images.

Filter mesh size experiments

Seawater conditions

Heavy rain leading up to and during the experimental period delayed the commencement of experiments and reduced the salinity of the source seawater from Bullock Creek. Experiments were commenced once the water had returned to a salinity above 20 ppt. Bullock Creek plankton monitoring over the period of low salinity (data not presented) indicated that zooplankton diversity was lower than during higher salinity periods and tended to be dominated by rotifers rather than copepods. The delayed start to experiments also meant that the average temperatures were relatively low (Table 5), which may have slowed the development of plankton communities in the mesocosms.

Zooplankton communities that developed in mesocosms in the two experiments were markedly different, reflecting the very different zooplankton profiles in the source water at their commencement, three weeks apart. Experiment 1 source water was dominated by copepods (3,100 kL^{-1}). Rotifers were present at a very low, undetected level, and only became apparent over time in the mesocosms. Conversely, at the start of Experiment 2, rotifers strongly dominated the zooplankton community in the source seawater, and there was only a comparatively low abundance of copepods (140,000 and 2,000 kL^{-1}, respectively). Coincident with the commencement of the second experiment, additional rain runoff reduced source seawater salinity to 27 ppt (Table 5), and increased turbidity levels, which may have been a trigger for the rise of the rotifer population in Bullock Creek. Raw seawater turbidity level in Experiment 2 was twice that in Experiment 1; 24.23 and 12.05 NTU respectively.

Table 5. Mean and range of water quality parameters during filtration Experiments #1 and #2. Dissolved oxygen was consistently above 90% saturation.

	Temperature (°C)		pH		Salinity (ppt)	
Experiment 1	22.1	(20.6-24.2)	8.1	(7.9-8.3)	30.5	(30-31)
Experiment 2	21.4	(17.9-23.4)	8.1	(8.0-8.3)	27.5	(27-28)

Effect of filtration on turbidity

Seawater turbidity was more intensively measured during Experiment 2 and the impact of filtration on turbidity was statistically assessed. RDF filtrate turbidity was significantly ($P<0.05$) reduced by the 20 µm mesh, but not affected by the other mesh sizes tested (Figure 8). After filling the mesocosm tanks, turbidity rapidly dropped and by day 7 was on average 1.3 NTU with no significant differences among treatments. It is apparent that the turbidity was caused by a high load of material with the majority of particles of a size able to pass through a 20 µm mesh filter. These particles however readily settled out, even in continuously aerated tanks.

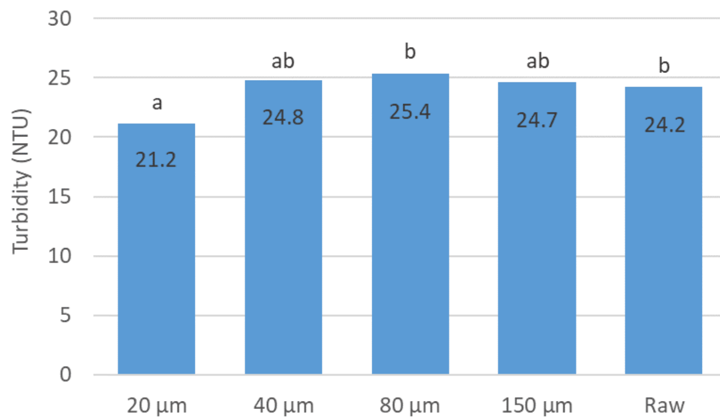

Figure 8. Turbidity of RDF filtrate directly after filtration through different mesh sizes in Experiment 2. Bars with the same letters are not significantly different (P>0.05). Values are the mean (n = 4).

Effect of filtration on zooplankton

The proliferation of zooplankton groups, particularly copepods and rotifers, in mesocosms after adding treated seawater was a clear indication that the conditions presented in mesocosms was well suited to the development of reproductively active populations within the time frame of the experiment. This supports the use of the post-treatment mesocosm approach as a sensitive method for investigating the longer-term impact of water treatment on zooplankton community development. The results presented in Table 6 are those derived from day 14 samples of Experiment 1 and day 7 samples for Experiment 2. In Experiment 1, the plankton bloom appeared to progress in a relatively steady manner for the entire 14-day test period, but in Experiment 2, the blooms of a high proportion of test tanks had apparently crashed by day 14. It is thought that the high initial rotifer population across all treatments except the smallest filter mesh in this experiment (Table 6) contributed to development of unstable bloom conditions before the end of the experiment. Rotifers typically have the capacity to rapidly bloom and then exhaust food (microalgae) when they rapidly decline. The higher start-up seed of Experiment 2 probably reached this end point in a shorter time frame, leading to the observed bloom crash by day 14. Mid experiment samples were taken to provide a contingency for that potential outcome.

Barnacle nauplii consistently occurred in the Bullock Creek source seawater at the farm, as evidenced by their low abundance in the concentrated filter backwash. As a result of their very low abundance in both experiments no useful data was generated for the impact of filtration on barnacles.

Only the 20 µm mesh dramatically reduced the passage of copepod life stages, as evidenced by their non-detection in seawater immediately after filtration (day 0 mesocosm results, Figures 9 and 10), and very low or non-detection in mesocosms 7 to 14 days later (Figures 9 and 10, Table 6). Additionally, in Experiment 1 there was a significant, positive correlation between filter mesh size and copepod and nauplii abundance in mesocosms, where mesh size was approximately doubled at each increment. The same relationship was less evident in Experiment 2, and the lower abundance of these two groups in the source water, compared with Experiment 1, may have been a factor contributing to these results. By day 7 in experiment 2, however, copepods were detectable in all but the 20 µm mesh treatment, and remained at very low levels (Figure 10).

Filtration at and above 40 µm did not appear to affect the relative abundance of the copepod orders present in the source waters. In Experiment 1, the relative proportions of the three main copepod orders was similar among the 40, 80 and 150 µm and raw treatments (Figure 9). Though copepods were not identified to genus or species level, the result of these larger mesh sizes indicated that broader level diversity was maintained filtered water, which is important for establishment of sustainable copepod populations in production ponds.

Table 6. Abundance (individuals kL^{-1}) of the most prevalent zooplankton groups after 14 days (Experiment 1) and 7 days (Experiment 2) in mesocosms for 5 different levels of filtration. Values are mean ± s.e. (n = 4). In Experiment 2, sample counts of some treatments were well below the threshold for valid statistical analysis. Within rows, filter treatments with the same superscript are not significantly different (P>0.05).

	20 µm	40 µm	80 µm	150 µm	Raw
Experiment 1. Day 14					
Total copepods	256 ^{a} ± 341	19,760 ^{b} ± 3308	29,673 ^{b} ± 3410	82,170 ^{c} ± 5970	120,212 ^{d} ± 7638
Nauplii	0 ^{a} ± 2	6926 ^{b} ± 2479	33,976 ^{c} ± 5825	74,557 ^{d} ± 8376	136,862 ^{e} ± 11500
Polychaete larvae	30 ^{a} ± 40	94 ^{ab} ± 71	14 ^{a} ± 39	335 ^{ab} ± 169	537 ^{b} ± 215
Gastropod veliger	0 ^{a} ± 0.7	0 ^{a} ± 0.6	1062 ^{b} ± 476	1618 ^{bc} ± 539	3270 ^{b} ± 769
Rotifers	119,615 ^{a} ± 58,443	14,379 ^{a} ± 17,547	21,457 ^{a} ± 22,402	13,079 ^{a} ± 16,231	53,651 ^{a} ± 36,995
Experiment 2. Day 7					
Total copepods	0 -	667 -	1333 -	1333 ^{a} ± 471	66,670 ^{b} ± 13,608
Nauplii	0 -	0 -	167 -	0 -	25,000 ± 15,960
Polychaete larvae	69 -	167 -	0 -	500 -	8330 ± 8330
Gastropod veliger	0 -	167 -	1000 -	1170 ^{a} ± 569	25,000 ^{b} ± 8330
Rotifers	0 ± 0	30,240 ^{a} ± 16,970	90,170 ^{a} ± 31,480	38,830 ^{a} ± 18,700	616,670 ^{b} ± 39,675

It is possible that the abundance of fragile organisms and early life stages are not accurately recorded in sample count data. The eggs and trochophore larvae of both molluscs and annelids may not preserve well in alcohol, either distorting or disintegrating, and not have been readily identifiable in the preserved samples (Steedman 1985). Similar to crustacean larvae, the later larval stages of molluscs and polychaetes, the veliger and nectochaete stages respectively, did appear to maintain structure in the preservative and were readily identifiable. It was therefore possible that the egg and trochophore stages of these organisms passed through small filter mesh sizes without detection in the day 0 samples but were subsequently identified in mesocosms after they had progressed to the next larval stage. However, there is only direct evidence that polychaete larvae were able to pass through the 20 µm mesh (Figure 9 and 10).

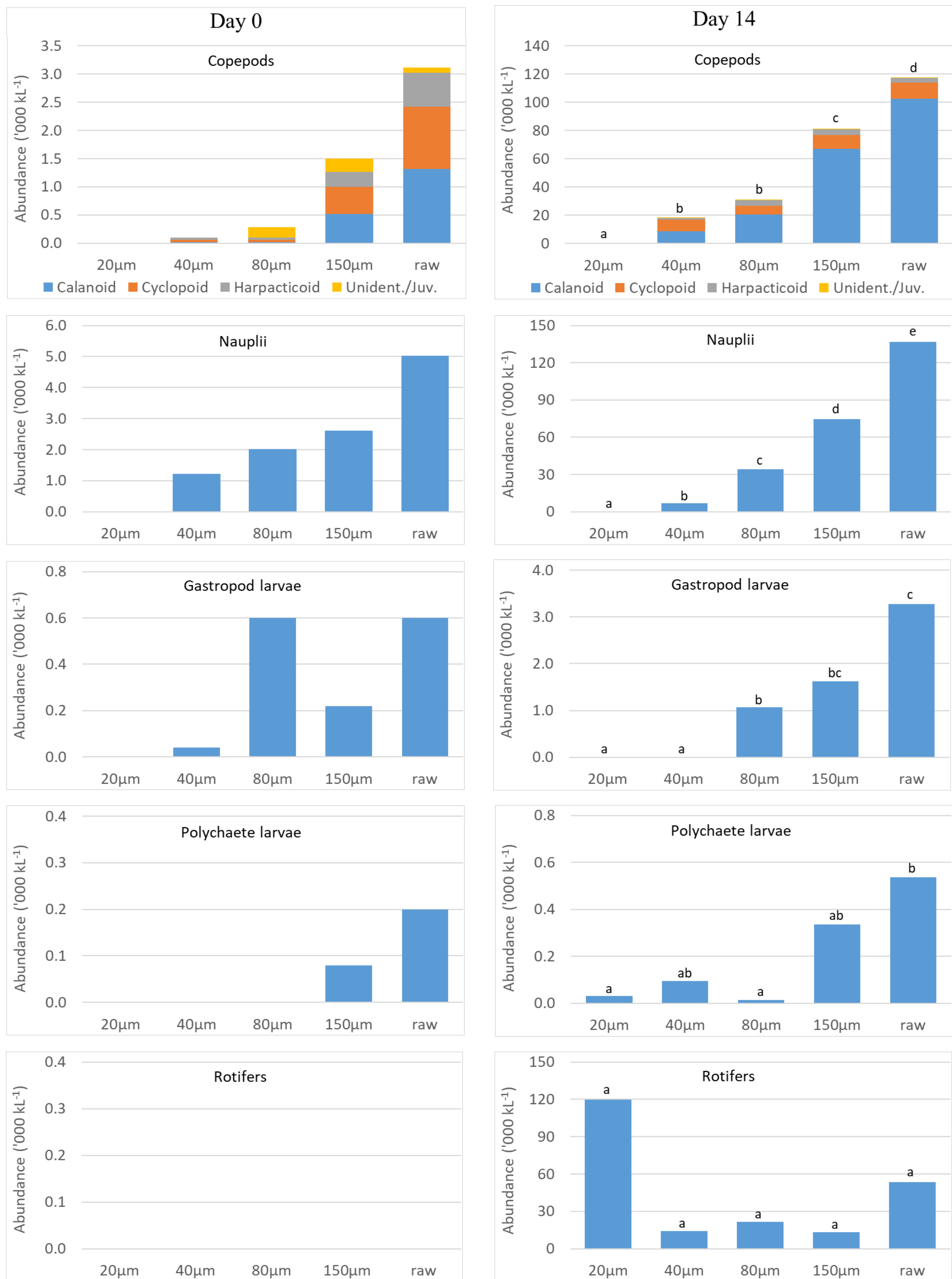

Figure 9. Experiment 1 abundance (individuals kL^{-1}) of selected zooplankton groups in seawater from each filtration treatment immediately upon exit of the filter (n = 1) and after 14 days of culture (n = 4). Day 0 charts (left column) are a pooled count of treated water and Day 14 charts (right column) are the average of four culture tanks. Within a chart, bars with the same letters are not significantly different (P>0.05).

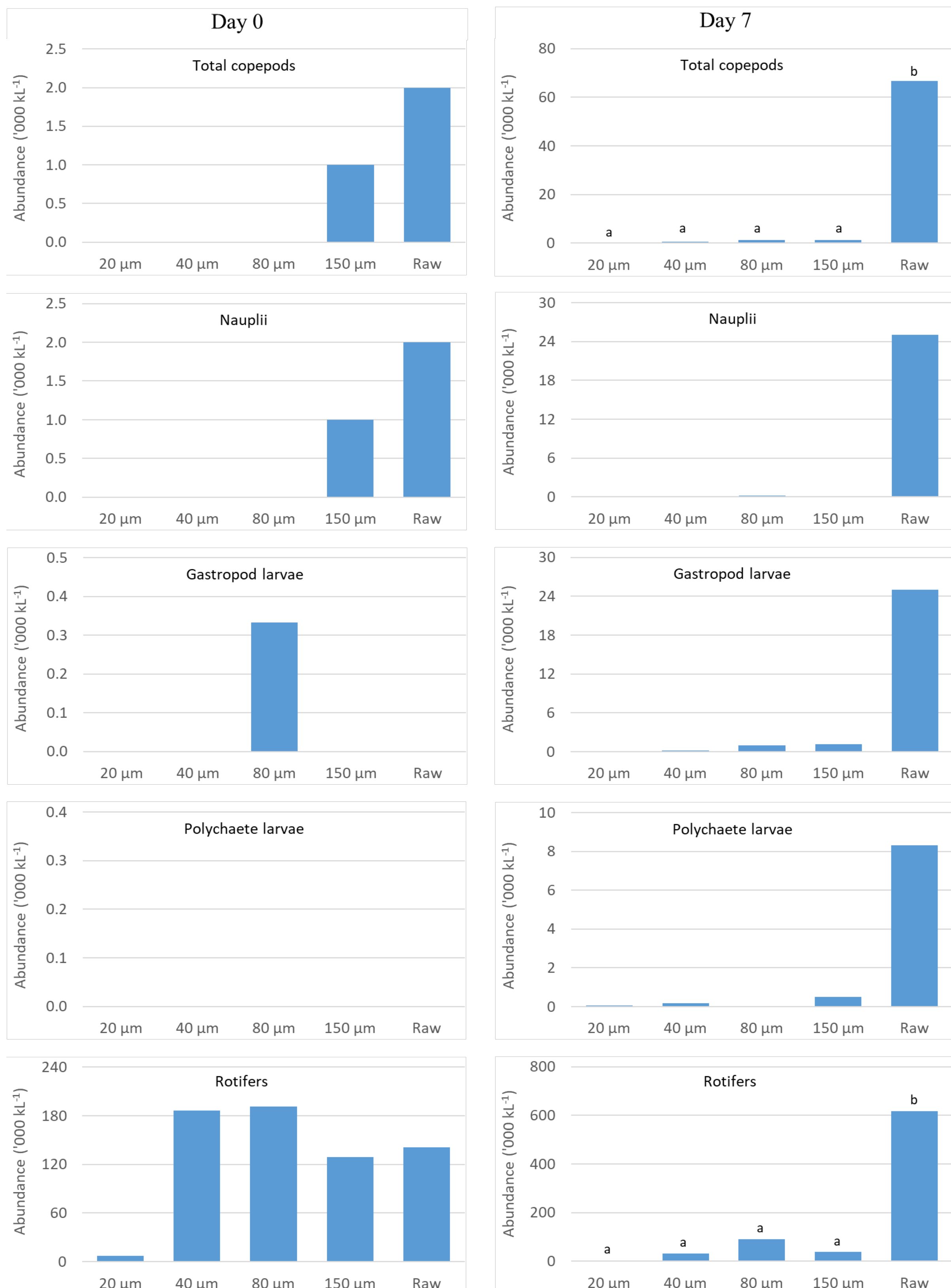

Figure 10. Experiment 2 abundance (individuals kL^{-1}) of selected zooplankton groups in seawater from each filtration treatment immediately upon exit of the filter (n = 1) and after 7 days of culture (N = 4). Day 0 charts (left column) are a pooled count of treated water and Day 7 charts (right column) are the average of four culture tanks. Within a chart, bars with the same letters are not significantly different (P>0.05). Due to very low sample counts not all zooplankton groups could be statistically analysed.

Both copepod adults and nauplii present post-filtration were found to have an average minimum body dimension considerably larger than 40 µm but were nevertheless present in the filtrate of the 40 µm mesh treatment (Figure 11). There are several potential explanations for this: 1) a small proportion of plankton may have been able to bypass the filter mesh; 2) the post-filter seawater system was contaminated (this was considered unlikely due to care taken to wash equipment between treatments); 3) the mesh was a nominal rating only and actual aperture size was larger; and 4) copepods at all life stages are somewhat flexible and may compress and contort as they are forced through an aperture. Explanations 1 and 2 above were preventable and in the context of the experiments, very unlikely. The RDF was new with no design characteristics that would appear to allow water to deviate from the intended flow path through the filter mesh. Furthermore, the filter mesh inserts were meticulously installed and checked for sealing against the housing and the system was back-flushed every 5 minutes to prevent excessive pressure build up within the drum. Unlike some large-scale rotating drum filters, the one used in the experiment did not rely on a flexible seal against a face of the rotating drum structure on the water supply side to prevent water leakage around the side of the drum. Additionally, to prevent contamination of the post filter water distribution system being a significant factor, the order of treatment application always progressed from smallest mesh size to the largest, and then the raw seawater treatment was done last.

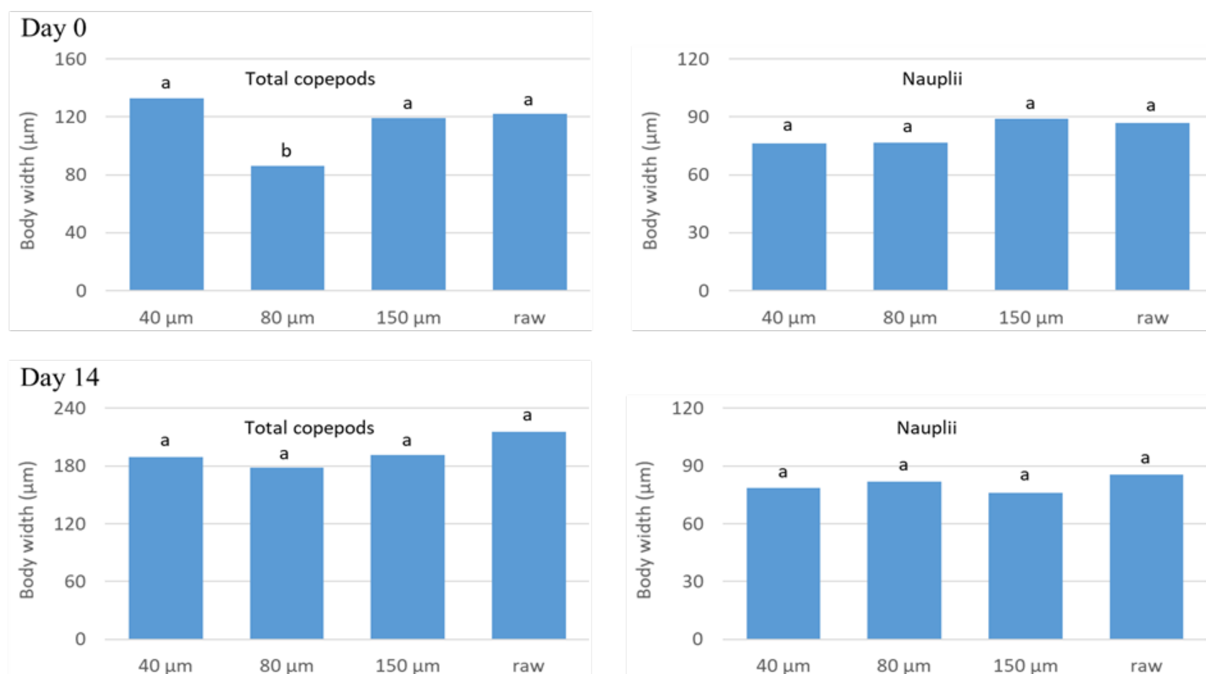

Figure 11. Mean body width of all copepod groups combined and nauplii for each filter treatment on day 0 (top row) and after 14 days in mesocosms (bottom row) in Experiment 1. There were no specimens detected in the 20 µm mesh treatment. Bars with the same letters are not significantly different (P>0.05).

As a consequence of the experimental precautions, the factor thought most likely to have contributed to the observed presence of copepods and nauplii in the 40 µm filtrate was mesh geometry. The 20, 40 and 80 µm meshes used in the RDF had a 3-dimensional structure with a triangular aperture geometry due to the dutch weave construction (Figure 7). The height and base dimensions of the triangular aperture well exceeded the nominal mesh size rating (Table 4). Notably, the weave and aperture configuration of the mesh used in these experiments appears to be common for RDF mesh made from stainless steel thread. This material and method of construction is most likely generally used since it is robust and durable under prolonged use in abrasive and somewhat corrosive environments.

From an anatomical perspective, the prosome (main body) of copepods and nauplii is not jointed to permit articulated bending, but their thin, chitinous exoskeletons are relatively flexible. It is therefore possible they could remain viable after compressive forces contort them, as would happen when a pressure gradient and water flow forces them through apertures that are smaller than their dimensions would otherwise permit. This coupled with the fact that plankton are appreciably concentrated within the drum filter, may mean that by chance a proportion of animals could be appropriately aligned with the seawater flow, in the right position, to squeeze through a smaller aperture and remain viable. To put this into perspective, only a quite small proportions of the raw seawater's copepod and nauplii abundance were present in the 40 µm filtrate (3.2% and 24% respectively). Even for the 80 µm mesh treatment, the proportion of copepods passing through was low (9.0%) and lower than could be expected given their average body width of 92 µm, and range of body widths from 60 to 143 µm. For the 40 µm and 80 µm mesh sizes, nauplii, or eggs dislodged from adults in the filtration process and then passing through the filter, may have been the main contributors to the copepod populations observed in mesocosms days later.

The many-fold increase in abundance of copepods by Day 14 (Figure 9 and Figure 10) indicates that upon exiting the filter, regardless of the mesh size, many were sufficiently viable to establish a thriving population. However, in Experiment 1, the significantly lower final abundance for the 40, 80 and 150 µm mesh treatments compared with the unfiltered treatment indicates that the initial population restriction by filtration had a strong influence on the population abundance attained after two weeks. The rate of copepod population growth after filling is important for prawn farms seeking to promote strong blooms of zooplankton prior to stocking post-larvae. Typically ponds are stocked with prawns 1 to 2 weeks after filling, during which time the zooplankton, predominantly copepods, can rise to over 100,000 kL^{-1} (Coman et al. 2003). This suggests that a longer pre-stocking period may be necessary when using filtered water to fill ponds. This advice is tempered by differences in zooplankton dynamics in tanks and large earthen ponds with varying designs (e.g. lined, clay or sand bottoms) and management alternatives (e.g. use of organic fertilisers which stimulate copepod blooms), and the farm's overall reliance on a surplus zooplankton resource to achieve high survival of stocked post-larvae. For example, such reliance may be reduced by other management strategies, such as initially applying more artificial starter feeds. Copepod abundance on the monitored commercial farm is discussed in section 4.9.

The 20 µm mesh did not prevent the passage of rotifers through the filter. Small numbers were present in RDF filtrate (mesocosm day 0) in both experiments, even though Experiment 1 they were not detected until 14 days later (Figure 9 and Figure 10). A different type of rotifer with different body dimensions occurred in each experiment. Experiment 1 had a bdelloid type rotifer with mean adult dimensions 163 ± 12 x 42 ± 6 µm. The rotifer in Experiment 2 was a monogononta type with mean dimensions for adults of 93 ± 8 x 63 ± 6 µm and eggs 43.8 ± 1.4 x 35.5 ± 0.3 µm. In Experiment 2, the abundance of rotifers in the source seawater was extremely high which likely increased the probability of a small number getting through alive. Rotifer abundance in the 20 µm filtered seawater was 5% of that in the raw source seawater. In this experiment it was notable that there were no rotifers detected on day 7 of mesocosm culture, either because those that passed through the mesh became non-viable, or because conditions which presented in these mesocosms were not conducive to their proliferation.

Part B. Monitoring intake water filtration on a commercial prawn farm

Zooplankton composition in the farm intake

A total of 30 zooplankton groupings (Table 7) were identified for samples of GCMA's seawater supply and distribution system. Copepods were by far the most consistently abundant taxonomic group, regardless of sampling location. All nauplii except barnacle nauplii were grouped together during identification and counting, however their size and morphology was consistent with the majority being copepod nauplii. Glass shrimp were non-quantitatively sampled opportunistically with a dip net, targeting aggregations when observed.

Table 7. Zooplankton taxonomic and other general groups identified across all samples and the sub-group names used throughout the report.

Crustacea		Other taxa	
Major group	Sub-group	Major group	Sub-group
Copepoda	Calanoid Cyclopoid Harpacticoid Poicilostome Unidentified juvenile	Rotifera	Rotifers
		Annelida	Polychaete larva/juveniles
		Mollusca	Mollusc veliger
Nauplii	Unspecified nauplius	Ctenophora	Comb jellies
Barnacle	Barnacle nauplius Barnacle cypris	Chordata	Appendicularian Ascidian larva Fish larva
Decapoda	Sergestid shrimp Crabs Other decapods	Platyhelminthe	Flatworms
		Nematoda	Nematodes
Percaridia	Amphipods Isopods	Chaetognatha	Chaetognaths
		Echinodermata	Echinoderm larva
Ostracoda	Ostracods	Tintinnida	Tintinnid ciliates
Unspecified Crustacea	Other crustaceans	Cnidaria	Jellyfish
		Unidentified egg	All eggs

A broad range of small faunal species were extracted from the Logan River by the large pumps (Table 7). Most of these were obligate inhabitants of the plankton community, which either spend their whole life history in the water column, or were the larval life-stage of a meroplanktonic species in which pre-settlement larvae are planktonic before transitioning to a benthos oriented phase as juveniles and adults, e.g. barnacles, polychaetes or crabs. Some of the crustacean species life-stages identified have an epibenthic existence (e.g. small crabs and amphipods), or associate with large particulate matter. Water turbulence in the river pump intake zone may entrain these animals. Their abundance was relatively low and the best record for their presence is the RDF backwash (Table 8 and Table 9). The backwash also contained large amounts of coarse material and many other organisms concentrated from thousands of litres of raw seawater (Table 8 and Table 9).

The order Decapoda was mostly represented by crab larval stages, unidentified decapod larval stages, and *Lucifer* sp., a small, <10 mm, type of decapod prawn (top left Figure 12). Isopods and amphipods were also a component of the >3 mm size animals (bottom row Figure 12). With the

exception of crab larvae, decapods occurred sporadically and in low abundance (Table 8 and Table 9). It is not clear whether the naupliar stages of decapods were present in greater numbers, as nauplii were not attributed to taxa. Glass shrimp, *A. sibogae*, juveniles and adults were occasionally observed in the intake seawater receiving pond in low numbers but were not detected by the quantified plankton sampling.

There were significant differences in abundance over time for all groups over the two seasons monitored, however there were no consistent patterns to their occurrence identified (Table 10). This finding is consistent with that of a study of zooplankton in Moreton Bay that found little seasonal abundance variability in copepod and other planktonic groups populations (Greenwood 1980). The same study also found that meroplankton, including larvae of decapods and polychaetes, contributed a greater proportion to the total zooplankton in summer but examples of this broad group of plankton were present throughout the year. It is therefore considered that there is potential for presence of high risk crustacean groups in farm source water at any time throughout the prawn production cycle and risk management therefore, needs to be constantly maintained. The state of knowledge for the potential WSD transmission risk at high taxa resolution, particularly below the level of Class, does not provide for distinguishing changes to risk associated with shifts in population dominance among related taxa. For example, different copepod or crab species may have different associated WSSV or other disease transmission risk, but this is unknown.

Weather events also have strong potential to impact the abundance of various taxa. For example, rain events that cause a rapid drop in salinities in the lower reaches of estuaries are expected to markedly reduce species with salinity tolerance ranges closer to normal seawater than freshwater. Similarly, species with a preference for low salinity would be expected to become more abundant. The GCMA farm experienced such rainfall events during monitoring, which led to a drop in salinity at times to near zero, but there is insufficient resolution in the data around rainfall times to define the impacts. As a general rule, farms including GCMA, tend to stop pumping water into the farm during these periods.

The list of zooplankton taxa identified in this project included representatives of the same major groups as were found in a recent extensive survey of plankton in Moreton Bay (Pausina et al. 2019). This is not surprising since the seawater intake for GCMA is located only a short distance from the southern end of Moreton Bay and tides would promote substantial mixing of bay and lower Logan River waters. Copepod abundance in particular showed considerable variation between sampling days, from <3000 individuals kL^{-1} to almost 50,000 individuals kL^{-1} (Table 8). Copepods and nauplii of unidentified taxa were consistently the most abundant groups in the farm intake seawater, and these were present on every sampling day over the course of both production seasons.

Zooplankton in estuaries are known to pulse in abundance over short (weekly) and longer (yearly) time frames (Pausina et al. 2019), and tidal influences can move populations on much shorter (hourly) time frames. Diurnal vertical migration of demersal species is also a well-known process that affects zooplankton abundance in marine and estuarine systems. Sampling the farm intake seawater was not standardised for tidal influences or time of day, and this may have contributed to some of the abundance variability observed. This was unavoidable in the farm studies because sampling the intake seawater could only be conducted while the pumps were operating and their operating time was not consistent on a daily basis.

Table 8. Season 1 frequency and abundance of identified plankton groups at different sampling locations. Frequency is percent occurrence across sample days and abundance (value in brackets) is the arithmetic mean number of individuals kL^{-1} across all sampling days. Juvenile and adult Sergestid shrimp were present in most farm waters but were not a component of the volumetric plankton samples and so are not listed here. Sample location 10-12 is the average of three production ponds.

Major Group	Sub-group	1 (intake)	3 (exit filter)	4 (backwash)	6 (exit Res1)	9 (end channel)	10-12 (ponds)
Copepods	Calanoid	100 (10,396)	100 (6850)	100 (455,000)	100 (4,784)	100 (7,457)	92 (9,241)
	Cyclopoid	100 (4767)	100 (2303)	88 (173,750)	100 (1739)	100 (2289)	88 (889)
	Harpacticoid	100 (2663)	100 (2375)	100 (180,750)	100 (1352)	100 (1131)	88 (2743)
	Poicilostome	46 (96)	93 (242)	75 (9250)	58 (100)	33 (38)	25 (198)
	Unident. juv. copepod	54 (546)	50 (353)	88 (44500)	67 (177)	67 (228)	63 (491)
	Total copepods	*100 (18,467)*	*100 (12121)*	*100 (863,250)*	*100 (8155)*	*100 (11146)*	*100 (22074)*
Nauplii	Unspecified nauplius	100 (18,608)	100 (14792)	100 (391,500)	100 (30140)	100 (17464)	100 (38133)
Barnacles	Barnacle nauplius	92 (2613)	93 (2725)	100 (138,750)	75 (1908)	50 (1165)	88 (19495)
	Barnacle cypris	23 (121)	21 (32)	13 (2500)	0 (0)	8 (183)	25 (207)
Other crustaceans	Crab larva	38 (104)	21 (28)	75 (4750)	8 (3)	0 (0)	0 (0)
	Amphipod	38 (42)	0 (0)	75 (10,500)	17 (4)	0 (0)	0 (0)
	Ostracod	31 (92)	43 (89)	63 (9500)	25 (13)	25 (80)	0 (0)
	Isopod	0 (0)	7 (7)	13 (500)	8 (4)	0 (0)	0 (0)
	Unident. crustacean	0 (0)	0 (0)	38 (5500)	0 (0)	0 (0)	0 (0)
Rotifers	Rotifers	15 (467)	14 (228)	0 (0)	33 (2075)	33 (1962)	50 (181112)
Annelids	Polychaete larva/juv.	92 (2108)	93 (2339)	100 (65,500)	92 (934)	100 (1071)	88 (3431)
Molluscs	Mollusc veliger	92 (908)	64 (378)	88 (37,500)	100 (5823)	100 (4455)	75 (521)
Ctenophores	Comb jellies	67 (2425)	43 (1435)	88 (49,500)	75 (2059)	50 (368)	63 (629)
Less common groups	Unident. egg	67 (5983)	57 (11371)	100 (74,500)	67 (4707)	67 (4703)	88 (4388)
	Appendicularian	92 (504)	71 (575)	50 (3750)	67 (2295)	42 (5011)	13 (312)
	Fish	8 (4)	0 (0)	13 (250)	0 (0)	0 (0)	0 (0)
	Flatworm	31 (217)	29 (114)	88 (12,000)	50 (608)	33 (435)	75 (3075)
	Nematode	0 (0)	7 (10)	0 (0)	8 (16)	0 (0)	38 (127)
	Chaetognath	42 (100)	29 (42)	50 (1750)	25 (37)	33 (71)	0 (0)
	Echinoderm larva	8 (17)	0 (0)	13 (2500)	0 (0)	0 (0)	0 (0)
	Tintinnid	15 (21)	7 (3)	38 (2000)	42 (1528)	33 (396)	38 (1490)
	Ascidian larva	8 (33)	7 (3)	13 (500)	42 (233)	25 (56)	13 (182)
	Jellyfish	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	13 (0)

Table 9. Season 2 frequency and abundance of identified plankton groups at different sampling locations. Frequency is percent occurrence over sample days and abundance (value in brackets) is the arithmetic mean number of individuals kL^{-1} across all sampling days. Juvenile and adult Sergestid shrimp (a decapod), were present in most farm waters but were not a component of the volumetric plankton samples and so are not listed here. Sample location 10-12 is the average of three production ponds.

Major Group	Sub-group	1 (intake)	3 (post-filter)	4 (backwash)	6 (post Res1)	9 (end channel)	10-12 (ponds)
Copepods	Calanoid	100 (10,787)	63 (640)	100 (715,750)	100 (12,752)	80 (4442)	100 (148,180)
	Cyclopoid	100 (4462)	13 (200)	88 (286,000)	100 (4572)	80 (2097)	80 (11,193)
	Harpacticoid	88 (2942)	25 (300)	100 (210,750)	90 (823)	70 (1062)	70 (893)
	Poicilostome	13 (200)	0 (0)	38 (4000)	0 (0)	10 (53)	20 (146)
	Unident. juv. copepod	0 (0)	0 (0)	25 (21,000)	10 (254)	0 (0)	10 (157)
	Total copepods	*100 (17,850)*	*75 (500)*	*100 (1,183,500)*	*100 (18,091)*	*90 (5981)*	*100 (15,7805)*
Nauplii	Unspecified nauplius	100 (11,637)	100 (4112)	100 (639,750)	100 (10,595)	100 (4907)	100 (228,743)
Barnacles	Barnacle nauplius	88 (700)	0 (0)	100 (88,000)	60 (939)	50 (121)	70 (31,224)
	Barnacle cypris	0 (0)	0 (0)	0 (0)	0 (0)	10 (53)	30 (1798)
Other crustaceans	Crab larva	13 (200)	0 (0)	25 (10,000)	10 (84)	0 (0)	0 (0)
	Amphipod	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Ostracod	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Isopod	25 (200)	0 (0)	25 (21,000)	0 (0)	0 (0)	0 (0)
	Unident. crustacean	0 (0)	0 (0)	13 (80,000)	10 (338)	20 (79)	10 (1462)
Rotifers	Rotifer	25 (4500)	13 (4000)	75 (63,333)	60 (1610)	80 (4893)	90 (70,869)
Annelids	Polychaete larva/juv.	88 (614)	13 (200)	88 (60,571)	90 (11,678)	90 (792)	90 (2614)
Molluscs	Mollusc veliger	100 (1062)	38 (200)	88 (238,571)	100 (6033)	100 (7453)	80 (3420)
Ctenophores	Comb jellies	50 (575)	0 (0)	88 (58,750)	60 (127)	40 (111)	0 (0)
Less common groups	Unident. egg	0 (0)	0 (0)	0 (0)	10 (127)	0 (0)	0 (0)
	Appendicularian	75 (633)	0 (0)	63 (15,600)	60 (5141)	30 (983)	20 (788)
	Fish	0 (0)	0 (0)	13 (2000)	10 (42)	20 (185)	10 (1349)
	Flatworm	0 (0)	13 (200)	0 (0)	10 (84)	0 (0)	0 (0)
	Nematode	13 (400)	0 (0)	0 (0)	20 (52)	10 (212)	10 (552)
	Chaetognath	25 (350)	0 (0)	13 (8000)	20 (296)	20 (371)	0 (0)
	Echinoderm larva	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Tintinnid	50 (1525)	13 (9400)	25 (60,000)	50 (1449)	30 (52,685)	40 (40,648)
	Ascidian larva	25 (150)	0 (0)	0 (0)	0 (0)	20 (185)	0 (0)
	Jellyfish	13 (200)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 10. Abundance (individuals kL^{-1}) of the most commonly encountered zooplankton groups in the raw farm intake seawater for different sampling days during production Seasons 1 and 2. Within each of the group columns abundance varied significantly (P<0.05) among sample dates. The column 'Grouped crustaceans' is a combination of similarly sized small crustaceans; crab larvae, amphipods, isopods, ostracods, and the larval stages, except nauplii, of unidentified crustacean taxa.

Season 1							
Date	Total copepods	Unspec. nauplii	Barnacle nauplii	Polychaete larvae	Mollusc veligers	Grouped crust'ns	Rotifers
25/09/18	13,650	23,550	1500	1150	1650	250	0
02/10/18	9200	18,400	1000	400	550	50	0
16/10/18	2850	1150	450	950	300	200	0
23/10/18	45,000	91,600	2600	800	800	200	5600
30/10/18	21,200	5800	0	2000	200	0	0
13/11/18	48,500	11,900	8200	2700	1500	1500	0
20/12/18	21,600	14,000	3000	1200	3200	0	0
15/01/19	29,200	17,000	3800	7400	400	200	0
12/02/19	8400	4600	1000	300	100	100	0
12/03/19	6600	7900	2000	2000	1200	100	0
26/03/19	6200	10,800	3200	0	0	0	0
09/04/19	9200	16,600	4600	6400	1000	200	0
Season 2							
Date	Total copepods	Unspec. nauplii	Barnacle nauplii	Polychaete larvae	Mollusc veligers	Grouped crust'ns	Rotifers
01/10/19	26,000	5200	800	600	600	0	0
15/10/19	15,800	8600	1000	800	600	0	0
29/10/19	8000	4200	0	600	1000	0	0
12/11/19	25,600	13,600	800	0	600	400	200
26/11/19	22,200	26,200	400	400	2400	0	0
07/01/20	19,600	16,500	500	900	2700	200	0
29/01/20	21,000	5200	1000	800	400	0	0
05/03/20	4600	13,600	400	200	200	0	8800

Figure 12. Examples of larger size fraction inhabitants of the farm intake water that included decapods, amphipods and other crustaceans. These organisms were included in a single grouping for statistical analyses referred to as 'grouped crustaceans'.

Only metazoans (multi-celled organisms) within the farm samples were assessed. Protozoans (single celled organisms) were present but generally not well preserved in the ethanol preservative used and were either destroyed or highly distorted. The exception was a *Favella*-like tintinnid which has a relatively large, semi-rigid lorica (outer cuticle), not dissimilar to rotifers in appearance. It was often sufficiently preserved to enable identification, so these were identified and counted in a small number of samples. This tintinnid was only identified at low abundance (<1000 kL^{-1}) in the unfiltered farm supply seawater, but on several occasions reached over 100,000 kL^{-1} in the farm supply channel and production ponds. With a relatively large size (mean 175 x 86 µm) and a filter feeding mode of life (Patterson and Burford 2001), these protozoans may have a biosecurity risk similar to that of small, filter feeding metazoans such as rotifers.

Zooplankton size in the farm intake seawater

The body size of metazoan zooplankton in the raw, unfiltered seawater ranged from 60 µm to greater than 10 mm in total length (Table 11). However, when considering appropriate mesh sizes to retain particular plankton groups, it is more relevant to consider their minimum body dimensions. This measurement extended over a narrower range across all zooplankton groups with large overlaps among diverse groups (Table 11). Additionally, the minimum body widths stated in Table 11 should not be considered to directly indicate the maximum filter mesh aperture sizes that would retain the organism, as discussed in section 4.7.

Table 11. Length and width of zooplankton groups identified in the raw farm intake water. Copepod sizes are for adults and late juvenile stages.

	n	Total length (µm) mean ± sd	(min - max)	Body width (µm) mean ± sd	(min - max)
Calanoid copepods	116	605 ± 273	(209 - 2092)	164 ± 69	(94 - 484)
Cyclopoid copepods	105	549 ± 109	(291 - 972)	138 ± 26	(86 - 211)
Harpacticoid copepods	100	545 ± 157	(261 - 1425)	117 ± 28	(61 - 377)
Poicilostome	10	477 ± 139	(336 - 777)	155 ± 57	(94 - 300)
Unspecified nauplius	134	166 ± 65	(62 - 341)	83 ± 27	(37 - 171)
Barnacle nauplius	100	241 ± 57	(93 - 502)	191 ± 63	(60 - 420)
Barnacle cypris	15	424 ± 146	(245 - 648)	240 ± 35	(175 - 309)
Crab larva	28	926 ± 336	(329 - 1491)	690 ± 453	(264 - 1461)
Amphipod	12	1760 ± 1084	(750 - 4414)	284 ± 86	(157 - 378)
Ostracod	8	342 ± 177	(180 - 618)	202 ± 108	(101 - 375)
Unident. crustacean	25	3537 ± 2937	(814 - 13,741)	449 ± 302	(205 - 1641)
Rotifers	13	156 ± 21	(103 - 184)	46 ± 11	(39 - 82)
Polychaete larva/juv.	34	504 ± 273	(173 - 1471)	134 ± 44	(54 - 257)
Mollusc veliger	81	205 ± 71	(105 - 533)	161 ± 59	(58 - 367)
Appendicularian	32	908 ± 347	(271 - 2075)	116 ± 50	(40 - 282)
Fish	5	2407 ± 752	(1738 - 3700)	430 ± 92	(295 - 549)
Nematode	6	1190 ± 438	(663 - 1900)	62 ± 14	(39 - 74)
Chaetognath	35	3998 ± 1244	(2144 - 7088)	175 ± 47	(90 - 311)

Biosecurity risk of zooplankton groups

A hierarchy of disease transmission risk associated with the various plankton groups that were identified at the GCMA farm was created to assess the potential impact of water treatment on water biosecurity (Table 12). The theoretical risk ranking for each organism group was based on two criteria:

1. Level of an organism's relatedness to Penaeid prawns - an indicator of the probability the organism could host *P. monodon*-relevant diseases; and
2. Life history attributes and population dynamics that may influence an organism's interaction with sources of pathogens, and its capacity to deliver a high contamination load into the farm.

Findings of a range of published scientific reports contributed to the determination of the risk ranking by providing evidence related to the capacity of different aquatic organisms to host, accumulate, or otherwise associate with viral pathogens, particularly but not limited to WSSV (Department of Agriculture and Water Resources 2019; OIE 2019; Oakey and Smith, 2018; Glanville et al., 2017; Oidtmann et al., 2017; Porchas-Cornejo et al., 2017; Haryadi et al., 2015; Sanchez-Paz et al., 2015; Macias-Rodriguez et al., 2014; Mendoza-Cano et al., 2014a; b; Aquavet, 2013; Desrina et al., 2013; Responsible Aquaculture Foundation 2013; Desrina et al., 2012; Valeriano Corre et al., 2012; Chang et al., 2011; Oidtmann and Stentiford, 2011; Martorelli et al., 2010; Esparza-Leal et al., 2009; Ma et al.,

2009; Overstreet et al., 2009; Walker and Mohan, 2009; Zhang et al., 2008; Liu et al., 2007; Sánchez-Martínez et al., 2007; Zhang et al., 2006; Vijayan et al., 2005; Yan et al., 2004; Chen et al., 2000; Supamattaya et al., 1998). Table 12 only provides guidance since the characteristics of *P. monodon's* viral and bacterial pathogens vary widely, and in most cases their mechanisms of persistence and transfer in the environment are not known.

Table 12. Hypothetical disease transmission risk from highest (1) to lowest (9) for organisms present in the intake water at the GCMA prawn farm over the monitoring period.

Risk rank	Organism group	Explanation
1	Penaeid prawns	Larvae, juveniles and adults of a number of genera within the family Penaeidae.
2	Non-penaeid decapods	Larvae, juvenile and adult life stages; e.g. crabs, Sergestid shrimp (glass shrimp), Palaemonid shrimp.*
3	Amphipods and isopods	Small planktonic or epibenthic peracarid crustaceans with predatory and/or scavenger modes of life.
4	Copepods, ostracods and feeding crustacean larval stages	Many species feed on microplankton; predatory and filter feeding species.
5	Non-feeding crustacean nauplii	The first life stage of various crustacean species, e.g. copepods and barnacles.
6	Rotifers, polychaetes and mollusc larvae	Filter feeders or benthos feeders that occur at high density in natural waters and ponds.
7	Chaetognaths and ctenophores	Predators of zooplankton, including larval stages of crustaceans; these tend to occur at low frequency.
8	Other zooplankton	Larval to adult forms of a diverse range of non-crustacean, micro- to meso-plankton groups not specified above; predatory and filter feeding forms that occurred at low frequency.
9	Phytoplankton	WSSV can remain viable for an extended period if associated with an algal cell.

** Palaemonid shrimp were not detected in the farm intake water but were found within the farm water distribution system*

Farm filter mesh geometry

Over the two production seasons monitored, three configurations of filters were studied, generating useful data for pre- and post-filtered seawater. This adds greatly to the value of the study by providing a broader range of assessments for the performance of different screen mesh options under commercial farm conditions. In Season 1, only the two RDF units installed pre-season and operated during the first half of the production cycle were studied. Both of these filters had a mesh of stainless steel thread with a Dutch (Cross) weave and a nominal filter mesh rating of 80 µm. Under microscopic examination the mesh was found to have the following measurement specifications; warp and weft threads of approximately 224 µm and 167 µm diameter respectively, with the warp threads approximately 866 µm apart (Figure 13). At the microscopic level it becomes apparent that this mesh is quite 3-dimensional, making assessment of aperture size more complicated than a simple measurement of the distance between threads. Precise measurement was therefore not possible. However, it was estimated that the triangular aperture created by the warp and the weft threads, as seen in Figure 13, had a base and length of roughly 165 µm and 250 µm, respectively.

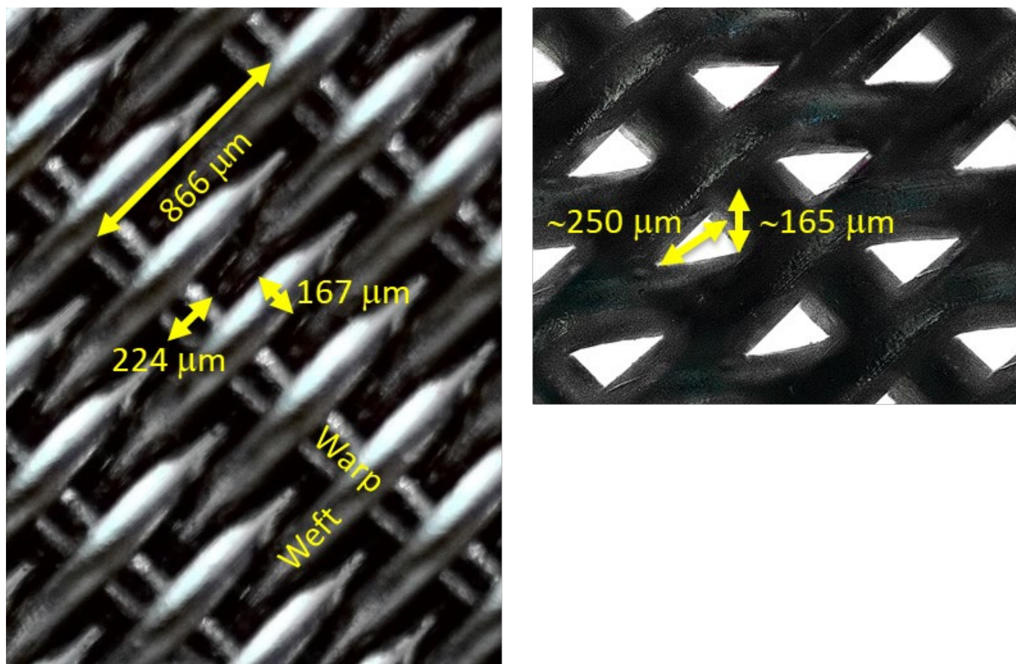

Figure 13. Profile and angular perspective of dutch (cross) weave stainless steel mesh as used on the original rotating drum filters, showing approximate dimensions as determined with a microscope. Left image view is from directly above the mesh and the right image view is angular to the mesh allowing apertures to be visible.

The third rotating drum filter installed at around half-way through the first season was from a different manufacturer than the original two, and also had a mesh rated as nominally 80 µm. The mesh of the new filter had a polymer thread in a plain weave construction, and presented a more 2 dimensional, square aperture to the flow of water (Figure 14). The aperture size was measured to have 80.7 ± 1.2 µm side length. It is difficult to directly compare the apertures of the two different meshes used in the first season because the geometries are very different, and for the stainless steel mesh the path through is not a straight line (Figure 13). The best way to compare the performance of these meshes is to analyse the sizes of organisms in their filtrate. This comparison is covered in the following section.

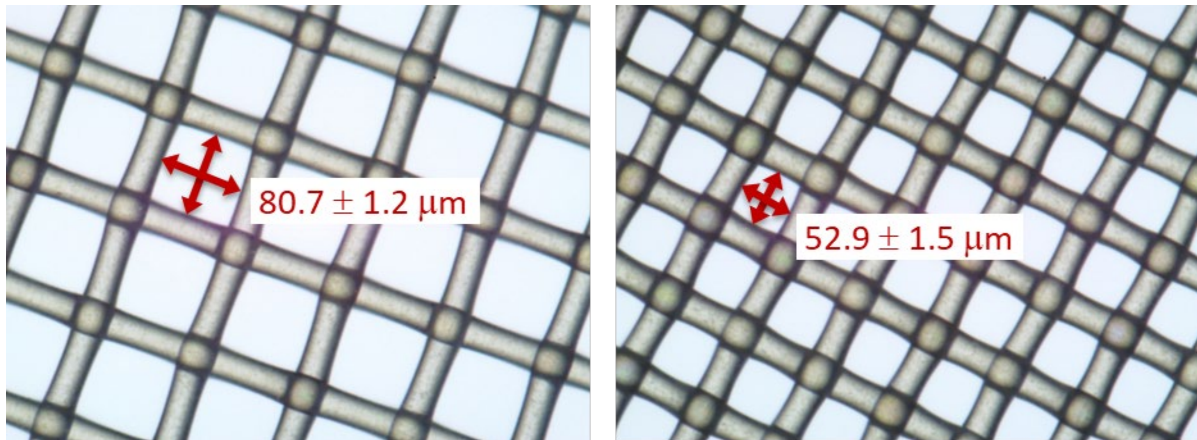

Figure 14. Polymer mesh of 80 µm nominal size rating used on the third filter in Season 1 (left) and 50 µm mesh used on all filters in Season 2 (right).

During the inter-season period of 2019, GCMA modified the filter system by replacing the mesh on all existing filters with 50 µm rated polymer mesh and they also installed a fourth drum filter with the same mesh. The nominal 50 µm rated mesh was of the same weave as the previous 80 µm mesh and under magnification can be seen to have the same geometry (Figure 14) with a measured square aperture side dimension of 52.9 ± 1.5 µm.

Performance comparison of different filter types

All filters operated at GCMA reduced the influent zooplankton population, however the extent of abundance reduction of the various identified groups was influenced by both the mesh type and the nominal mesh size rating of the RDF. Data from Season 1 enabled direct comparison of filter performance of the original stainless steel mesh filters with a nominal 80 µm rating and the new polymer mesh filter of the same nominal rating. Direct comparison was made possible because both filters operated concurrently for half of the season.

The stainless steel mesh, did not significantly impact abundance of six of the seven most dominant plankton groups identified, with only a statistical impact on mollusc veliger larvae (Table 13). In contrast, abundance of six of these seven plankton groups had significantly lower abundance in the filtrate of the polymer mesh filter ($P<0.05$) (Table 13). Only rotifer abundance was not different between the two mesh types. Rotifers had the smallest body dimensions of all organism groups measured, with a minimum body dimension average of 46 ± 11 µm (Table 11), much smaller than the nominal mesh size rating of the filters.

Table 13. Mean abundance (individuals kL^{-1}) of seven dominant plankton groups in the farm's raw intake water (sampling location 1) and RDF filtrate. In Season 1 two filters are compared, the original and the new filter installed during the season (sampling locations 3 and 5 respectively). In Season 2 there is just one type of filter. Mean values are bias-corrected back-transformed from the general linear model Ln(count+1) values. The % value in brackets is the filtrate abundance as percent proportion of the pre-filter abundance.
** denotes abundance in filtrate was significantly different from the pre-filter seawater;*
▲ denotes abundance in filtrate of the new filter was significantly different to that of the original filter;
ns denotes no significant difference. All significance levels at P<0.05.

Season 1	Pre-filter (Loc. 1)	Post-original stainless steel 80µm filter (Loc. 3)			Post -new polymer 80 µm filter (Loc. 5)		
Total copepods	27,432	20,241	(74%)	ns	853	(3.1%)	*▲
Unspecified nauplii	26,848	21,785	(81%)	ns	5675	(21%)	*▲
Barnacle nauplii	12,462	8245	(66%)	ns	102	(0.8%)	*▲
Polychaete larvae	3078	2206	(72%)	ns	66	(2.2%)	*▲
Mollusc veligers	1674	207	(12%)	*	17	(1.0%)	*
Grouped crustaceans	756	134	(31%)	ns	23	(7.5%)	*
Rotifers	1185	944	(80%)	ns	825	(70%)	ns
Season 2	**Pre-filter (Loc. 1)**	**Post 50 µm filter (Loc. 3)**					
Total copepods	77,148	609	(0.8%)	*			
Unspecified nauplii	19,969	4402	(22%)	*			
Barnacle nauplii	2039	6	(0.3%)	*			
Polychaete larvae	4338	32	(0.7%)	*			
Mollusc veligers	2356	22	(0.9%)	*			
Grouped crustaceans	26	0	(0.0%)	*			
Rotifers	202	94	(47%)	ns			

The relative performance of the two polymer mesh sizes used at GCMA over the course of the project, 80 µm in Season 1 and 50 µm in Season 2, were compared for commonly occurring plankton groups using calculated percent remaining values; the group's abundance in the RDF filtrate as a proportion of the pre-filter abundance. The pattern of percent remaining among the seven dominant plankton groups for both mesh sizes was similar, but with a trend towards higher abundance reduction by the 50 µm mesh (Figure 15 and Table 13). It is apparent from the values that a high proportion of the smallest organisms in the list, nauplii and rotifers, were still passing through the 50 µm mesh (Figure 15 and Table 13). Perhaps the most noteworthy difference between the two mesh sizes was for the Grouped crustaceans, a grouping for statistical purposes that includes decapod and other larvae, amphipods, isopods and ostracods. Even though the overall abundance of this group was low in the intake water, there was a functionally significant difference in the impact of each mesh size with a small number in the filtrate of the 80 µm mesh (7.5%) and none detected in the 50 µm mesh filtrate.

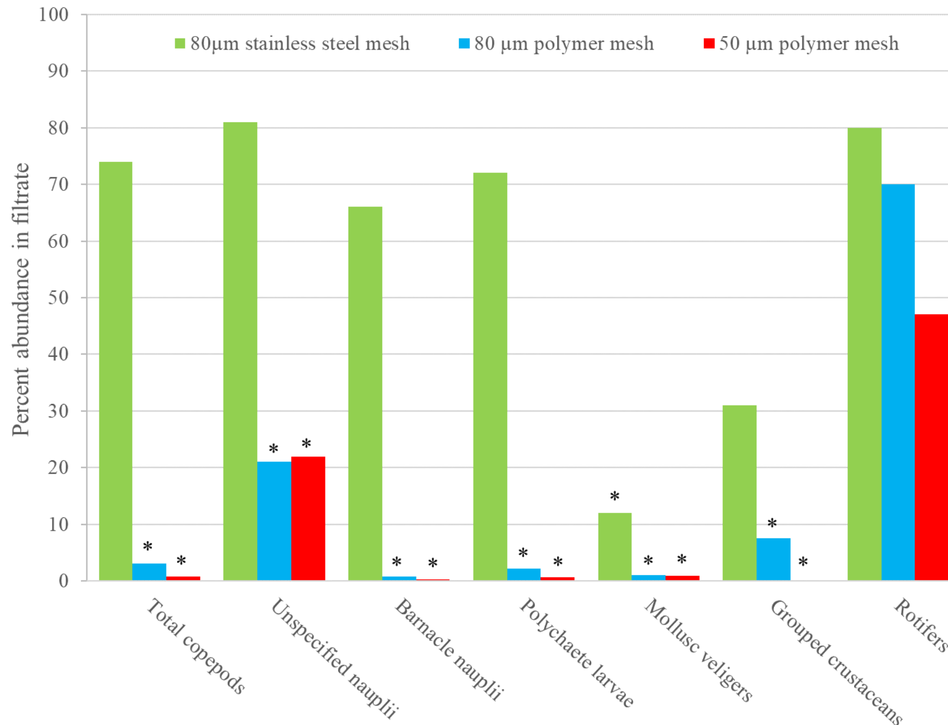

*Figure 15. Percent abundance of commonly occurring zooplankton groups in the RDF filtrate for each of the three filter mesh types used at GCMA. Percent abundance is the group's abundance in the RDF filtrate as a proportion of the pre-filter abundance. * denotes group abundance significantly reduced by the filter (P<0.05)*

Filter backwash content

The performance of the filter meshes, and their capacity to reduce biosecurity risk, was also qualitatively assessed by analysing the composition of filter residue. The abundance of organisms in the filter residue, as retrieved by sampling the backwash, was not quantifiable due to the pulsing nature of the backwash from multiple filters at the collection point and the unknown volume of filtered water that it represents. However, it was apparent that even for the 80 µm stainless steel mesh filter which had the least impact on zooplankton abundance, the backwash contained a high abundance of the larger bodied life stages of crabs, amphipods, prawns and shrimp that were recorded only infrequently from intake water samples (Figure 15). The selective removal of larger organisms by the RDF, and therefore their biased concentration in the filter residue, is indicated by the shift towards a larger body size fraction compared with the raw intake water (Figure 16). The 20 L of backwash water sampled at the onset of a RDF backwash cycle potentially represented the residue of many kilolitres of filtered seawater. Filter residue is therefore a sensitive detection method for rarer organisms occurring in the farm source water.

Filter mesh aperture size versus zooplankton body size

Zooplankton with larger body sizes than the nominal filter aperture size were consistently present in the RDF filtrate of all filters used. Few organisms in the raw Logan River seawater had a spheroid shape; almost all were more elongate, with body width less than the body length. It is therefore more relevant to consider the body width dimension when assessing a filter's capacity to remove particular groups of zooplankton.

Analysis of the body size distribution for organisms in the raw intake and RDF filtrate in both seasons indicates a trend towards smaller body width in the filtrate (Figure 16 and Figure 17). However, the size distribution also reveals that a substantial proportion of the zooplankton in the filtrate have a body width greater than 1.5 x the nominal aperture rating of the filter mesh; size classes > 125 µm in Season 1 (Figure 16) and > 75 µm in Season 2 (Figure 17).

The physical characteristics of these planktonic organisms may explain why comparatively large bodied specimens can pass through smaller apertures. The main body of most of the organisms identified had a semi-rigid exoskeleton or similar outer covering. Manual manipulation of preserved specimens under the microscope revealed their pliable, somewhat elastic body forms, which could be readily distorted with light compressive force and then rebound to the original shape after pressure was released. Such organisms may therefore pass through apertures smaller than their body dimensions when forced by a water pressure gradient across the filter mesh.

If there is any probability that an organism will pass through mesh of a certain aperture each time it comes in contact with the mesh then the artificially concentrated density of organisms within the drum of the RDF will greatly increase the frequency of it happening. The continuous turbulence at the water/mesh boundary caused by the rotation of the drum filter may also act to increase the frequency that organisms entrained in the water flow are contacting the mesh. Additionally, it is also possible that the behaviour of some organisms may promote their passage through the filter, when their rheotaxis (active orientation within a current) and limb and body movements are taken into account. To minimise the likelihood of organisms passing through a mesh filter, their concentration in the volume of water within the drum filter and the pressure gradient across the mesh, should therefore be minimised with regular, or even continuous, backwashing.

In both seasons a low abundance of copepods in the RDF filtrate had a minimum measured dimension that was much larger (3 x) than the filter mesh aperture. It seems unlikely that these could have compressed to such an extent as to allow passage through the mesh and remain intact and of normal appearance. This finding was particularly evident in Season 2 when copepods with a body width of >250 µm (5 x larger than the mesh aperture), were found in the filtrate on multiple occasions (Figure 17). A more likely explanation for the large copepods is that there was a low level of filter mesh bypass occurring. Damage to the integrity of the mesh is one possibility. Another possible route for water bypass could have been a poorly sealed part of the RDF structure. The RDFs used have a flexible seal between the filter housing and the rotating drum which presses against a plate on the rotating drum. If the drum becomes slightly off-set, the seal wears or solid material wedges in between the seal and the rotating plate, it is possible for water containing a concentrate of filterable material to escape directly into the filtrate. This situation may be very difficult to detect during routine maintenance checks. In single-pass single treatment systems such as that operated at GCMA during the study period ensuring consistent system performance is critical to achieving the desired biosecurity outcomes.

There is potential for the distortion and compression that an organism experiences as it is forced through an aperture smaller than its body dimension, to disrupt internal organs or break appendages, rendering it incapable of surviving and/or reproducing. The presence of an intact organism in a preserved sample of the filtrate does not necessarily mean that it was viable. Viability tests for the organisms in the farm RDF filtrate were not directly assessed, however the plankton monitoring of locations further along the farm water distribution system provided a good indication of plankton that persisted. These locations contained water filtered days to weeks previously. Plankton populations within the farm are discussed in detail in section 4.9. Post-filter zooplankton persistence was also investigated in Part A of this project, where mesocosm cultures of filtrate were undertaken on a small scale to directly assess the viability of various organism groups.

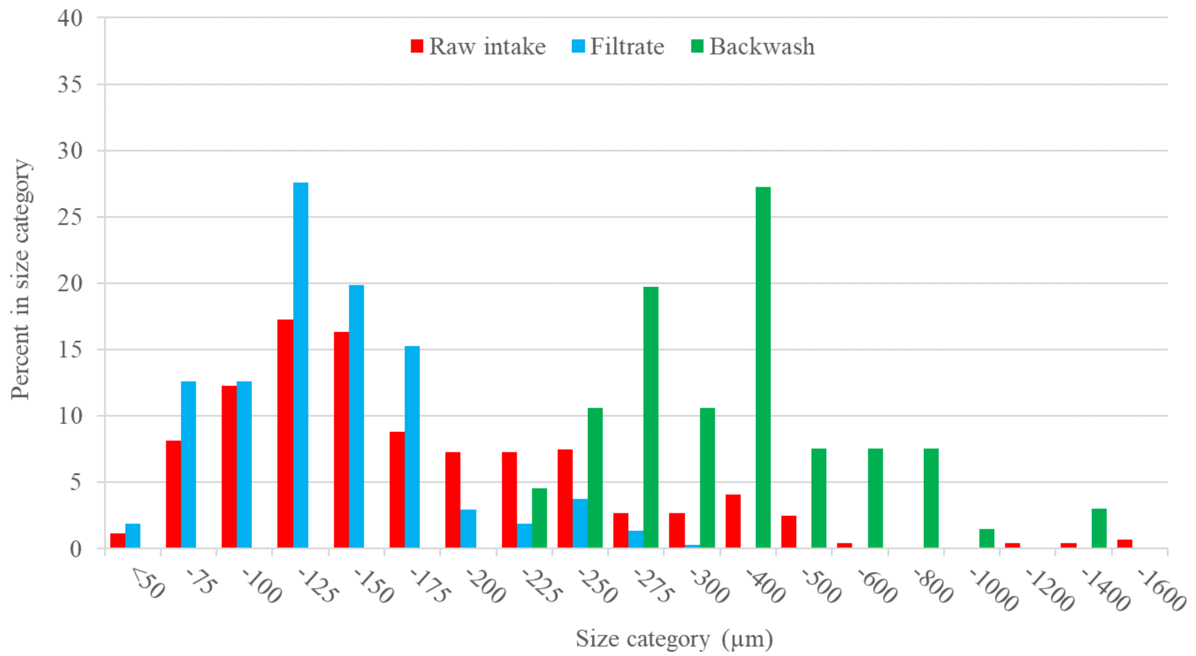

Figure 16. Distribution of organism body width (all zooplankton combined) before and after filtration and in filter backwash in Season 1 at GCMA when 80 µm stainless steel mesh was used.

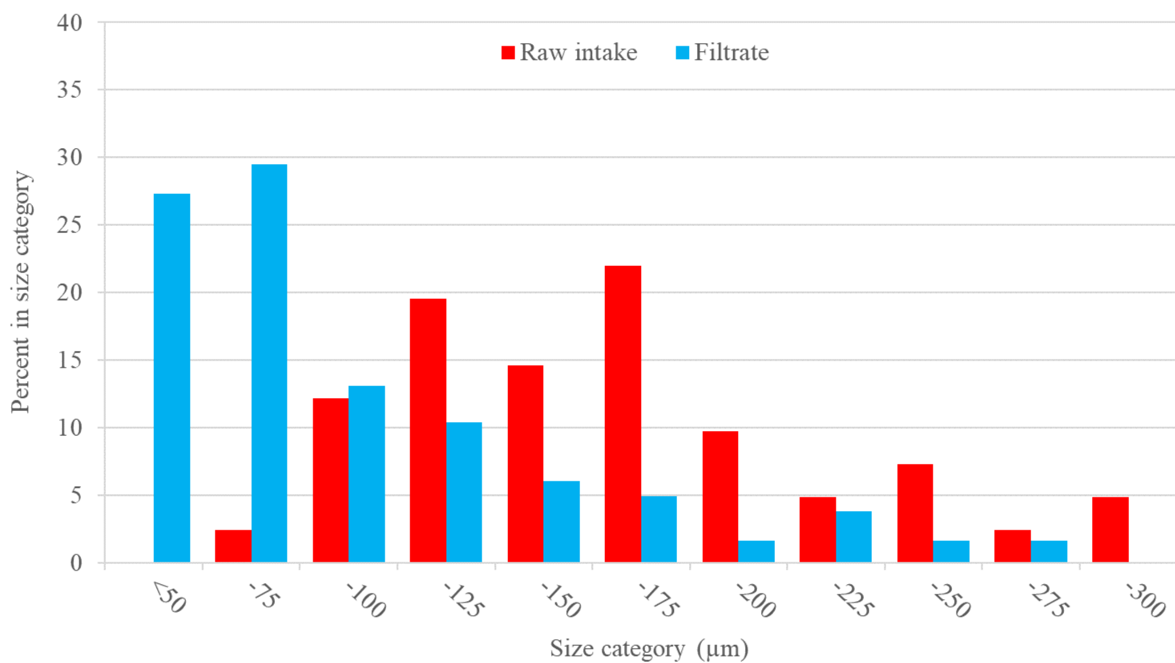

Figure 17. Distribution of organism body width (all zooplankton combined) before and after filtration in Season 2 at GCMA when 50 µm polymer mesh was used.

Impact of filtration on copepods

Copepods are an important component of plankton blooms in production ponds, so the impact of filtration on their entry into the farm was assessed separately to other zooplankton groups. Two population characteristics were assessed, abundance and body size, for copepods in the filtrate entering the farm relative to the raw intake seawater.

The abundance of adult and late juvenile stage copepods was greatly reduced by both the 80 µm stainless steel mesh and the 50 µm polymer mesh. In both cases abundance levels were reduced by filtration to ~ 1-3% of that in the raw intake seawater (Table 13 and Figure 15). At this high removal rate there was strong potential for selective retention of different copepod groups due to interspecific differences in average body size and body proportions. Season 2 mean abundance values (Table 14) show that there may have been a trend towards shift in the relative proportions of the three dominant copepod Orders caused by filtration with 50 µm mesh, with cyclopoids the most heavily impacted by filtration.

Table 14. Proportion of each copepod Order in raw seawater (pre-filter) and RDF filtrate for Season 2 when a nominal 50 µm mesh filter was in operation.

	% Calanoid	% Cyclopoid	% Harpacticoid
Pre-filter	72	18	10
Filtrate	81	2	17

In this investigation nauplii were not taxonomically identified, but it was apparent that the great majority had morphologies consistent with those described for the main copepod orders present as sub-adults and adults. In Season 2, the ratio of mean abundances of unspecified nauplii to late copepod stages was 8: 1 (4402 and 609 kL^{-1}, respectively). Filtration using either the 80 µm stainless steel mesh or 50 µm polymer mesh reduced the nauplii abundance in filtrate to around 20% of that in the pre-filter water (Figure 15), but due to their high natural abundance there was still an average of 4000 to 6000 kL^{-1} remaining in the filtrate which entered the farm (Table 13). Assuming that a large proportion of the unspecified nauplii are of copepod origin, it is evident that the majority of copepods entering the farm do so at the nauplius stage.

Filtration skewed the body size distribution of the combined zooplankton population by selectively removing the larger sizes from the filtrate (Figure 16 and Figure 17). This pattern was also evident for copepods when comparing raw pre-filter seawater and filtrate for all copepod groups combined, or just Calanoid copepods, the most abundant group with largest average body size (Table 15).

*Table 15. Mean (± standard deviation) copepod total length and body width in raw pre-filter seawater and RDF filtrate for Seasons 1 and 2. * denotes pre-filter and filtrate mean sizes significantly different for the dimension group (P<0.05).*

		Season 1		Season 2	
		All copepods	Calanoids only	All copepods	Calanoids only
Total length (µm)	Pre-filter	624 ± 236	706 ± 340	579 ± 264	620 ± 216
	Filtrate	505 ± 102 *	481 ± 81 *	535 ± 204	549 ± 216
Body width (µm)	Pre-filter	155 ± 63	190 ± 84	162 ± 51	179 ± 43
	Filtrate	124 ± 26 *	128 ± 25 *	155 ± 51	158 ± 52

Comparing Season 1 and Season 2 filtrate copepod size determined that the size of copepods in the filtrate determined that mean copepod body width was significantly larger in Season 2, by around 30 µm (Table 16). This is unexpected since a smaller mesh size was used in Season 2 (50 µm) than in Season 1 (80 µm). The result is difficult to explain in light of the finding that there was no significant difference (P>0.05) in pre-filter copepod mean size between the seasons, body widths of 155 and

162 µm for Seasons 1 and 2, respectively. Since the filtration greatly reduced overall copepod abundance (Figure 15) this result may indicate potential selective removal of copepod species, based on some characteristic other than body width. There is however insufficient data for such analysis. The result does however confirm that in this case the reduction in filter size to 50 µm did not seem to impact the late juvenile to adult staged copepod populations entering the farm over and above that produced by the original 80 µm filter.

*Table 16. Mean (± standard deviation) copepod total length and body width in RDF filtrate for Seasons 1 (using 80 µm mesh) and 2 (using 50 µm mesh). * denotes sizes were significantly different between seasons (P<0.05).*

	All copepods		Calanoid copepods only	
	TL (µm)	BW (µm)	TL (µm)	BW (µm)
Season 1	505 ± 102	124 ± 26	481 ± 81	128 ± 25
Season 2	535 ± 204	155 ± 51 *	549 ± 216	158 ± 52 *

Zooplankton abundance within the farm

The average abundance of most zooplankton groups changed after they entered the farm in RDF filtrate and progressed through the seawater distribution system (Figure 18 and Figure 19; Table 8 and Table 9). The most common groups present in the filtrate persisted throughout the distribution system to eventually enter the production ponds. In both seasons total zooplankton showed a trend for increasing abundance from levels in filtrate to levels in production ponds. When interpreting the zooplankton population data for the various sampled locations it should be recognised that the abundance values were derived from periodic sampling events which did not follow 'plugs' of water progressing through the farm distribution network. On each sampling day, seawater in the RDF filtrate had no direct relationship with seawater further down the farm's distribution system, and each sampling location was independent of the others. Despite this, the derived averages over time still provide informative data on the zooplankton population characteristics for the sampled locations. Given that locations 6 and 9 represented bodies of seawater that had been within the farm for longer periods (days to weeks), their abundance data are good indications of which groups formed reproductively viable populations in the reservoirs and channels of the farm. It is likely that copepods, barnacles, polychaetes, gastropods, and rotifers were all reproducing within the farm, and particularly in the production ponds.

Since the production ponds received little to no exchange for the first two months once filled, organisms that proliferated there were likely initiated by organisms in the RDF filtrate in September and October each year (see Table 8). Copepods were well represented in RDF filtrate at this time and their abundance would have expanded from there with *in situ* reproduction. It is clear that the moderate abundance of barnacle larvae in RDF filtrate at that time also flowed through to the production ponds, colonising the plastic liners of channels and ponds at high density, as observed within several weeks of commencing seawater flow into the farm.

Of those common groups identified in the plankton, only copepods and rotifers have life histories that are entirely planktonic, i.e. they are members of the holoplankton. Other common groups, barnacles, polychaetes, gastropods and most other crustaceans are meroplanktonic. They exist in the plankton as larval stages before either permanently attaching to the substrate or adopting a strong association with the benthic environment. This means that for an enclosed water body without water inflow, it would be expected that the larval planktonic population would decline in the short term as the first generation progressed through development. Finer timescale sampling however could have been necessary to detect such patterns

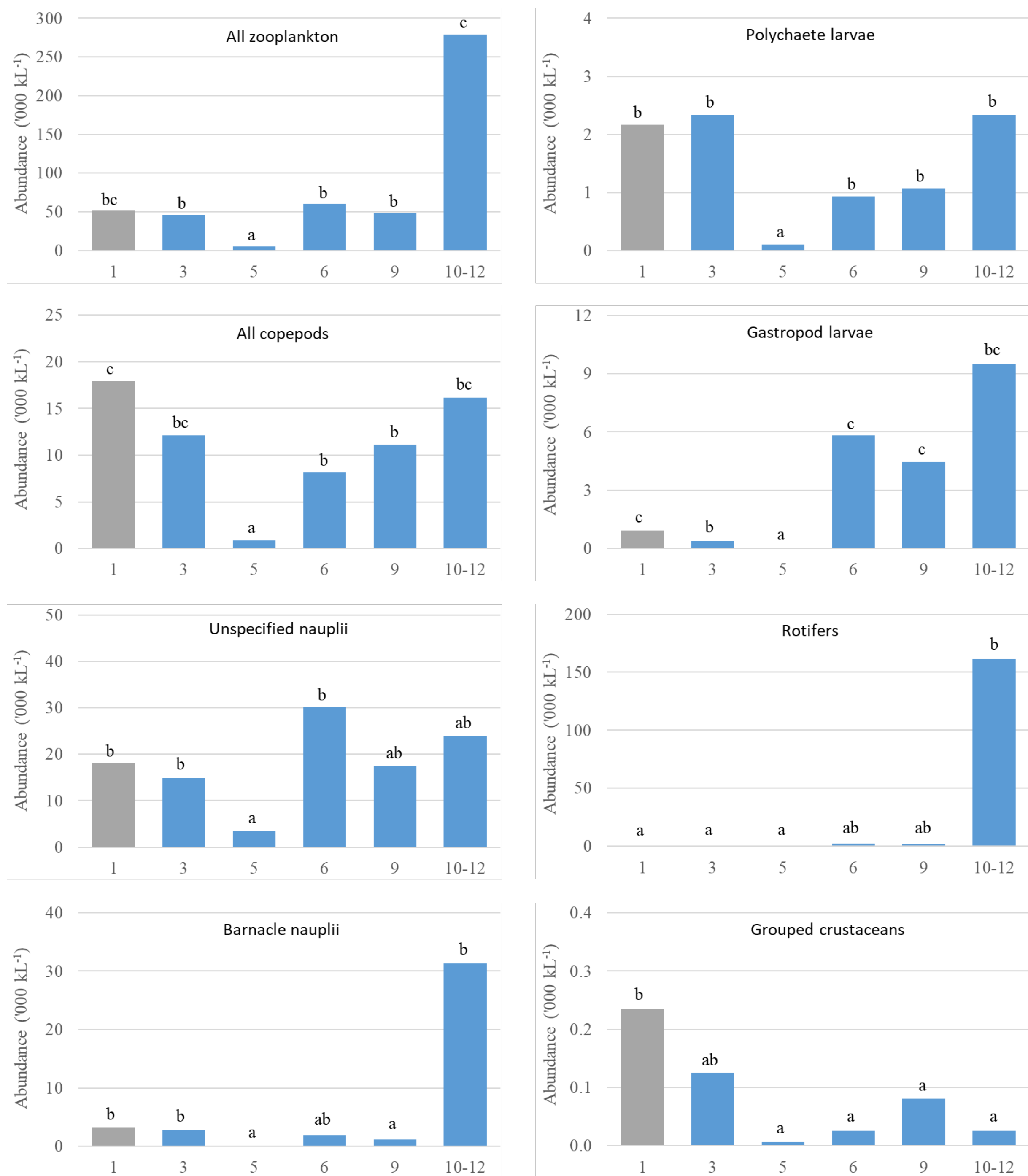

Figure 18. Mean population abundance of selected zooplankton groups at different locations within the farm for Season 1. Location codes are: 1 – raw intake; 3 – RDF filtrate; 5 – 2nd filter (Season 1 only); 6 – exit of 1st reservoir; 9 – end of main channel; 10,11,12 – average of 3x production ponds (as per Table 3 and Figure 6). Bars with the same letters are not significantly different (P>0.05).

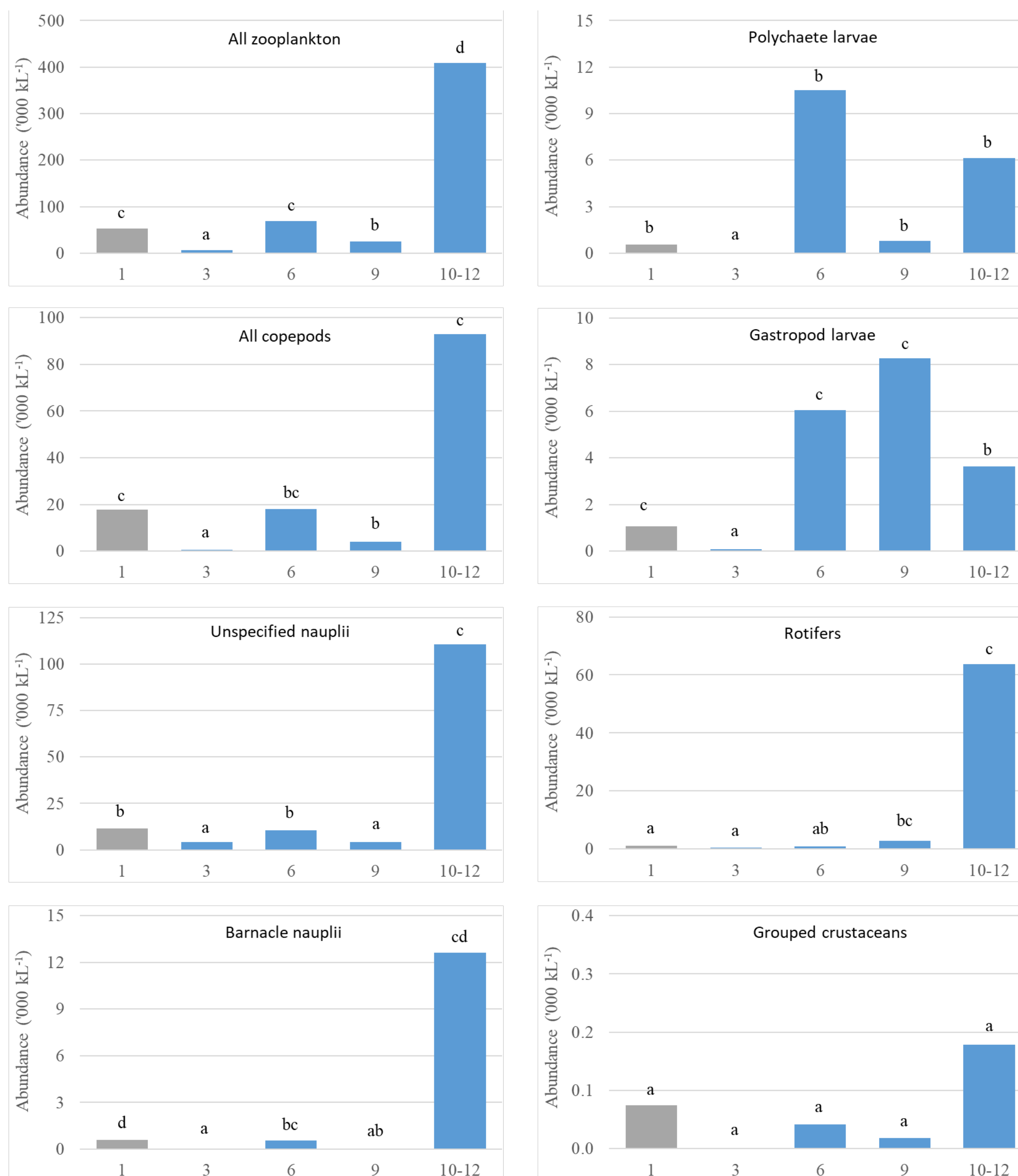

Figure 19. Mean population abundance of selected zooplankton groups at different locations within the farm for Season 2. Location codes are: 1 – raw intake; 3 – RDF filtrate; 6 – exit of 1st reservoir; 9 – end of main channel; 10, 11,12 – average of 3x production ponds (as per Table 3 and Figure 6). Bars with the same letters are not significantly different (P>0.05).

Copepod abundance within the farm

Copepods were present in the raw intake water on all sampling days assessed but exhibited high variability in abundance throughout the seasons, ranging from 2850 to 48,500 kL^{-1} in Season 1, and 4600 to 26,000 kL^{-1} in Season 2 (Table 8). Abundance at the different sample points within the farm were similarly highly variable over time, however the relative proportions of the three main copepod Orders in the water distribution system were somewhat consistent at around 65:20:15 (%) (Figure 20). It is therefore apparent that conditions within the farm do not consistently favour the dominance of, or conversely inhibition, any one type of copepod.

Filtration reduced copepod abundance entering the farm in Season 1 by non-significant levels (74%), and on average the abundances at different points within the farm were not significantly different (Figure 18 and Table 13). In the latter part of that Season the 80 µm stainless steel and polymer filters were operating concurrently and it is not possible to discern the differential impact of each filter type on the copepod population downstream (Figure 20). However, in Season 2 filtration significantly reduced copepod abundance (Figure 20). The significant increase in copepod abundance at the two downstream sampling points indicates that the relatively low abundance entering the farm does not constrain rapid population expansion (Figure 20). It should be noted that variable seawater flow at the intake and through the farm distribution system means that the period of time for water to reach sampling locations 6 and 9 (Figure 6) is not consistent and fluctuates throughout the production cycle.

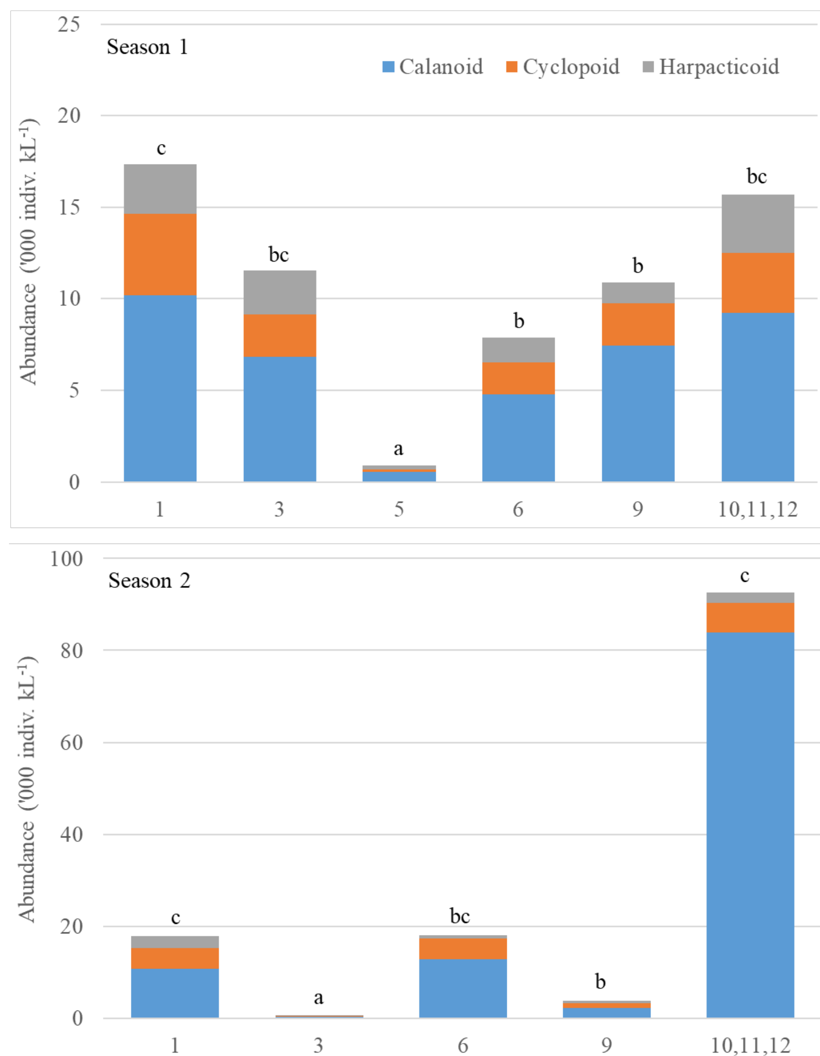

Figure 20. Copepod mean (n=10-14) abundance at different locations in the farm water distribution system in the first (top) and second (bottom) seasons. Location codes (as shown in Figure 6) are: 1 – raw intake; 3 – RDF filtrate; 5 – 2nd filter (Season 1 only); 6 – exit of 1st reservoir; 9 – end of main channel; 10, 11, 12 – average of 3x production ponds. Within each chart, the total copepod value of bars with the same letter are not significantly different (P>0.05).

Influent water filtration in Season 1 did not significantly reduce the copepod abundance (Figure 18) so the treatment would not be expected to have a significant impact on the rate of copepod population growth in production ponds at the start of the season. However, at the start of that season, which was the first to have all farm intake seawater filtered, the slower rate of filling ponds meant that there was often only a short period between filling and stocking. Consequently, there was an abbreviated period for blooms to grow. On the sampling day that coincided with stocking of one of the monitored ponds copepod abundance was only at 25,600 kL^{-1} which is considered relatively low.

In Season 2, when the filtration system had a highly significant impact on copepod abundance entering the farm (Figure 20), at the time of stocking the three ponds monitored, copepods were also at a level considered low. The one pond for which sampling and stocking occurred on the same day, pond sampling location 11 (Figure 6), copepod abundance was only 6,500 kL^{-1}. Once prawn post-larvae are stocked, their grazing pressure typically rapidly reduces copepod abundance (Coman et al. 2003) so abundance during the first weeks post-stocking is not an indication of potential copepod population growth. Pond copepod population did however exhibit a capacity to attain high density over the remainder of the production season, with abundance estimates varying over a broad range, but regularly exceeding 100,000 copepods kL^{-1} (Figure 21).

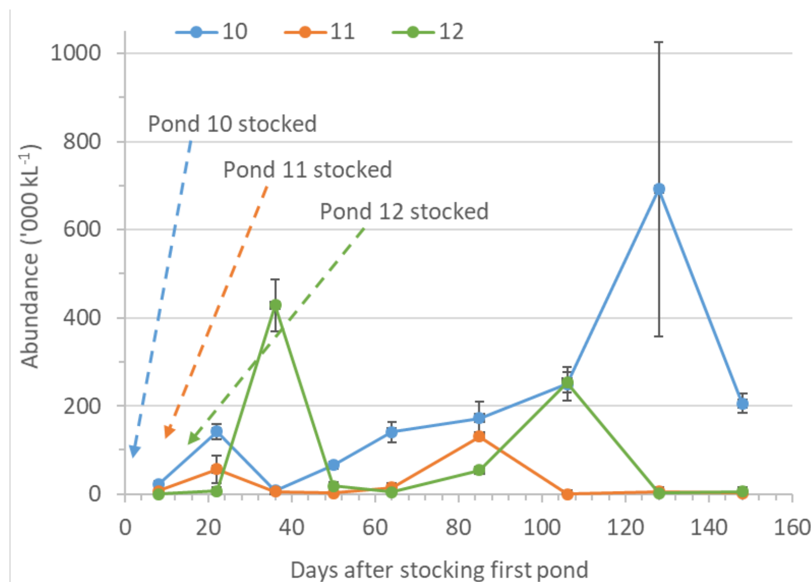

Figure 21. Copepod abundance in three production ponds in Season 2. Time 0 is 23/09/2019. Data points are the mean (± standard deviation bars) of three samples from different locations within the pond. Pond numbers refer to the sampling location plan (see Figure 6).

Phytoplankton blooms within the farm

Phytoplankton abundance in the raw intake seawater was low during Season 1 and filtration did not significantly affect abundance of the main phytoplankton groups identified (Table 17). It is therefore likely that phytoplankton diversity entering the farm was not impacted by filtration. As the filtered seawater made its way through the water distribution system there was a significant trend for diatoms and dinoflagellates to increase in abundance (Figure 22). The seawater reaching sampling locations 6 (after the first reservoir) and 9 (the end of the main supply channel) had by then passed through one, and two reservoirs, respectively. This created an extended residence time for these sampling locations, and hence more time for phytoplankton bloom development. For diatoms, most of the increase was in solitary centric and pennate forms, such as *Cyclotella* and *Nitzschia*, though short-chain diatoms such as *Melosira* and *Chaetoceros* also increased.

Table 17. Mean abundance (cells L^{-1}) (n = 6) of phytoplankton groups in the raw intake seawater (pre-filter) and in RDF filtrate in Season 1. Within each phytoplankton group across the locations, bars with the same letter are not significantly different (P>0.05).

	Diatom chain cells	Diatom solitary cells	Diatoms total	Dinoflagellates	Cyanobacteria
Pre-filter	811 ± 463	636 ± 220	3614 ± 738	389 ± 106	474 ± 255
Filtrate	636 ± 220	2569 ± 496	2994 ± 446	369 ± 152	719 ± 440

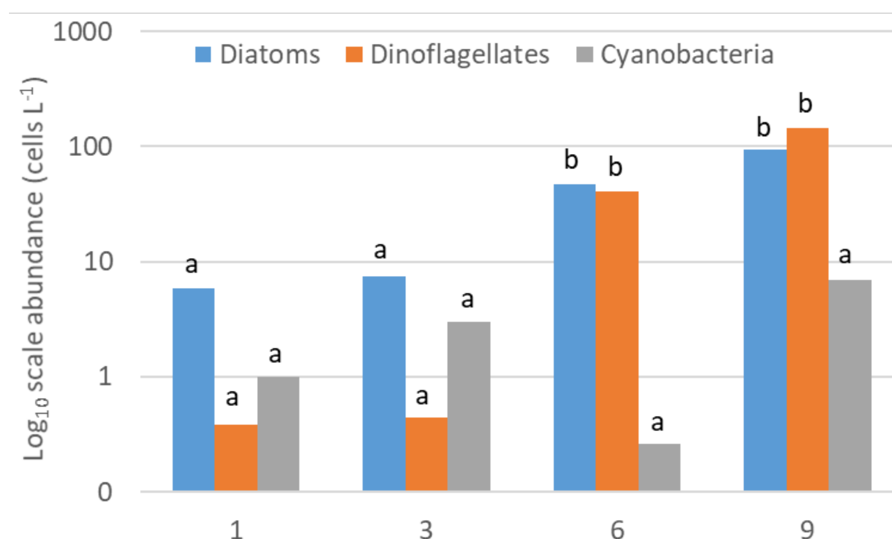

Figure 22. Average (cells L^{-1}) (n = 6) abundance of the three main phytoplankton groups at each sampling location in the farm water distribution system in Season 1. Location codes (as shown in Figure 6) are: 1 – raw intake; 3 – RDF filtrate; 5 – 2nd filter (Season 1 only); 6 – exit of 1st reservoir; 9 – end of main channel; 10, 11, 12 – average of 3x production ponds. Within each phytoplankton group columns with the same letters were not significantly different (P>0.05).

Seawater turbidity variations within the farm

In Season 1 raw intake water ranged widely in turbidity over the season, from 20 to over 100 cm, with a mean of 57.6 ± 5.6 cm (approximately 12.4 ± 2.0 NTU). Intake water clarity on sampling days in Season 2 was substantially lower with an average of 30.5 ± 6.3 cm (approximately 40.8 ± 13.6 NTU).

Water turbidity was not significantly affected by filtration in either Season 1 or Season 2, indicating that most of the particulate material present in the farm intake zone of the Logan River readily passes through 50 µm mesh. Despite the high turbidity entering the farm, by the time water had exited the first reservoir turbidity had greatly decreased and remained very low throughout the seawater distribution system (Table 18). It is assumed that much of the suspended particulate material was inorganic settleable solids that remained in Reservoir 1, the first point in the water distribution system that experiences a low water velocity and extended residence time.

Table 18. Turbidity column readings for raw intake seawater and RDF filtrate in Seasons 1 and 2. Values are means ± se (n = 21 and 10 respectively for Seasons 1 and 2) in cm for all sampling occasions over the season. Within a column means with the same superscripts were not significantly different (P>0.05).

Farm location	Season 1 (80 µm mesh)	Season 2 (50 µm mesh)
2 (pre-filter)	57.6 ± 5.6^{a}	30.5 ± 6.3^{a}
3 (RDF filtrate)	59.9 ± 5.6^{a}	30.9 ± 6.1^{a}
6 (reservoir 1 exit)	100.4 ± 7.6^{b}	110.0 ± 2.3^{b}
9 (end of channel)	99.0 ± 8.9^{b}	115.2 ± 1.9^{b}

WSSV test results

In Season 1, a total of 25 filter backwash samples taken over the course of the production cycle were submitted to Biosecurity Queensland. All were negative for WSSV (Table 19). On six occasions in this season, between 6 November 2018 and 12 February 2019, glass shrimp were collected from aggregations observed in channels and the second reservoir within the farm (5 samples) and from location 2, the pre-filter pond (1 sample). These were the only times that aggregations that could be sampled were found. All glass shrimp samples, consisting of 30-50 animals, were found negative for WSSV by Biosecurity Queensland.

In Season 2, filter backwash samples taken regularly between 1 October 2019 and 5 March 2020 all returned WSSV negative test results (Table 20). Additional plankton samples collected from two key points within the farm water distribution system, the exit of the first storage reservoir and the end of the channel on farm 2 (locations 6 and 9 respectively Figure 6) were also negative for WSSV (Table 20). No glass shrimp were found in the farm water distribution system in Season 2.

The lack of detectable WSSV in multiple farm samples up to 5 March 2020 indicates that if there was an elevated virus load in the Logan River that contributed to the April 2020 disease outbreak on prawn farms, it likely occurred after this sampling time. Following confirmation of the WSD outbreak in mid-April, FRDC project 2019/214 was initiated to investigate WSSV status of crustaceans in and around the three prawn farms operating in the Logan River region, as well as other locations in Moreton Bay. This project sought to provide vital information on the source and extent of the viral load associated with the local environment and the farms and the results reported here contributed to the epidemiological determinations of that project.

Table 19. Season 1 (2018/19) summary of WSSV test results of samples submitted to Biosecurity Queensland.

Date	Sampling location[1]	Location code[2]	Sample type[3]	WSSV test result
Season 1 (2018/2019				
25/09/18	Filter backwash	GC04	plankton	neg
25/09/18	Filter backwash	GC04	plankton	neg
25/09/18	Filter backwash	GC04	plankton	neg
02/10/18	Filter backwash	GC04	plankton	neg
02/10/18	Filter backwash	GC04	plankton	neg
09/10/18	Filter backwash	GC04	plankton	neg
09/10/18	Filter backwash	GC04	plankton	neg
16/10/18	Filter backwash	GC04	plankton	neg
23/10/18	Filter backwash	GC04	plankton	neg
30/10/18	Filter backwash	GC04	plankton	neg
06/11/18	Filter backwash	GC04	plankton	neg
06/11/18	End Farm 1 channel	GC07	glass shrimp	neg
13/11/18	Filter backwash	GC04	plankton	neg
13/11/18	Reservoir 2	GC08	glass shrimp	neg
20/11/18	Filter backwash	GC04	plankton	neg
27/11/18	Filter backwash	GC04	plankton	neg
27/11/18	Reservoir 2	GC08	glass shrimp	neg
11/12/18	Filter backwash	GC04	plankton	neg
20/12/18	Filter backwash	GC04	plankton	neg
20/12/18	Intake pond	GC02	glass shrimp	neg
03/01/19	Filter backwash	GC04	plankton	neg
15/01/19	Filter backwash	GC04	plankton	neg
29/01/19	Filter backwash	GC04	plankton	neg
29/01/19	Reservoir 2	GC08	glass shrimp	neg
12/02/19	Filter backwash	GC04	plankton	neg
12/02/19	End Farm 1 channel	GC07	glass shrimp	neg
26/02/19	Filter backwash	GC04	plankton	neg
12/03/19	Filter backwash	GC04	plankton	neg
26/03/19	Filter backwash	GC04	plankton	neg
09/04/19	Filter backwash	GC04	plankton	neg
23/04/19	Filter backwash	GC04	plankton	neg

[1] Filter backwash (GC04) is intake filter residue which is a concentration of water borne particles and organisms that have been pumped into the farm raw water receiving basin from the Logan River.
[2] Sample coding: GC## = GCMA farm and sample location reference (see Figure 6);
[3] Glass shrimp, *Acetes sibogae,* samples contain adults (~3cm) and juveniles.

Table 20. Season 2 (2019/20) summary of WSSV test results of samples submitted to Biosecurity Queensland.

Date	Sampling location[1]	Location code[2]	Sample type	WSSV test result
01/10/19	Filter backwash	GC04	plankton	neg
15/10/19	Filter backwash	GC04	plankton	neg
29/10/19	Filter backwash	GC04	plankton	neg
12/11/19	Filter backwash	GC04	plankton	neg
28/11/19	Filter backwash	GC04	plankton	neg
07/01/20	Filter backwash	GC04	plankton	neg
07/01/20	Reservoir 1 exit	GC06	plankton	neg
07/01/20	End Farm 2 channel	GC09	plankton	neg
29/01/20	Filter backwash	GC04	plankton	neg
29/01/20	Reservoir 1 exit	GC06	plankton	neg
29/01/20	End Farm 2 channel	GC09	plankton	neg
18/02/20	Reservoir 1 exit	GC06	plankton	neg
18/02/20	End Farm 2 channel	GC09	plankton	neg
05/03/20	Intake pond	GC01	plankton	neg
05/03/20	Filter backwash	GC04	plankton	neg
05/03/20	Reservoir 1 exit	GC06	plankton	neg
05/03/20	End Farm 2 channel	GC09	plankton	neg

[1] Filter backwash (GC04) is intake filter residue which is a concentration of water borne particles and organisms that have been pumped into the farm raw water receiving basin from the Logan River.
[2] Sample coding: GC## = GCMA farm and sample location reference (see Figure 6).

Microbiome analysis of prawn farm supply water

Microbiome samples of the farm's water distribution system did not consistently provide sufficient DNA for valid analysis and a number of samples were rejected from the dataset. The sample collection method used, though simple, was one successfully implemented by another group working in a similar situation and initial testing had indicated it was adequate for the purpose. It is apparent however that the method did not account for the very high variability in microbial load that was experienced. Despite this technical difficulty the study successfully determined the bacterial and archaeal populations for a total of 28 pooled and individual water samples, with days 2 and 4 collected from three farm locations 2 (pre-filter pond), 6 (reservoir 1 outlet) and 9 (Farm 2 channel end) (Figure 6; Table 3) well represented within the microbiome sequence dataset. These three sample collection points encompassed the start, middle and end of the water distribution system within the farm and were therefore able to be used to describe the microbial populations present throughout the pond water supply system.

The microbial populations within the farm water distribution system were found to be highly diverse. Statistical analysis of the bacterial alpha diversity values within each sample, indicated the within-sample diversity was not influenced by location and sampling time, or measured environmental (e.g. wind speed, rainfall, salinity, water temperature and turbidity) and biological (e.g. phytoplankton and zooplankton) factors. The water distribution system maintained a diverse microbial community across the farm which is considered to be an indicator of system health as the maintenance of a high

level of microbial diversity, has been shown to be a characteristic of healthy aquatic ecosystems (Zeglin et al., 2015; Comte et al., 2013).

The majority of water samples collected in this study were found to contain microbial assemblages which are commonly associated with coastal marine environments. Many of the microbial populations observed could not be taxonomically classified to a known genus, as marine bacteria are often unable to be isolated and cultivated. The analysis therefore focussed on the populations classified at higher taxonomic levels (phylum, class or family). Approximately 90% of samples were dominated by the bacterial phyla Proteobacteria and Bacteroidetes. Within the phylum Protoeobacteria, the SAR11 clade within the class Gammaproteobacteria and members of the order Rhodobacteriales within the class Alphaproteobacteria were present in all samples. The SAR11 clade is one of the most abundant bacterial chemoheterotrophic groups found in marine environments, often representing 25-50 % of the total planktonic cells (Morris et al., 2002; Henson et al., 2018). These aerobic, free-living microorganisms cannot fix carbon and instead use organic compounds, such as dissolved organic matter to satisfy their carbon requirements. The other highly abundant taxon, Rhodobacterale that sits within the Class Alphaproteobacteria, are known to form close associations with phytoplankton (Meyer et al., 2017).

The final sampling location at the end of the supply channel network (location 9), possessed the highest number of unique bacterial taxa (119 highly abundant genera) at all time points assessed (Figure 23). Despite this divergence in total microbial community profile, the most dominant taxonomic groups with very high abundance showed some similarities across time and sampling location (Figure 23). Particularly at the first sample collection point, the pre-filter pond receiving Logan River water from the supply pumps (location 2), but also to a lesser extent in the downstream samples, the bacterial populations consistently contained a high abundance of taxa belonging to Alphaproteobacteria from the order Rhodospirillales. This order includes photosynthetic and sometimes chemo-organotrophic aquatic bacteria as well as chemoheterotrophs involved in sulphur and carbon biogeochemical cycling and symbiosis with aquatic micro- and macro-organisms (Pujalte et al., 2014).

From a farm stock health perspective, it is relevant that the abundance of known and potential pathogens belonging to the *Vibrio* genus were very low in the communities within the farm water distribution system. This suggests that *Vibrio* were not selected for and enriched by the on-farm conditions and management practices employed in this aquaculture system.

The complete report for the microbiome investigation is included in Appendix D.

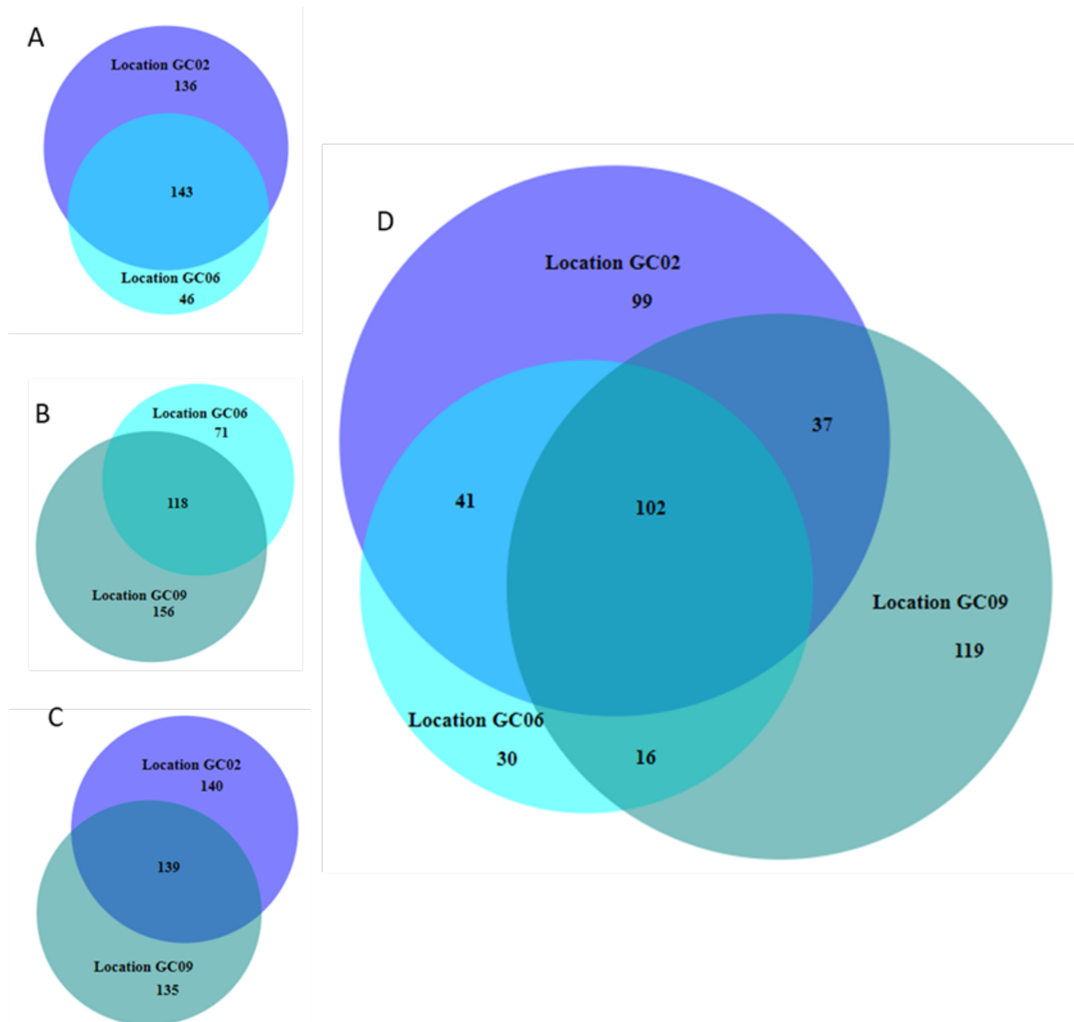

Figure 23. Venn diagram comparison of the numbers of highly abundant microbial populations, classified at genus level, found to be either shared between each location: (A) GC02 and GC06; (B) GC06 and GC09 and (C) GC02 and GC09, or (D) found to be unique to each location at all time-points of sample collection (collection days 2, 4, 6, 18 and 19).

Disease risks and filtration in perspective for Australian prawn farmers

WSSV in perspective

The WSD outbreak that occurred in the Logan River region in the 2016/17 production season may have been the stimulus for this project, but it is important to note that the prawn farming industry has been developing increasingly biosecure systems in recent years, particularly in the hatchery sector. Prawn farming experts and epidemiologists seem to agree that the greatest disease introduction risk for farms is via vertical transmission; that is, via contaminated broodstock transferring pathogens to the next generation. In this regard, the industry continues to make progress on broodstock domestication and application of pathogen screening technology for PL production (Sellars et al., 2019). There is however accumulated evidence for the capacity of various waterborne organisms, particularly zooplankton, and particulate matter to be vectors of WSSV (Esparza-Leal et al. 2009) (See also section 4.3). There is a paucity of information on environmental source routes for endemic viruses like Gill-Associated Virus (GAV), Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) and Yellow Head Virus genotype 7 (YHV7). A pathogen surveillance project is currently underway that may provide information on the prevalence of these viruses in Queensland coastal zones (CRCNA project, Biosecurity in northern Australian prawn aquaculture).

The absence of WSD emergence, and indeed any WSSV detections in Biosecurity Queensland's environmental surveillance program, or other disease outbreak on the studied prawn farm during the period of project farm sampling, meant there was no opportunity for the present project to contribute to associated epidemiological investigations. The outbreak in the Logan River region in April 2020 occurred several weeks after cessation of farm sampling. The project did however collect and test zooplankton (including all crustacean groups and other taxa – Table 1) concentrates from the raw water entering the farm in the first season, and test these for WSSV. Consistent with the absence of an outbreak on any farm in the region during this time, all samples tested WSSV negative.

The 2016/17 WSD outbreak and the one on Logan River farms in 2020, as well as the most recent finding that WSSV still endures in natural crustacean populations in Moreton Bay (Biosecurity Queensland 2020), is clear demonstration that environmental waters used to supply aquaculture facilities can be a high-risk source of pathogens for farms. For most disease issues experienced on Australian prawn farms, it is ultimately difficult to ascertain the precise source and transmission route of the pathogen. Even for the WSD outbreak in 2016/17, there is still no clear proof of the route of infection into the farms that were affected. In reality, there may be multiple routes by which a pathogen can potentially infect a farm that uses outdoor ponds filled with seawater directly sourced from estuaries. Some may be readily controlled, such as restricting the movement of personnel and equipment between farms, but others such as the avian route are more intractable. There is, however, strong evidence for the potential of farm intake waters to be a significant vector. Viral pathogens that have caused substantial losses on prawn farms in other countries commonly occur in environmental waters adjacent to the farming areas (Oidtmann et al. 2017; Thitamadee et al. 2016; Santos and al. 2013). In many cases effluent water from farms can also be considered a significant source of contamination for adjacent farms and may contribute to the persistence of the pathogen in the environment.

Of the viral pathogens most important to prawn aquaculture, WSSV is considered to have an unusually broad host range, including most decapods and other crustaceans, and has an unusually long period of viability outside a host (Oidtmann et al., 2017) (See also section 4.3). WSSV also can have an extended period of viability when associated with non-host vectors, including microalgae, which depending on conditions, can be more than a week (Liu et al., 2007). Other, less virulent,

prawn viruses of interest in Australia, including those identified in the exotic disease hazard list (Department of Agriculture and Water Resources 2019), have a much narrower host range and shorter free viability period (Oidtmann et al., 2017), but should not be underestimated in their potential to cause production losses. Importantly though, actions taken to reduce risks of WSSV will also afford protection from these and other, yet to be identified, pathogens.

There is sufficient evidence in the scientific literature that almost any inhabitant of the plankton can act as a vector for WSSV in a farm's intake water, under particular circumstances (see Section 4.3 and associated references). It is generally considered that high environmental loads of WSSV can increase the risk of non-host vectors transmitting this virus, yet evidence from the Australian WSD outbreak in 2016 is that environmental loads of the virus remained at low, difficult-to-detect levels throughout the episode. Even at the height of the Logan River disease outbreak, extensive sampling of crustacean hosts conducted by Biosecurity Queensland in the local vicinity of affected farms found low prevalence in natural crustacean populations (Biosecurity Queensland, 2020).

There is strong evidence, at least for WSSV, that the greatest horizontal infection route risk for development of disease in prawns is via ingestion of contaminated tissue (Raja et al., 2015). The potential for disease to develop from other contact transmission routes is far lower (Hamano et al., 2015; Raja et al., 2015). Given the high disease risk associated with ingestion of contaminated material, filtration of influent environmental water can be considered an effective approach to removal or reduction of potentially diseased or contaminated organisms that may be directly or indirectly ingested by prawns. Influent water pathogen transmission risk and the risk of disease occurrence are correlated with the abundance of risk vectors it carries, so logically, to achieve the highest degree of protection, a filtration system should remove all vectors if it is the only control method. However, within the operating constraints of large production area farms, this is not achievable, and in fact, may not be desirable when other factors important to prawn pond management are considered, e.g. necessity for stable phytoplankton blooms.

A hierarchy of disease transmission risks

A theoretical viral disease transmission risk hierarchy for waterborne organisms that routinely occur in farm intake seawater was proposed in the present study (Table 12). The intention of this was to provide some basis on which to assess the relative effectiveness of influent water treatment in terms of biosecurity threat risk reductions. While a broad range of scientific studies were used to provide evidence of transmission for formulation of the ranking (Section 4.3), there is insufficient information to fully quantify these risks for the circumstances experienced on Logan River prawn farms, or indeed on other Australian prawn farms. For example, there is an important difference between organisms having the capacity to transmit a virus under high viral load conditions, and the probability of these risks occurring in natural waters when there is low or undetectable prevalence. The risk hierarchy developed in this study was primarily focussed on WSSV, which has the largest known host range of viral pathogens affecting *P. monodon*, and less on other exotic and endemic pathogens due largely to the lack of information about host range for other viruses and diseases of importance to prawn farms in Australia.

This hierarchy places organisms with the closest taxonomic relationship to black tiger prawns as the highest risk due to them having the greatest likelihood of carrying pathogens that readily transfer across the species. Within the farm, these organisms also have the greatest potential to amplify the pathogen load and then infecting prawn stock via the ingestion route. The latter point suggests that monitoring for, and control of, non-stock crustacean populations within the farm are also important biosecurity management procedures for farms.

Filtration to reduce disease risks

Prawn farms in Australia typically apply a flow-through water management approach to maintaining appropriate conditions in intensive production ponds. With little on-farm water storage capacity this approach requires consistent inflow of seawater sourced from adjacent coastal waters, particularly during first fill of the farm and in the latter half of the production cycle. Critical operating parameters for farm influent water treatment systems include high peak inflow rate, high volume demand over extended periods, frequent high water turbidity due to suspended particulate matter, high microorganism load and variable water qualities such as pH and salinity. Alternative batch-wise water treatment to improve biosecurity would necessitate a large portion of farm area be devoted to seawater pre-treatment and storage, which is not considered feasible for larger farms. In this scenario, a storage reservoir would be regularly filled and treated to achieve the desired level of disinfection. If chemical disinfection were applied the water would then need to be rendered safe or suitable for use in the production system. This is an approach commonly employed by the hatchery sector, where ozonation or chlorination practices are used. Where this is not feasible for a pond farm to implement, for practical or business management reasons, the most practical approach is a single-pass water treatment operated on a flow-through basis.

The present study shows how mesh-based mechanical filtration can effectively remove most risk organisms that are of a size that would be directly ingested by prawns. However, the level of risk reduction depends on the performance characteristics of the filtration system. As stated in a report on the Mozambique and Madagascar WSD outbreak, “From a biosecurity standpoint, WSSV risk decreases with screen size”. This report recognised that the more potential carriers that are removed, the lower the risk of transmission of a disease-initiating viral load (Responsible Aquaculture Foundation, 2013). From this perspective, the present study shows how the implementation of influent water filtration at the GCMA prawn farm can be considered to have reduced biosecurity risk. The top three risk groups identified in farm source water (penaeids and other decapods, and epibenthic crustacean species), were largely excluded from entry, particularly by the 50 µm screen used in the 2019/20 season. Additionally, the investment in upgrading the original 80 µm mesh to 50 µm mesh seems to be justified by this risk reduction, whereby larger crustacean species considered the highest risk were more effectively excluded by the smaller sized mesh.

The present study’s use of small replicated mesocosms supported the work being simultaneously undertaken at much larger scales, and under commercial constraints at the GCMA farm. This provided opportunity to more robustly investigate multiple variables in a more controlled manner. As an example, Jung et al (2012) used 1,000 L tanks to assess the effects of crude oil and dispersant on natural plankton communities. In the present study, an array of 21 tanks provided for statistically valid comparisons of five filtration levels at a time. The response of the biota remaining in the treated seawater within the mesocosms was expected to approximate the influence of similar sized filters if used to fill a pond. Copepod proliferations were observed in these experimental mesocosms, with the populations continuing to include representatives of the three main types naturally present in the source seawater (i.e. Calanoid, Cyclopoid, Harpacticoid). This is a good indication that diversity was maintained for ecosystem health, despite their different body sizes. Of relevance to the current study, was the capacity of this mesocosm approach to identify viable passage of an organism through the micron mesh when their abundance in raw intake seawater was very low. A clear example was in Experiment 2 where, given sufficient time, rotifers bloomed in seawater treated previously with a 20 µm screen size that should have, and on initial inspection did, exclude such animals from the RDF filtrate.

For single pass filtration systems, with no additional treatment step, malfunction is a constant risk with critical repercussions. Damage to the filter mesh is an obvious point for failure and the smaller the mesh size the less robust it is. Even a small, partial tear in the filter mesh will allow passage of potentially contaminated particulate matter into the farm water distribution network. At GCMA during this study, there was an instance at the end of the 2018/19 production season when the mesh

ruptured, and unfiltered water was released into the farm inflow. In this case it was assessed that a breakdown of the backwash pump overnight had led to a pressure build up against the mesh and a weak point had failed. This was rectified upon detection, but presumably not before high risk organisms had the opportunity to enter the farm's water distribution system. Another potential RDF failure point is the seal against the rotating drum that is critical to preventing raw supply water from bypassing the filter. This study identified the presence of large copepods in the RDF filtrate that could not possibly have passed undamaged through a 50 µm aperture, indicating a low level of filter bypass could have been occurring. The potential for incomplete sealing is a design feature that farms should consider when investing in and installing RDF systems.

After harvest at the end of Season 2 a small number of penaeid prawns, glass shrimp and Palaemonid shrimp were found within the farm's water distribution channels, as part of the 2020 WSD outbreak investigation project FRDC 2019/214. This is clear evidence that wild, high WSSV risk species were still able to colonise the farm during the 2019/20 season. Some of the school prawns (*Metapenaeus macleayi*) and banana prawns (*Penaeus merguiensis*) found were over 75 mm total length so if they had entered the farm at a small, early stage, which is most likely, may have been in the farm water supply system for some months, well before the WSD outbreak in the region.

The occurrence of introduced prawns and shrimp does not necessarily mean that they passed through the 50 µm filter and remained viable. Rather, the small number could have by-passed the filter screens in a manner mentioned above. Also, their occurrence does not render the use of the filter system ineffective in reducing biosecurity risk. Presumably without the filtration, there would have been far greater numbers invading the farm and greater probability of a disease outcome. However, clearly if the filter malfunctioned at a time when there was a high WSSV prevalence in the adjacent environment the chance of sufficient load entering the farm to instigate a disease outbreak is unacceptably high. Unfortunately, periods of high WSSV load in the adjacent environment are not predictable and farms need to assume that high risk is ever-present.

Achieving consistent complete effectiveness of single-pass systems is not a trivial undertaking and will require good design and constant vigilance. Whether taking a physical or chemical approach to treatment, or both, all systems will have a constant risk of significant reduction in effectiveness or failure. Ideally a water treatment system would have some type of safeguard or alert system in place in the event of such failure.

A multistage filtration system would be one approach to reducing the risk of filter failure or malfunction. An example is a system with a primary filter that excludes larger sized material such as a 150 µm mesh screen, followed by a secondary filtration stage that further reduces the size of material entering the farm. Failure at either of these steps individually would not allow raw water to enter the farm. Alternatively, the primary filtration step could be followed by chemical disinfection or other seawater treatment process, e.g. Polychaete-assisted sand filters (PASF) (Palmer 2010). It is worth noting that the seawater treatment at GCMA farm was effectively a two-stage process, whereby seawater was filtered and then settled. Water turbidity measurements at the farm in the present study showed that a large proportion of suspended particulate material exiting the filters did not enter the ponds, as it settled in the first reservoir prior to entering the main pond supply channel. Presumably, the settled material included debris of crustacean origin, such as animals damaged by the filters and crustacean exuvia. The flip side to this benefit is that the reservoir could potentially become a source of contaminated detritus, and benthic feeding organisms that then disperse within the farm.

Farm ecosystem and operational considerations

Early in the farm monitoring program it became apparent that some crustaceans entering the farm could establish quickly and form persistent reproductive populations. Barnacles are a good example, with dense aggregations observed on submersed solid structures within three weeks of filling ponds.

In a previous plankton study at the same farm (Coman et al. 2006) also noted that barnacle reproduction in ponds maintained high nauplii counts throughout the production season.

Glass shrimp, *Acetes sibogae*, were observed shoaling at the end of channels and corner of a reservoir after around two months of operation in the 2018/19 and 2019/20 seasons. Additionally, at the end of the second production season a small number of metapenaeid prawns and palaemonid shrimp were found in channels. It is most likely these species entered the farm as eggs or larvae. Their presence may provide a crustacean population susceptible to pathogen amplification and ultimately create a source of highly contaminated tissue that finds its way into the production ponds, triggering disease in stock otherwise resistant to low level, incidental contact with pathogens.

The relative difficulty in excluding crustacean nauplii by filtration raises a critical question: What is the biosecurity risk of the earliest life stages of high risk vector species? To almost entirely exclude nauplii by filtration requires an effective mesh size of around 20 µm or below, as demonstrated by the experiments conducted in this project. In that experimental work, a 40 µm mesh allowed over 20% of nauplii-stage larvae to pass through. Rotifers were also relatively unrestricted by this 40 µm mesh size. Notwithstanding filtration technology advancements in the future RDF seawater treatment at commercial scale appears to be limited to 40 to 50 µm mesh for practical and economic reasons. However, if it was practical to filter to smaller sizes, perhaps the additional installation and maintenance costs could be justified by further risk reductions, particularly given that pathogens such as WSSV, GAV and IHHNV can be vertically transmitted (i.e. from adults to eggs) (Sellars et al., 2019; Joseph et al., 2015; Cowley et al., 2002). In balancing the potential costs and benefits of such a pursuit, it should also be recognised that elevated levels of filtration could bring additional benefits, such as the exclusion of fouling organisms like barnacles.

A high filtration impact on plankton was shown in the most recent work with PASF at BIRC (Palmer et al. 2018). This work used constructed sand beds stocked with cultured polychaetes to fill ponds and treat fully recirculated pond seawater during intensive culture of *P. monodon*. Whilst the PASF in this work was challenged by the organic loading towards the end of the crop, it appeared to slow copepod bloom development, change the assemblage of algal species in the first few weeks after filling, and greatly reduce barnacle fouling. Importantly, the fine sand filtration of influent seawater and the associated ecosystem effects on natural feed organisms may have yielded lower survival of the particularly young (PL 13) prawn seedstock used, and would otherwise require a modified management regime that allows for a longer period before stocking for bloom development after fill and/or a greater reliance on artificial feeds more suited to small prawns.

Peak flow rate is another important filter system design consideration. If it restricts water availability at critical times during production, particularly towards the end of the cycle, then the filter system could possibly be a disadvantage for operational needs and stock health. Deteriorating water quality in production ponds is a contributor to disease susceptibility and emergence. Even in the absence of exotic disease agents, endemic diseases can heavily impact stock under sub-optimal pond conditions.

Intake water is not just a source of pathogenic viruses and bacteria. It is also the primary source for harmful algal species and other plankton that can adversely impact pond conditions, by forming dense blooms or creating pond conditions sub-optimal for the stock (Mann, 2017; Alonso-Rodríguez and Páez-Osuna, 2003). Filtration alone is unlikely to prevent entry and proliferation of harmful or otherwise undesirable algal species due to their small cell size. In this study there was no evidence that 20 µm filtration inhibited the occurrence of even relatively large diatom species such as the long chain forming *Helicotheca* sp.

The present study highlights the trade-off between the demands of pond management for high production and farm biosecurity. It is widely recognised that zooplankton blooms, particularly in the post-stocking period, are highly advantageous as a food source promoting stock health and fast growth (Abu Hena and Hishamuddin, 2014; Gamboa-Delgado, 2014; Porchas-Cornejo et al., 2011; Coman et al., 2003; Chen and Chen, 1992). Additionally, zooplankton also have a strong role as

herbivores, detritivores and micro-predators, and thus in maintaining pond ecosystem stability (Burford, 1997). However, the primary component of this zooplankton resource is crustacean, the copepods, and may present disease transmission risks. In a fully biosecure farm, influent seawater would receive treatment that completely removes the organisms that may be vectors of disease. But this works against balanced farm ecosystems and established uses of natural zooplankton blooms. The remediation of such heavily impacted seawater, which otherwise carries a range of beneficial microbiota, should therefore be an important research and development strategy that supports the most stringent biosecurity measures for influent seawater. Such remediation would be an added cost for farm management and could entail re-stocking the water with phytoplankton and zooplankton that have biosecure credentials. To date, full water disinfection has not been achieved by prawn farms in Australia and it remains to be seen how remediation of the seawater could be actioned, or even whether such methods are necessary.

In Season 1 of this study, when the filters had 80 µm mesh installed, the relative abundance of zooplankton in the RDF filtrate entering the farm seawater distribution system, compared with that in the raw source seawater, was unlikely to have greatly affected production pond bloom development. In Season 2, when the smaller 50 µm was used, the far greater rate of zooplankton exclusion, is considered to have had a significant impact on zooplankton bloom development prior to stocking post-larvae (Section 4.9). With no opportunity to compare ponds with and without filtration however the impact cannot be quantified. In both seasons, filtration was a critical control point for inflow rate, delaying pond filling and reducing the period for plankton bloom establishment. This work at GCMA has nevertheless provided demonstration that fine filtration of all influent seawater can service successful prawn production levels, and therefore be practical even for relatively large farms by Australian standards.

Healthy microbiomes

This project's survey of the influent water bacteriome is the first known attempt to catalogue the changes that occur as influent water progresses through the farm distribution and storage system to flow into the production ponds. Even in the absence of a chemical disinfection step, which would be a major disrupter of this microbiome, it can be expected that the water bacterial community will still change significantly from source water to pond entry, given the changed environmental conditions and residence time within the farm's distribution network. It is presently not known if these changes are positive, benign or negative in terms of promoting healthy productive pond environments.

Within the constraints of the present survey's operations, detailed information was generated with three instructive results for prawn farm bacteriome management:

1. Bacterial profiles changed dramatically as the water progressed through the farm, indicating that conditions within the farm are a strong driver for change and may overwhelm effects of influent seawater filtration.
2. Bacteriome diversity remained high throughout the seawater distribution system - high diversity is considered a critical characteristic of healthy aquatic systems.
3. No evidence was found for the selective advantage of genera that include pathogenic species, e.g. *Vibrio*, within the seawater distribution and storage system.

Reducing the risk of disease incursion by adopting heightened biosecurity processes, such as treatment of farm influent water, appears increasingly important for the future. But this is just one element that farms may need to adopt under heightened biosecurity threats. The inherent resistance of stock to disease, and the important role that a healthy pond microbiome plays in this, is another important element to be considered. New biosecurity processes should not compromise farm ecosystem health and stability. For example, a water treatment system that restricts availability of exchange water at critical times would be counterproductive. Results from this project indicate that RDFs are unlikely to impact the influent water microbiome in a severe manner, assuming mesh sizes no smaller than 40 µm are used. Chemical disinfection of the water, however, is potentially a major

disruptor of the natural microbiome and could therefore have more serious ramifications for establishing an optimal, productive microbial community in production ponds. In this instance remediation of the seawater may be necessary to restore vital functions.

Conclusion

This project has provided information and scope on parameters critical for the filtration of prawn farm influent seawater to reduce disease risk. The project combined robust experimental investigation of a range of filter mesh sizes with monitoring the impacts of a rotating drum filter system operated on a large commercial prawn farm. The results were placed into context with the perceived threats of viral pathogens that may threaten productivity of Australian prawn farms in the future.

Small, managed mesocosms were found to be a useful research approach to elucidate the true impacts of seawater treatment on plankton response and provided for direct comparisons of multiple factors not possible if investigations were restricted to monitoring outcomes on operational farms. Within the range of filter mesh sizes that may be considered by farms in the future, there is a large difference in proportions of different plankton groups retained by the mesh, and therefore excluded from the filtrate flowing into the farm. A 20 µm mesh size was shown in experimental work to exclude almost all metazoan plankton except small rotifers. However, 40 to 50 µm is presently the smallest mesh size considered practical for deployment of rotating drum filters on pond-based aquaculture farms.

A nominal mesh size as low as 40 µm was found to allow a significant proportion of crustacean nauplii to pass through. This permits founding populations of key plankton groups, particularly copepods, to establish in production ponds. However, the presence of crustaceans in the water entering the farm also means the biosecurity risk associated with crustacean vectors is not eliminated. It also means that barnacles and other potentially problematic small crustacean nauplii can be present in the filtered seawater, and hence may go on to colonise the farm.

Nominal mesh ratings provided by manufacturers are not a reliable indicator of filter performance for plankton, likely due to the flexibility of many organisms that permits their passage through comparatively small mesh sizes and retain viability. Mesh construction and geometry also has a significant effect on the maximum size of organisms that can pass through the mesh (Sections 3.1 and 4.4).

The farm-based work conducted at Gold Coast Marine Aquaculture's prawn farm during the 2018/19 and 2019/20 seasons provided commercial scale demonstration that influent water filtration can be a practical option for prawn farms to reduce the biosecurity risk associated with waterborne disease transmission. Rotating drum filtration systems were shown to significantly reduce the abundance of organisms in the high risk category for transmitting viral pathogens, particularly WSSV. In the absence of more detailed information on the transmission risks under different environmental scenarios, such risk assessments depend on assumptions of the potential for organisms to vector pathogens into the farm and instigate disease.

Implications

The outcomes from this project are directly relevant to marine pond farmers, particularly prawn farms who are considering improving biosecurity associated with using local environmental waters. Disease can have catastrophic impacts on production, as demonstrated by the WSD outbreak in Southeast Queensland and overseas. This research provides information to assist marine pond farmers to make sound decisions on actions to address emerging disease challenges.

Implementing influent water treatment on a prawn farm necessitates changes to farm management and is a substantial investment in initial capital and future operating. Getting the installation and operating parameters right the first time will reduce potential for costly malfunction and ensure the desired level of protection is achieved. Consistent disease risk management will lead to lower incidence of production losses and episodes of disruption to market supply.

Recommendations

Filtration is one of two common approaches to improving water biosecurity, the other being chemical disinfection. There was no opportunity to investigate different options for chemical disinfection within the current project. To have the highest level of influent water biosecurity it is recommended that a combination of filtration to remove the larger plankton and particulate material and chemical disinfection followed by extended settlement period to remove remaining disease vectors and potentially free virus from the water column (Aquavet 2013; Valeriano Corre et al. 2012; Park et al. 2004; Schuur 2003; Bratvold et al. 1999), depending on the disinfection process used. There is little local experience in farm scale disinfection of influent water and despite it being practised in prawn farms overseas there are a number of factors that require further information generated at the local level to ensure that treatment is effective, necessary permits can be attained and best practise guidelines are appropriate for Australian circumstances.

Prawn farmers considering implementing an RDF system for biosecurity purposes need to be aware that filter design and operation will have a large effect on the disease vector risk reduction achieved. In particular, project results indicate the following are critical considerations for maximising outcomes:

- Potential for filter drum bypass around flexible seals
- Resistance of filter mesh to mechanical damage
- Effectiveness of backwash system and its rate of duty cycle. The more often backwash is performed the better for filter performance
- Ability to easily inspect filter mesh integrity and quickly repair breaches
- A 40 to 50 µm mesh size is required to provide a high degree of exclusion of early life stage decapod species, considering that zooplankton with a minimum body dimension larger than the nominal filter aperture size can pass through and remain viable
- Only filter mesh sizes less than 40 µm have the capacity to exclude all crustacean life-stages
- At filter mesh sizes 40 µm and larger, copepods will form viable populations in production ponds, though the time taken to achieve abundances desirable at the time of stocking post-larvae will likely be extended
- Simple or plain weave mesh may provide a more preferable organism exclusion performance compared with weave designs that create more complicated aperture arrangements, such as twill weave

Regular collection of crustaceans found in and around farms and testing for WSSV, particularly for prawn farms on the Logan River, may provide ongoing feedback to farms on the virus load in the immediate area and a chance to respond to emerging risk before stock become symptomatic. It is recommended that filter residue be used for collection of material relevant to these tests. This water contains a range of organisms that are planktonic or only transient members of the plankton in high concentration. The mass of debris, consisting in large part of vegetative matter, is problematic for PCR based tests. Further refinement of sample processing in the laboratory could establish standardised protocols to ensure high reliability and sensitivity.

This project noted the presence of macro-crustaceans, shrimp and prawns, within the farm water distribution and storage system during and at the end of the production cycle. However, the dynamics of these species was not closely followed over the cycle and was not quantified. It is assumed that these species can enter the farm at an early life stage that can pass through 50 µm mesh and go on to colonise the waterways. As their presence represents a biosecurity risk there is value in undertaking on-farm investigation of their population dynamics with a view to implementing practical controls.

Epidemiological investigation of within-farm and environmental factors associated with the WSD outbreak that occurred in the Logan River region at the end of the 2019/20 production season would be useful to inform the industry of predictive conditions associated with periods of heightened risk. While two outbreak events may not provide for high predictive power, investigations over successive outbreaks, if they continue to recur, could provide the local industry with improved capacity to protect farms.

Extension and adoption

This project provided real-time feedback and technical detail surrounding the effectiveness of filtration as a biosecurity measure during the early implementation phase for the prawn farming industry. Information was provided to the participating farm through regular meetings, and other farms were consulted and provided with emerging results. This feedback from the first season's work advised GCMA on ways forward to enhance risk reduction measures in the future. Feedback from the second season's work provided further information and direction as this and other farms in the Logan River fortify their operations against WSSV.

A presentation summarizing the results from the 2018/19 season was given to the APFA Symposium in Brisbane on 31 July 2019, which generated healthy discussion in the following days and months with prominent farm managers. A presentation detailing the overall results from the project was also given at the APFA Webinar on 13 August 2020. This slide presentation was recorded and has been made available for access via the APFA website to provide a quick reference to the results in a simplified format.

In the lead up to the 2020/21 production season meetings with APFA and Biosecurity Queensland were undertaken that ensured both parties were presented with the findings of the project that were directly relevant to the decisions being made regarding biosecurity measures conditional to operation in the Logan River region.

This work also stimulated a series of DAF-funded research aimed at chemical treatments such as ozonation (DAF Strategic Reserve project – "A new approach to health and biosecurity for coastal aquaculture"), and alternative ways to rebuild healthy farm ecosystems following complete disinfection of pond influent (DAF Innovation project – "Biosecure domestication and outdoor pond culture of copepods"). This project has provided the cornerstone of work in the "Health and Biosecurity" research focus area identified as the highest priority for DAF RD&E Strategy 2020-2030, and results from this project's work will continue to inform future important biosecurity measures for the Australian prawn farming industry.

This report will be a comprehensive source of information for farms considering seawater treatment options, enabling informed decisions on these disease mitigation investments. Farmers and researchers will have ongoing access to it through a link on the APFA website and DAFs eResearch archive.

Glossary

Bacterioplankton	The bacterial component of the plankton.
Filter residue	Material retained by the filter to be discarded in the backwash.
Filtrate	Water that has passed through the filter mesh, i.e. the filtered water.
Microbiome	A collective term for all microscopic organisms in a particular environment. The microbiome of a prawn production pond includes zooplankton, phytoplankton, protists, bacteria, Archaea, fungi and viruses.
Microplankton	Phytoplankton and zooplankton in the size range 0.02 to 0.2 mm (20 to 200 µm).
Mesoplankton	Phytoplankton and zooplankton in the size range 0.2 to 2 mm (200 to 2000 µm).
Meroplankton	Aquatic organisms with both planktonic and benthic phases in their lifecycle. In crustaceans, typically the animal has planktonic larvae which settle to the substrate as it transitions to the adult form. Examples are penaeid prawns and barnacles.

Project materials developed

In addition to this report the project has two presentations originally delivered to APFA R&D Symposia in 2019 and 2020 that are accessible from the APFA website.

Appendix A. List of project participants

Name	Agency	Role
David Mann	DAF	Principal Investigator and leader of technical operations (Bribie Island Research Centre)
Paul Palmer	DAF	Co-investigator (Bribie Island Research Centre)
Stephen Wesche	DAF	Co-investigator (Biosecurity Queensland, Brisbane)
Tom Gallagher	DAF	Technical operations (Bribie Island Research Centre)
Hazra Thaggard	DAF	Molecular laboratory (Bribie Island Research Centre)
Diane Ouwerkerk	DAF	Molecular laboratory (EcoSciences Precinct, Brisbane)
Rosalind Gilbert	DAF	Molecular laboratory (EcoSciences Precinct, Brisbane)
Rebecca Ambrose	DAF	Molecular laboratory (EcoSciences Precinct, Brisbane)
David Mayer	DAF	Biometry (EcoSciences Precinct, Brisbane)
Kerri Chandra	DAF	Biometry (EcoSciences Precinct, Brisbane)
Noel Herbst Alistair Dick Daryl Herbst Joe Boontang	GCMA	Advisory and on-farm assistance

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Appendix C. Biosecurity Standard Operating Procedure (SOP) for project activities.

Queensland Government

BIO-SECURITY Standard Operating Procedure (SOP)

Working on commercial prawn farms

Farm intake-water treatment project (FRDC 2017-238)

Responsible Officers:	Position:	Phone:
1. David Mann	Project Investigator	3471 0923
2. Paul Palmer	Project Co-investigator	3471 0950
3. Tom Gallagher	Technician	3471 0935

1. Scope

Biosecurity is a set of measures designed to protect a specified area or zone from the introduction and spread of diseases within it.

It is the responsibility of staff undertaking project activities on commercial farms to ensure that they are aware of and adhere to all biosecurity procedures.

It is the responsibility of staff undertaking project activities on commercial farms to ensure that on-farm project operations do not affect the biosecurity status of farms or areas outside of the farms.

Biosecurity procedures outlined in this document are particularly directed at managing the threat posed by white spot syndrome virus (WSSV), but have relevance to all disease agents of concern to prawn farms.

2. Purpose

The purpose of this SOP is to:

- Reduce potential for infectious disease to be inadvertently introduced onto or spread within the prawn farms participating in the project.
- Reduce potential for infectious disease to be inadvertently transferred from a farm to other areas, including the Bribie Island Research Centre.

The procedures outlined particularly relate to project staff and project operations but may also be relevant to farm staff interacting with project staff, operations or equipment.

Compliance with this SOP will provide a high level of confidence that on-farm project operations do not adversely impact the biosecurity of the farms participating in the project and areas outside of those farms.

What are considered biosecurity risks?

- All aquatic animals and plants, or other aquatic organisms, present on the farm or being moved to or from the farm.
- Any water that has been in contact with or in close proximity to aquatic animals and plants. This includes seawater that has not been disinfected to an approved standard.
- Staff and equipment that have been in contact with animals, plants or seawater either on- or off- farm.

The risk profile for different biosecurity risk items varies but ALL biosecurity risks need to be managed appropriately. This document outlines the general procedures for achieving this.

3. Place and time

The biosecurity procedures in this document will apply to project staff, and farm staff when interacting with the project, and relate to accessing prawn farms, conducting project activities on farms and removing potentially contaminated materials from farms. The procedures also relate to biosecurity of staff outside of the farms before and after accessing the farms.

This Biosecurity SOP will apply to all activities related to the conduct of the 'Farm intake-water treatment' project (FRDC 2017-238). The project period is nominally between June 2018 and October 2019 but any activities that may occur outside of this will also be applicable.

4. Risks

Any disease agents known to be a potential risk to prawn aquaculture whether recorded in the farming area or not. White spot syndrome virus (WSSV) is considered to have the highest potential for devastating impact on farms.

Diseases that can be transmitted via water or materials of biological origin are the highest biosecurity risk. Project staff and equipment contacting either of these two potential vector types on farms are considered biosecurity risks. However, even without directly contacting farm water or biological materials when at the farm staff and equipment is considered to have incurred a biosecurity risk for transfer of disease agents.

Disease agent transmission within farms, e.g. between ponds, and between farms are considered risks to be controlled.

5. Procedures

i. Personnel

It is anticipated that there will only be three DAF project staff that undertake project activities on farms. All three have experience working on pond-based aquaculture farms and are familiar with biosecurity principles and practises. All project staff will be inducted into the biosecurity procedures that the project requires. Project staff will also liaise with farm staff regarding biosecurity procedures of project operation.

ii. Timing of farm visits

There are multiple commercial prawn farms participating in the project and movement between farms as well as movement between farms and the Bribie Island Research Centre (BIRC) by project staff will be controlled as per the following:

- Only one farm can be accessed on any given day by a project staffer.
- Farm visitation by a project staffer will have at least a full day between visits to different farms, that is, a period greater than 36 hours between visits.
- At least one shower and change of clothes will occur between visits to different farms.
- Project staff will avoid accessing or interacting with the prawn husbandry systems at BIRC during the period of engagement in the project. If this does occur for a compelling reason an intervening period of greater than 36 hours applies before visiting a prawn farm, as above.
- Project staff will not access any husbandry area at BIRC on the same day as visiting a farm. When there is no interaction of the project staffer with crustaceans or crustacean husbandry systems at BIRC, a farm and BIRC can be visited on consecutive days provided there is a change of clothing and shower in between.

It is acceptable for project staff to access the office area of BIRC prior to visiting a farm. Accessed BIRC areas in this instance will be restricted to the PC2 Laboratories/DAF offices block at the front of the site (i.e. not beyond the second electronic gate or outside of the main office complex internally. Staff should only access the BIRC office after being at a farm if there has been a change of clothing and disinfection and hygiene procedures detailed below have

been followed. It is anticipated that the latter instance may be required when samples need to be stored. Project samples will be stored at BIRC in a containment specifically designated for this project.

BIRC has implemented a biosecurity management system based on the segregation of the site into discrete biosecurity compartments. The potential for transfer of disease agents between compartments is greatly reduced by physical and procedural barriers. DAF staff are not involved in the prawn culture work that is carried out at BIRC and the work is housed in separate buildings and compartments to the work undertaken by DAF staff. This ensures there is a clear separation between staff undertaking this project's on-farm work and the prawn culture work at BIRC.

iii. Vehicle access to farms

Where practical vehicles used by project staff will not enter the primary area of farm activity, that is, any area in proximity to production ponds or other zone adjacent to impounded waters or frequented by farm activity. It is anticipated that only rarely, if ever, will a project vehicle be required to enter the farming area. It can be parked outside the entrance gate or in car parks identified for general public vehicles.

If it is necessary to move the vehicle onto the farm (with farm management approval), then vehicle disinfection will be undertaken prior to visitation of another farm or aquaculture facility. The primary disinfection method will be putting the car through a car wash facility that sprays detergent solution all over the car including the underside. Any accumulated mud will be removed with a pressure water jet. An intervening period of greater than 36 hours before visiting another farm will apply.

iv. Equipment and materials

Items of equipment used to carry out project activities on farms that come into contact with farm waters or biological material, for example plankton nets and buckets, will be used only within one farm and not carried to other farms. Each farm will be equipped with an exclusive set of equipment necessary for project activities.

All used equipment and materials will be kept in a clean, hygienic condition, stored in a protected area that allows the equipment to dry and reduces the likelihood of incidental contact with water, water spray or farm staff.

All equipment and materials initially brought on to the farm will be decontaminated prior to arrival. Sampling nets and minor items of equipment will be new. For previously used devices, for example water quality meters, disinfection of surfaces with alcohol and disinfectant bath solution for submersible components, coupled with dry-out period of at least 1 week, will be used to ensure effective hygiene control.

Equipment that will be in contact with multiple ponds within a farm will be cleaned and bath disinfected between uses. Micromesh screens and nets used for sampling plankton in intake channels and ponds will be cleaned with freshwater and submersed in a Virkon® or similar disinfectant bath for the recommended treatment time. For Virkon® Aquatic a minimum contact time of 10 minutes is considered effective.

A disinfectant bath in a covered plastic tub will be maintained at each farm. Currently Virkon® is recommended for disinfection but similarly effective disinfectant chemicals may be used. Disinfectant bath water will be discarded only after an extended treatment period has elapsed, likely hours to days, in a manner recommended by the farm operator that minimises the chance of it contacting farm waters or equipment.

Water and plankton sampling for an individual farm will start with the lowest risk area and finish with the highest risk area. This is likely to be starting with treated water sampling and finish with sampling of production ponds.

v. Personal clothing

Water proof boots, e.g. gum boots, will be provided for each of the project staff to be used exclusively within a single farm, i.e. each of the farms has boots for staff to be used only on that farm. Other forms of waterproof clothing protection will be considered for specific project tasks, e.g. apron used to minimise potential for wetting of clothing during some sampling tasks.

Project staff will ensure a complete change of clothing between visits to different farms and laundering of clothing (washing machine and drying) before re-use.

vi. Plankton and water samples

All plankton samples will be treated with a preservative to ensure potential infectious agents are inactivated before leaving the farm. Preservatives to be used are ethyl alcohol at 70% or more final concentration and formaldehyde at over 5% final concentration as buffered formalin solution.

Samples will only be processed in laboratories equipped to effectively manage containment of the samples and the health and safety risks posed by the preserved samples. At BIRC samples will be processed in a PC2 certified laboratory.

Water adhering to the outside of sample containers after sample collection will be washed and container surface treated with disinfectant.

vii. White spot syndrome virus detection

In the event of a WSSV positive result from any samples collected from prawn farms or in the immediate vicinity of those farms, including samples collected by the project, the farms, or as part of the white spot environmental surveillance program, project operations and biosecurity procedures will be immediately revised to incorporate risk management advice provided by Biosecurity Queensland. The project will comply with Biosecurity Queensland directions regarding accessing farms and any activities undertaken on or near farms.

6. Record keeping

Project staff will record events and activities undertaken on the prawn farms:

- Staff name/s
- Farm entry and exit time
- Log of activities undertaken on the farm, in particular, ponds and other water bodies that were interacted with and the nature of the interaction. This record to include time, pond name, activity carried out, e.g. algae and zooplankton sampling.
- Samples taken and transported from the farm, their destination and any subsequent transfer of the samples to other locations. All samples to have a unique identifying code.
- Any occurrence relevant to biosecurity or staff safety.

7. Authority

The project principle investigator, David Mann, will oversee and be responsible for implementation of project-based and facility-wide biosecurity requirements.

The Biosecurity Plan will be approved by the project stakeholders, including DAF (BIRC and Biosecurity Queensland), all participating prawn farms and FRDC.

8. Related Biosecurity SOPs

BIRC SOPs relevant to project staff activities at BIRC

- BIRC SOP1-001 - General Biosecurity

9. Definitions

SOP	Standard Operating Procedure
BIRC	Bribie Island Research Centre
BQ	Biosecurity Queensland
FRDC	Fisheries Research and Development Corporation
WSSV	White Spot Syndrome Virus

10. Project contacts:

Event	Action	Contact (Name, Org. & Position)	Phone No.
Biosecurity issue or any issue related to project operations	Contact project staff - Principle investigator	David Mann (DAF, BIRC)	3471 0923
	Alt. Project coinvestigators	Paul Palmer (DAF, BIRC)	3471 0950
		Stephen Wesche (DAF, Biosecurity Qld)	3087 8086
	Project technician	Tom Gallagher (DAF, BIRC)	3471 0935

11. Biosecurity Standard Operating Procedure Approval

Approved by:	
Signature:	
Date:	

Appendix D. Microbiome analysis of prawn farm supply water.

Authors:

Rosalind Gilbert, Rebecca Ambrose, Hazra Thaggard, Kerri Chandra and Diane Ouwerkerk

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Figure 15. Heatmap of highly abundant bacteria and archaeal classes identified in water collected from location GC02, on three different days (collection days 2, 4 and 18). Classes which were identified as being significantly different between each collection day using differential abundance analysis (ancom) are marked with a superscript (most abundant on collection day 2; ** most abundant on collection day 4). Scale bar coloured according to a measure of relative abundance, Feature frequency (log_{10}). 32*
Figure 16. Heatmap of highly abundant bacteria and archaeal classes identified in water collected from location GC06, on five different days (collection days 2, 4, 6, 18 and 19). Classes which were identified as being significantly different between each collection day using differential abundance analysis (ancom) are marked with a superscript (most abundant on collection day 2; ** most abundant on collection day 6; and *** most abundant on collection day 18; #most abundant on collection day 19). Scale bar coloured according to a measure of relative abundance, Feature frequency (log_{10}). 33*
*Figure 17. Heatmap of highly abundant bacteria and archaeal classes identified in water collected from location GC09, on four different days (collection days 2, 4, 6 and 19). Classes which were identified as being significantly different between each collection day using differential abundance analysis (ancom) are marked with a superscript (*most abundant on collection day 2; ** most abundant on collection day 4; and *** most abundant on collection day 6). Scale bar coloured according to a measure of relative abundance, Feature frequency (log_{10}). 34*
Figure 18. Comparison of numbers of microbial populations (Features) classified at genus level present at each of the sample collection locations (GC02, GC06 and GC09) from at least three different sample collection time-points (from either collection days 2, 4, 6, 18 or 19 dependant on where sample data was available). The proportions of microbial communities are compared using Venn diagrams, showing the number of Features classified to genus level (% of total number of Features identified at the location) either shared between respective locations, or found to be unique to each location. 35

1. Introduction

Microbial communities (microbiome) present in aquaculture ponds can fulfil a range of essential roles in the pond-farm ecosystem including nutrient recycling, degradation of organic matter and in shaping the metabolic capacity of their prawn host. Some can infect and cause disease in prawns and other microorganisms may protect against disease (Moriarty, 1997, Bentzon-Tilia et al 2016, Kumar et al., 2019). Disease outbreaks caused by pathogenic bacteria have long been identified as one of the most serious challenges in aquaculture (Meyer, 1991). The monitoring and manipulating of the microbiome in aquaculture environments has great potential, both in terms of assessing and improving water quality, improving the gut health of farmed animals, as well as the prevention of disease (Bentzon-Tilia et al 2016, Dittmann et al., 2017). During production, microbial communities develop into complex ecological networks that vary according to host filtering mechanisms, environmental parameters and pond management practices.

It is only since the development of high capacity DNA sequencing and bioinformatics technology that microbial communities can be comprehensively studied. Aquaculture systems have received some attention in this regard but there is still only rudimentary understanding of the bacterial community structure and the complex roles bacteria play in culture system health and productivity.

This project investigated the changes that occur to the seawater microbiome after it enters the farm and progresses through the distribution and storage system to supply the production ponds.

2. Methods

Microbial diversity profiling is a way of identifying the relative proportion of microorganisms present in a mixed microbial community, in this instance water samples obtained from different locations across an aquaculture farm.

Following extraction of DNA from water samples, the bacterial 16S ribosomal RNA gene (16S rRNA) was amplified using PCR. The 16S rRNA is a component of the 30S subunit of the bacterial ribosome. Ribosomes are tiny particles of RNA and associated proteins found in the cytoplasm of living cells and are involved in the synthesis of proteins.

The 16S rRNA gene sequences are commonly used to study bacterial phylogeny and taxonomy (Logares et al., 2012;Pollock et al., 2018). This is because the 16S rRNA gene is present in all prokaryotes (unicellular organisms) and has highly conserved and highly variable regions. The DNA sequences in the highly conserved regions allow targeting of the gene across different organisms, whilst the highly variable regions assist with classification into different species.

2.1 Microbial DNA extraction from water samples

Water samples for microbial diversity profiling were collected from three locations (GC02, GC06 and GC09) across an aquaculture farm (Figure 1). Samples were collected once a week from the 25th September 2018 to 27th November 2018 and then once a fortnight until the 23rd April 2019. Three individual samples were collected from each location at each time point.
Water samples were collected following a method that had proven effective in providing sufficient DNA recovery in pre-study testing of different methods. In brief, the water sample was initially passed through a 11 µm filter and then a 20 mL volume was added to 30 mL absolute ethanol in a 50 mL Falcon conical centrifuge tube (Corning, USA). Samples were kept on ice overnight and then stored at -20 °C until DNA extraction.

Extraction of DNA was performed using the DNeasy® PowerSoil® kit (Qiagen). Briefly, samples were centrifuged at 3,200 x *g* (6 °C) for one hour. The supernatant was discarded and 60 µL of solution C1 was added to the dissolve the pellet. The resulting suspension was added to the PowerBead tube

and DNA was extracted as per manufacturer's instructions and eluted in a final volume of 60 µL. As well as the individual samples, a pooled sample, consisting of 20 µL of DNA extract from each individual sample (x3 = 60 µL), was prepared for each location and time point.

To determine whether the extracted DNA contained enough bacterial DNA for subsequent microbial diversity sequencing, the bacterial 16S rRNA gene was PCR amplified using the following pair of primers: 27F (5'GAGTTTGATCCTGGCTCAG3') and 1525R (5'AAGGAGGTGWTCCARCC3') (Lane, 1991) following the amplification conditions of Ouwerkerk and Klieve (2001). Individual samples (three per location) and pooled samples were tested. Amplified products were run on a 1% agarose gel stained with Midori green (NIPPON Genetics, Japan) and visualised with a UV Transilluminator. A band of ~1,500 base pairs (bp) in size is expected for samples testing positive for the presence of amplifiable bacterial DNA.

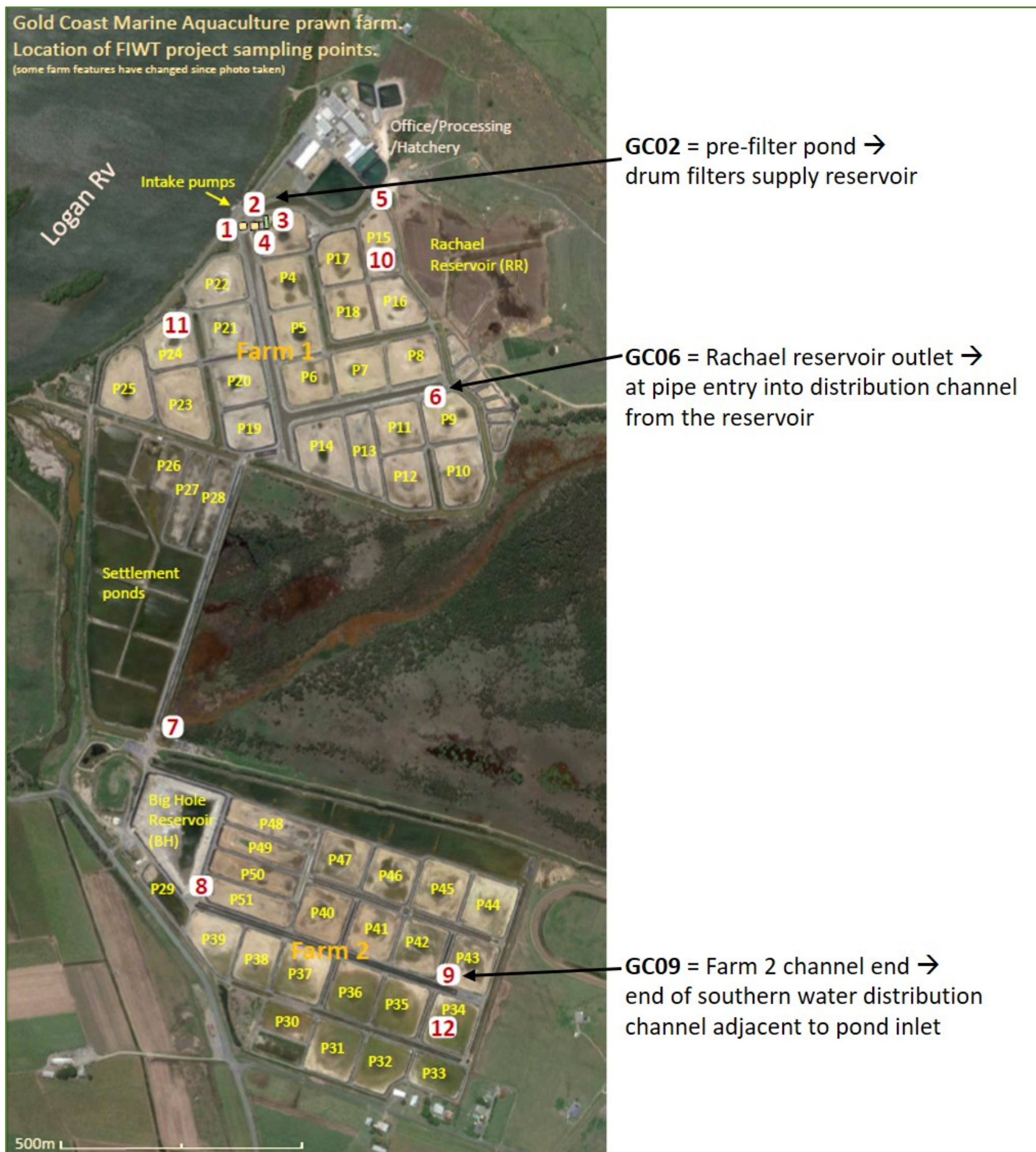


Figure 1. Map describing locations from which water samples were collected for microbiome analysis.

2.2 Sequencing and data storage

Microbial DNA, either from individual samples or microbial DNA pooled from three individual samples, were sent to the Australian Genome Research Facility (AGRF, St Lucia Qld) for microbial diversity sequencing. In brief, the submitted DNA samples were used as template in PCRs to prepare 16S rRNA gene barcoded amplicons of the 16S rRNA gene V3-V4 region using the forward primer 341F (5'-CCTAYGGGRBGCASCAG-3') and reverse primer 806R (5'- GGACTACNNGGGTATCTAAT-3') with overhang adapters. Individual sample indices (barcodes) and sequencing adapters were attached to the amplicons using the Nextera XT Index kit and the resulting barcoded amplicons normalised, pooled and sequenced using the Illumina MiSeq platform to obtain 300 bp paired end reads.
(https://static1.squarespace.com/static/5c6a2bfa11f7845bc7a99405/t/5df2d87aeeaf93748a1fa72e/1576710346786/SVG1908MPR_Service+Guide_Microbial+Profiling_print.pdf)

Sequencing data obtained from AGRF, consisting of .fastq files of forward and reverse paired end reads for each water sample were archived in a restricted access archive within the Queensland Government, *Department of Environmental Science, High Performance Computing facility (Apollo).*

2.3 Sequence analysis

Sequence data was analysed using the Quantitative Insights into Microbial Ecology (QIIME 2) software package (Version 2019.1) (Bokulich et al., 2018, Boylen et al., 2019). The raw sequence data obtained from AGRF (Forward and Reverse reads) was initially quality filtered and sequence reads less than 200 bp in length removed using Trimmomatic version 0.36 (Bolger et al., 2014). Sequences were then formatted for import into QIIME 2 and the DADA2 software used for modelling and correcting Illumina-sequenced amplicon errors (Callahan et al., 2016), the input sequences were further quality filtered, the forward and reverse reads merged, unique sequences (sequence variants) grouped, and chimeras removed. To analyse the microbial diversity the sequence data was sorted by sequence identity and a Feature table (the equivalent of the QIIME 1 Operational Taxonomic Unit (OTU) or Biological Observation Matrix (BIOM) tables) containing the counts (frequencies) of each unique sequence (Feature) present in each sample in the dataset, a representative sequences file and a FeatureData file which maps Feature identifiers in the Feature table to the sequences they represent, was created. A multiple sequence alignment using MAFFT v7 (Katoh and Standley, 2013) and a phylogenetic tree were created to relate the Features to one another and assign phylogenetic groups to the Feature table. Taxonomy was then assigned using a pre-trained Naïve Bayes classifier trained on the SILVA database (update 132, downloaded 20th February, 2019 from QIIME 2 Resources) (Yilmaz et al., 2014).

Feature tables were also filtered to retain only samples of interest, for example one Feature table contained all samples relating to a specific sample collection day (days 2 or 4 were the most complete datasets), whilst another Feature table contained samples from Farm sample collection locations GC02, GC06 and GC09 (Figure 1). This enabled more specific statistical analysis of metadata categories (for example, salinity, turbidity and total populations of zooplankton, dinoflagellates and cyanobacteria). To determine which populations were of high abundance in the water sample microbial community, the Feature table for each respective study was filtered to remove Features which were represented by only a limited number of sequences (low frequency). The sequences number used for filtering was determined using the third percentile threshold of frequency (presented in the QIIME 2 FeatureTable[Frequency] file) and was specific for each dataset.

2.4 Statistical analysis

For each study, alpha diversity measures (microbial diversity within a sample) and beta diversity measures (differences in diversity between samples), were calculated using QIIME 2 software. Alpha diversity analysis (microbial diversity within a sample) was determined on the basis of three measures: (1) counts of observed species (Observed Species); (2) Phylogenetic Diversity (PD); and (3) Shannon entropy of counts (Shannon). The three alpha diversity measures were analysed using a linear regression with collection day as the explanatory variable and grouped by location (GC02, GC06 and GC09) and sample type (pooled DNA sample or average of 2 or 3 individual samples). All regression and Analysis of Variance (ANOVA) were generated using GenStat v16 (VSN International, 2017).

Beta diversity measures (differences in diversity between samples), were calculated using the Unweighted and Weighted UniFrac tests within QIIME 2. Metadata was grouped into categories in order to minimise the number of categories used in statistical tests to evaluate the contribution of abiotic and biotic metadata (Table 1) and sampling factors (e.g. time, location, type) to the variation in community composition.

Table 21. Metadata groupings of on-farm measurements, such as Temperature and Salinity, as well as biological parameters, such as Total Zooplankton and Diatom counts.

Temperature max. (°C)		Salinity (ppt)	
20-25	Low	35.5-42.2	Low
25.1-30	Medium	42.3-49.0	Medium
30.1+	High	49.1-55.7	High
Rain (mm)		**Total Zooplankton (#/kL)**	
0-4.7	Low	>10^{3}->10^{4}	Low
4.8-9.5	Medium	>10^{4}->10^{5}	Medium
9.6+	High	>10^{5}->10^{6}	High
Wind speed (km/hr)		**Total Diatoms (#/L)**	
20-39	Low	>10^{3}->10^{4}	Low
40-59	Medium	>10^{4}->10^{5}	Medium
60+	High	>10^{5}->10^{6}	High
Water temperature (°C)		**Total Dinoflagellates (#/L)**	
21.2-24	Low	>10^{2}->10^{3}	vLow
24.1-26.8	Medium	>10^{3}->10^{4}	Low
26.9+	High	>10^{4}->10^{5}	Medium
		>10^{5}->10^{6}	High
Turbidity		**Total Cyanobacteria (#/L)**	
28-46	Low	>10^{0}->10^{2}	vLow
47-83	Medium	>10^{2}->10^{3}	Low
83+	High	>10^{3}->10^{4}	Medium
		>10^{4}->10^{5}	High

Additional non-parametric tests were undertaken to determine the special distribution of microbial communities, using the distance matrixes calculated by the Unweighted and Weighted UniFrac analysis, including: (1) Analysis of composition of microbes (ANCOM) (Mandal et al., 2015), a method for comparing the composition of microbes in two or more populations, with no assumptions of distribution and scaling to account for taxonomically diverse samples; and (2) Permutational multivariate analysis of variance (PERMANOVA), a distribution-free method of multivariate data analysis (Anderson, 2001), with significant differences calculated on the basis or $P < 0.05$. On occasion, to further validate initial indications of strongly significant effects, PERMANOVA was also calculated for the additional distance matrixes calculated using the Bray Curtis and Jaccard tests within QIIME 2. As most diversity metrics are sensitive to different sampling depths across different samples, for all beta diversity measures, the minimum sequence sample depth was used as a measure for even sampling (rarefaction) depth.

For the generation of Venn diagrams to visualise the comparison of microbial communities (for example, between locations), the on-line tool Venny (https://bioinfogp.cnb.csic.es/tools/venny/), or for coloured Venn diagrams, the online tool BioVenn (http://www.biovenn.nl/index.php) was used (Oliveros, J.C., 2007-2015; Hulsen et al., 2008).

3. Results

3.1 Preparation of Microbial DNA from water samples and sequencing data summary

Following DNA extraction from the 189 water samples, the DNA was used as template in a universal 16S rRNA gene PCR. This assisted in determining whether the samples contained adequate, amplifiable DNA for microbial diversity profiling. After PCR amplification, 81% of samples (individual and pooled) had no band or produced only a very faint band on agarose gel electrophoresis, indicating a very low concentration of bacterial DNA. These were considered poor candidates for sequencing being likely to fail completely or generate a low number of sequence reads.

Ultimately, 42 samples, including six negative controls, were selected to send as DNA to AGRF for bacterial diversity sequencing. These samples represented six sampling days in which 16S rRNA gene amplicons were able to be generated by PCR from one or more samples at each of the collection locations. Some of the samples submitted were only weakly positive via PCR, but it was decided to proceed with sequence submission to try obtaining a complete dataset for these days. There was the possibility that these samples could pass the AGRF sequencing laboratory's quality control (QC), based on PCR and indexing Fluorometry.

Following QC at AGRF, 14 samples, including the six negative controls, did not meet the minimum requirement of 0.20 ng/µL of usable PCR product to generate a sequencing output guarantee of 10,000 raw reads. The DNA concentration of samples (excluding negative controls) ranged from 0 – 1.16 ng/µL with a mean of 0.70 ng/µL (median 0.89 ng/µL, SD 0.40 ng/µL).

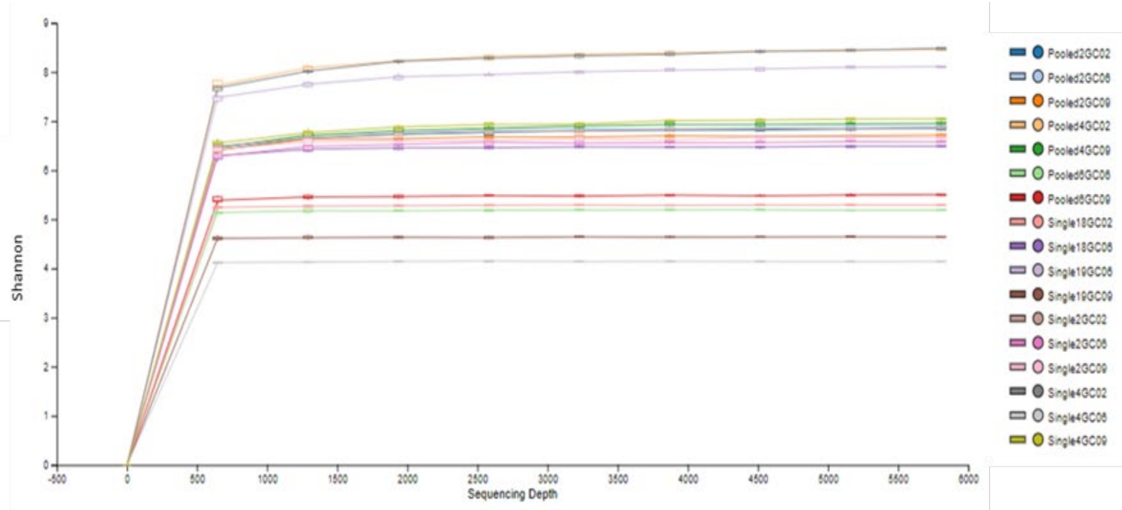
A total of 2,597,350 reads were obtained with a mean of 61,842 reads per sample (standard deviation 56,210; median 58,072; range 515-177,431). When the negative controls were removed from the data set, a mean of 71,646 reads per sample was obtained (standard deviation 54,852; median 63,966; range 515-177,431).

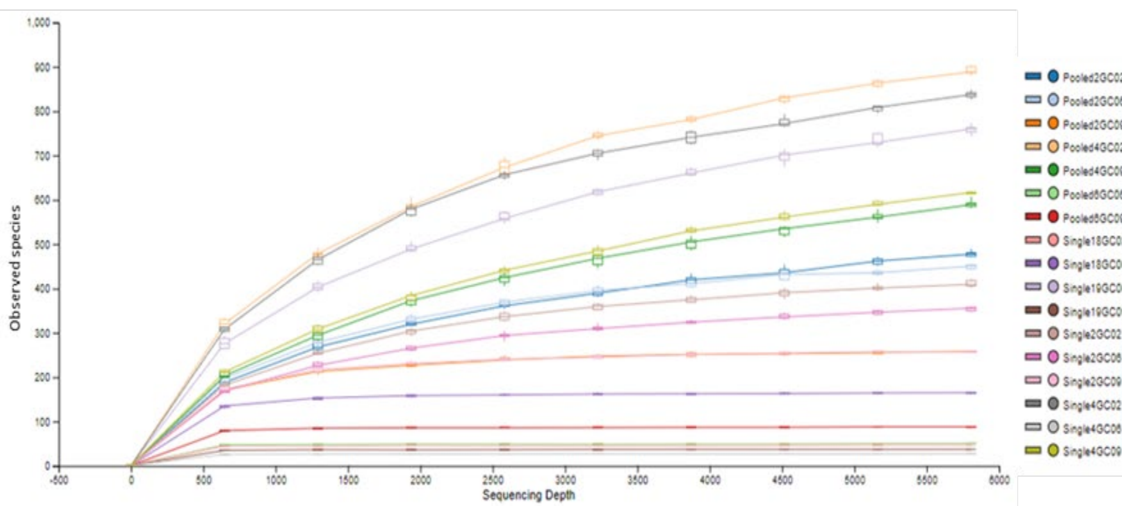
3.2 Microbial diversity (alpha diversity analysis)

For every water sample, the microbial population diversity within each sample (alpha diversity) was calculated using three alpha diversity measures (Faith Phylogenetic Diversity, Shannon and Observed

species; Figure 2.). Statistical analysis of these diversity measures on the basis of the type of sample (pooled or individual sample), the sample collection day (collection days 2, 4, 6, 18 and 19) and the location from which samples were collected (GC02, GC06 and GC09) indicated that all samples had an extensive microbial diversity. Using linear regression, interactions between collection day, sample collection location and the type of sample (pooled DNA or individual sample DNA) were all found to be non-significant ($P > 0.05$) (Table 2). Similarly, ANOVA of the most complete datasets obtained on sample collection days 2 and 4 (Table 3), showed no significant effects of sample collection day, location and sample type (pooled DNA and individual DNA sample) on the diversity within the water samples, as determined using the three diversity measures.

None of the sampling factors were statistically significant which indicates a null model so the data can be summarised by the mean diversity across the dataset (Table 4). This also shows that there was considerable variation in the microbial diversity found in specific sample groups as indicated by the relatively large standard deviations obtained for each diversity measure, for example, when considering all samples collected on the same day, from a specific location.





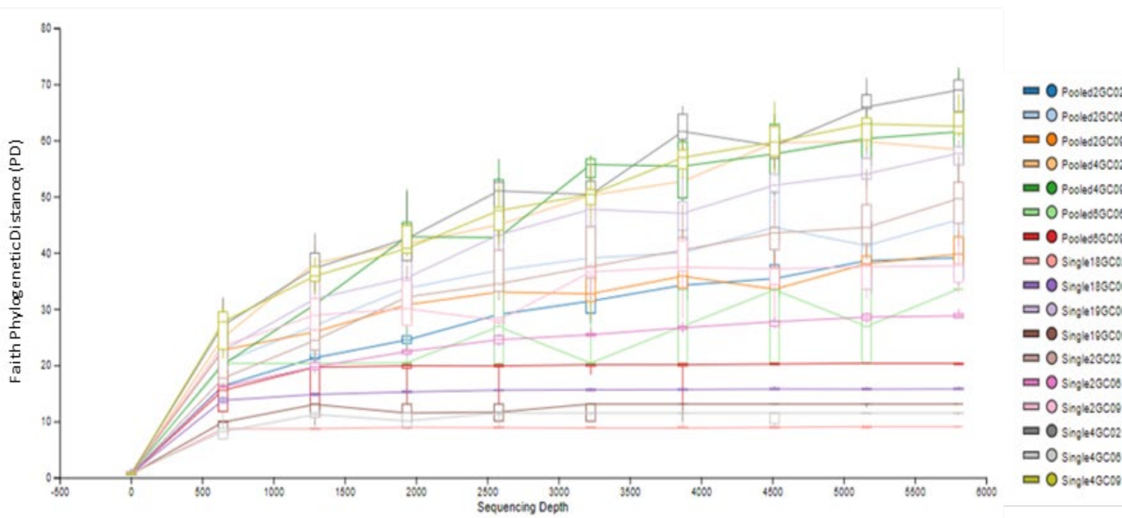


Figure 2. Statistical analysis of the diversity of individual samples, grouped according to the type of sample (pooled or individual sample), the sample collection day (collection days 2, 4, 6, 18 and 19) and the location from which samples were collected (GC02, GC06 and GC09). Results are presented for each alpha diversity measure (a) Shannon, (b) Observed species and (c) Faith Phylogenetic Distance (PD), with a minimum sampling depth of 5,807 sequences.

Table 22. Results from linear regression *analysis (accumulated analysis of variance) of microbial diversity within each sample (alpha diversity).*

Fixed effect	Alpha Diversity Measures (F Pr-values)		
	Faith PD	*Shannon*	*Observed species*
Collection day	0.097	0.378	0.301
Location	0.735	0.523	0.505
Sample type	0.943	0.985	0.894
Collection day + Location + Sample type	0.459	0.701	0.632

Table 23. ANOVA of samples from sample collection days 2 and 4 only.

Change	Degrees of freedom	Alpha Diversity Measures (F Pr-values)		
		Faith PD	*Shannon*	*Observed species*
Collection day	1	0.246	0.798	0.176
Location	2	0.122	0.218	0.220
Sample type	1	0.596	0.618	0.564

Table 24. Summary of microbial diversity (alpha diversity) measures from samples across the entire dataset.

Alpha diversity measure	Mean	Standard deviation
Observed species	380.1	287.6
Shannon	6.536	1.22
Faith PD	39.84	20.4

3.3 Microbial taxonomic summaries and beta-diversity analysis

3.3.1 Overview

The taxonomic composition of the bacterial and archaeal communities was determined for samples collected from three locations and for at least two collection time-points over a 31 week period. Due to the low concentrations of microbial DNA extracted from samples collected throughout the duration of the trial, the bacterial and archaeal communities present in many of the 189 farm water samples and 63 negative control samples could not be accurately determined. The six negative control samples selected and sent for analysis returned very low, unusable numbers of DNA sequences and did not contain sequences representative of those usually found in marine water samples. This indicated that the methodology used for DNA extraction, PCR and sequencing did not introduce contaminants into the final sequence dataset and they could be regarded as true negative controls and were removed from further sequence analysis. From the remaining sequence dataset, several samples sent for sequencing were removed on the basis of the very low sequence numbers obtained and a total of 28 samples were retained for further analysis.

The relative abundance of taxonomic groups was ascertained following clustering of the sequences (V3-V4 region of the 16S rRNA gene) from each sample into highly related groups termed Features (sequence variants), and a representative sequence for each Feature compared to a database of 16S rRNA gene sequences of taxonomically defined microbial groups (SILVA database update 132 (Yilmaz et al., 2014)). Taxonomy was assigned on the basis of 99% sequence homology and if the Feature sequence did not match any of the reference sequences in the database, then it was classified as either unassigned or classified to the highest taxonomic level possible, for example, Kingdom Bacteria.

When the negative control samples and low sequence number samples were removed, a total of 7,578 Features were identified across the 28 pooled and individual water samples analysed, representing a total of 1,366,386 16S rRNA gene sequences. When the dataset was normalised according to the minimum sequence depth obtained per sample (5,807 sequences), alpha rarefaction indicated there was a mean of 479 observed species identified per water sample. The final dataset of identified microbial Features (referred to as a Feature table) was adjusted to retain only Features of relatively high abundance (frequency > third percentile). The most abundant phyla classified across samples analysed from the farm were Proteobacteria (59%), Bacteroidetes (23%), Cyanobacteria ranging from (7%) and Actinobacteria (4%) (Figure 3). Further classification at lower levels of taxonomic classification (Figure 4) revealed the highly diverse nature of the microbial populations present at different locations and at different sample collection time points. When examined at the genus level of taxonomy at all time-points of sample collection, the microbial community present at Location GC09 had the highest level of genera (43%) present only at that site whilst the other two sites, GC02 and GC06, had 35% and 15% of genera unique to each location respectively. The number of genera shared between each sample collection location is contained within Figure 5.

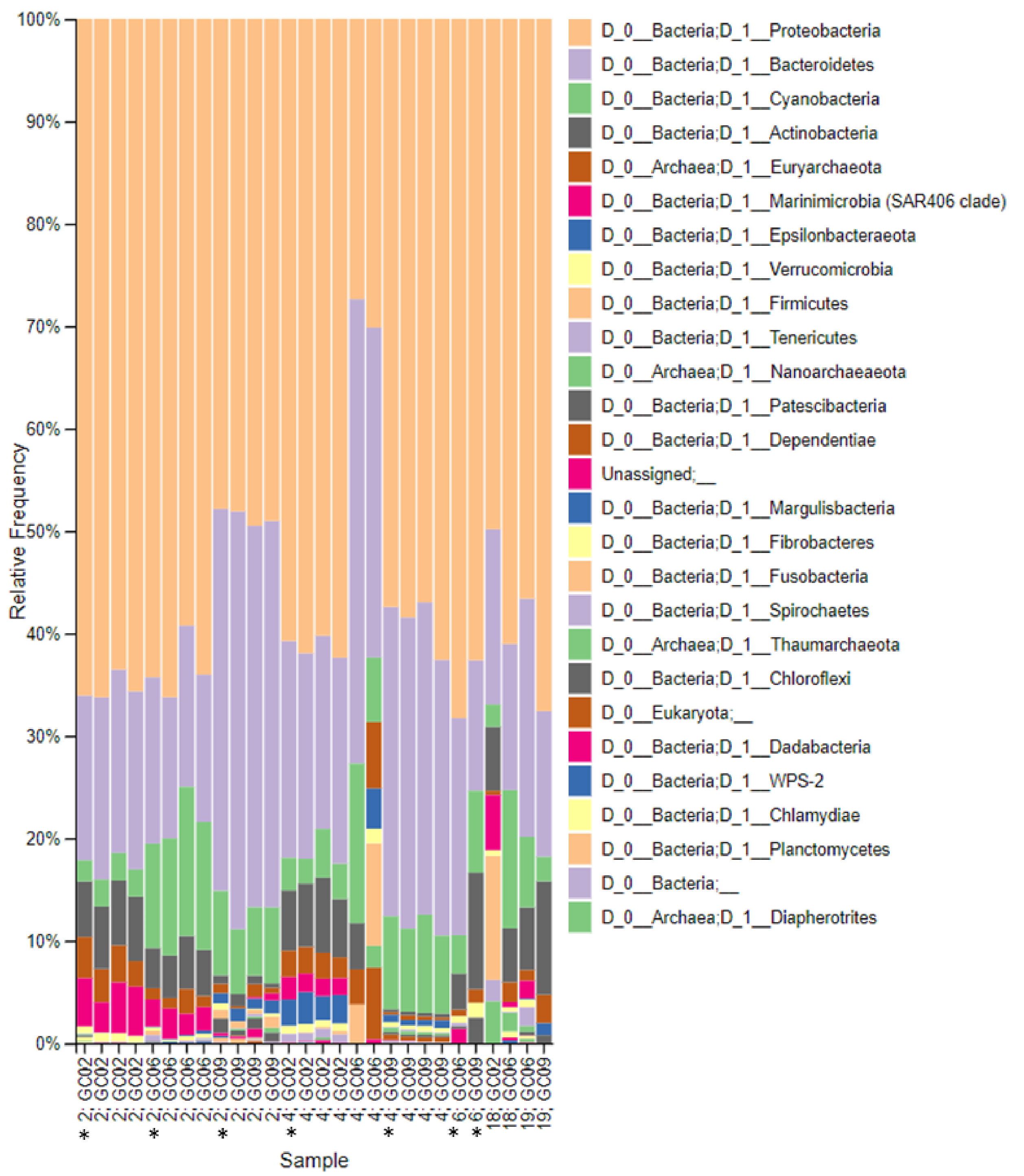
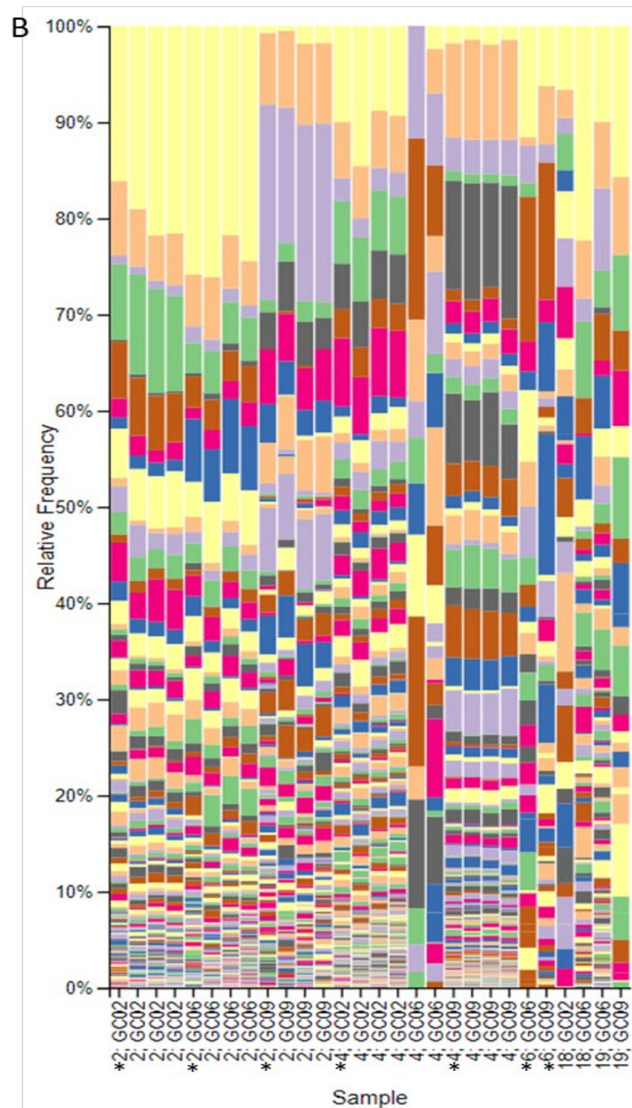


Figure 3. Stacked bar graph of highly abundant bacterial and archaeal populations (Features) classified at phylum level with 28 samples sorted according to collection days (Collection days 2, 4, 6, 18 and 19) and sampling locations (GC02, GC06 and GC09). Pooled DNA samples are marked with an asterisk.



D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__SAR11 clade;D_4__Clade I;D_5__Clade Ia
D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodobacterales;D_4__Rhodobacteraceae;D_5__HIMB11
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Cryomorphaceae;D_5__uncultured
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Flavobacteriaceae;D_5__NS5 marine group
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Oceanospirillales;D_4__Nitrincolaceae;D_5__Marinobacterium
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__SAR86 clade;__;__
D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodobacterales;D_4__Rhodobacteraceae;__
D_0__Bacteria;D_1__Cyanobacteria;D_2__Oxyphotobacteria;D_3__Chloroplast;__;__
D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhodospirillales;D_4__AEGEAN-169 marine group;__
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Flavobacteriaceae;D_5__uncultured
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Cellvibrionales;D_4__Halieaceae;D_5__OM60(NOR5) clade
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Methylophilaceae;D_5__OM43 clade
D_0__Bacteria;D_1__Cyanobacteria;D_2__Oxyphotobacteria;D_3__Chloroplast;D_4__Aureococcus anophagefferens;D_5__Aureococcus anophagefferens
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Oceanospirillales;D_4__Litoricolaceae;D_5__Litoricola
D_0__Bacteria;D_1__Marinimicrobia (SAR406 clade);D_2__marine metagenome;D_3__marine metagenome;D_4__marine metagenome;D_5__marine metagenome
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Flavobacteriaceae;D_5__NS4 marine group
D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Puniceispirillales;D_4__SAR116 clade;__
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__Flavobacteriaceae;D_5__Formosa
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Alteromonadales;D_4__Alteromonadaceae;D_5__Glaciecola
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Sphingobacteriales;D_4__NS11-12 marine group;D_5__uncultured Bacteroidetes bacterium
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Flavobacteriales;D_4__NS9 marine group;__

Figure 4. Stacked bar graph of highly abundant bacterial and archaeal populations (Features) classified at genus level with samples sorted according to collection days (Collection days 2, 4, 6, 18 and 19) and sampling locations (GC02, GC06 and GC09). Pooled DNA samples are marked with an asterisk.

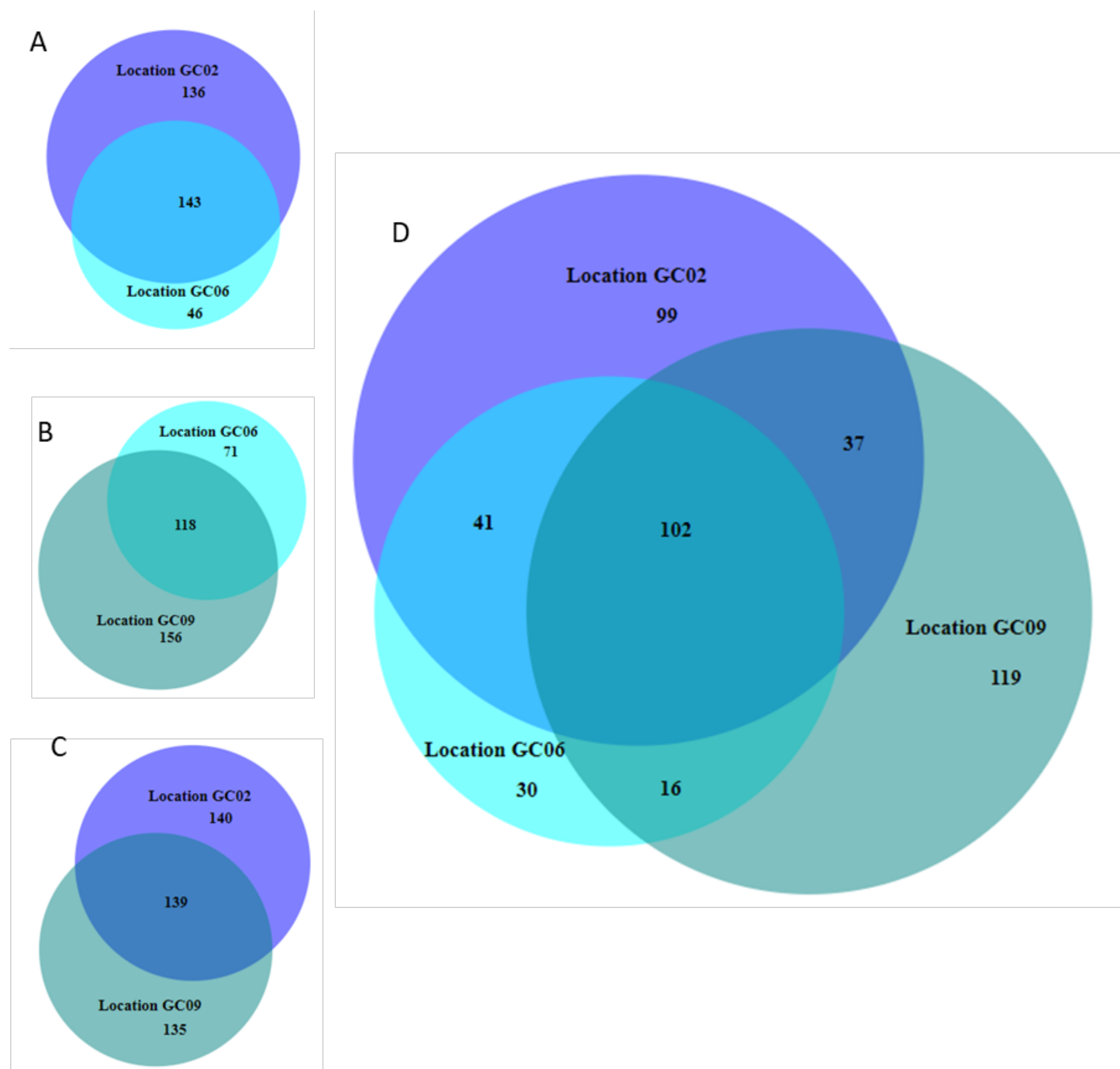


Figure 5. Venn diagram comparison of the numbers of highly abundant populations (Features), classified at genus level, found to be either shared between each location: (A) GC02 and GC06; (B) GC06 and GC09 and (C) GC02 and GC09, or (D) found to be unique to each location at all time-points of sample collection (collection days 2, 4, 6, 18 and 19).

Comparative analysis (Principal Co-ordinates analysis) of the relative composition of the microbial community undertaken with two qualitative measures based on community dissimilarity (Unweighted UniFrac and Jaccard) and two qualitative measures describing the relative abundance of microbes (Bray Curtis and Weighted UniFrac) all showed differences in microbial communities occurring with time and location (Figure 6 and Figure 7). PERMANOVA performed on four beta diversity metrics revealed significant overriding effects of time (collection day) and location on the bacterial community composition (Table 5). Considering this result, together with gaps in the overall dataset from problematic DNA extractions, low sequence numbers, and missed collection times and locations, a decision was made to sub-divide the data to enable the inclusion of replicate samples for more robust statistical analysis. Five data sub-divisions were identified: two being collection days 2 and 4, with each of these days incorporating a near compete set of sample locations; and three being each of the locations: GC02 (pre-filter pond, nearest to Logan River supply pumps); GC06 (Rachael reservoir outlet, pipe entry into distribution channel, Farm 1); and GC09 (channel end of southern water distribution channel adjacent to pond inlet, Farm 2).

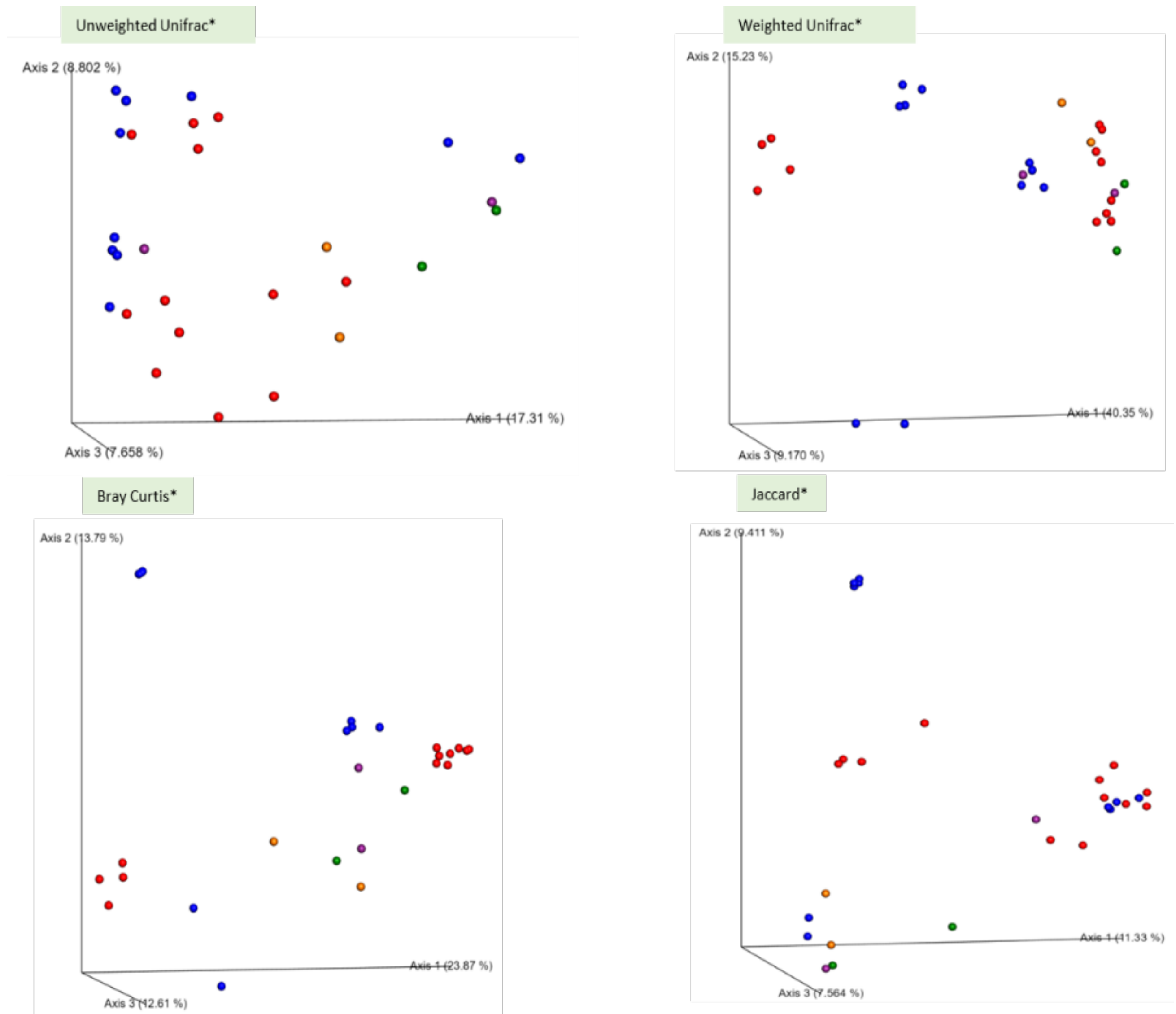


Figure 6. Changes in microbial communities with time: differences in bacterial and archaeal communities at each sample collection day (● day 2, ● day 4, ● day 6, ● day 18 and ● day 19). Principal co-ordinates analysis (PCoA) on the basis of the Unweighted Unifrac, Weighted Unifrac, Bray Curtis and Jaccard measures of dissimilarity is shown. Diversity measures found to be significantly different on the basis of time (collection day) are indicated with an asterisk and highlighted in green.

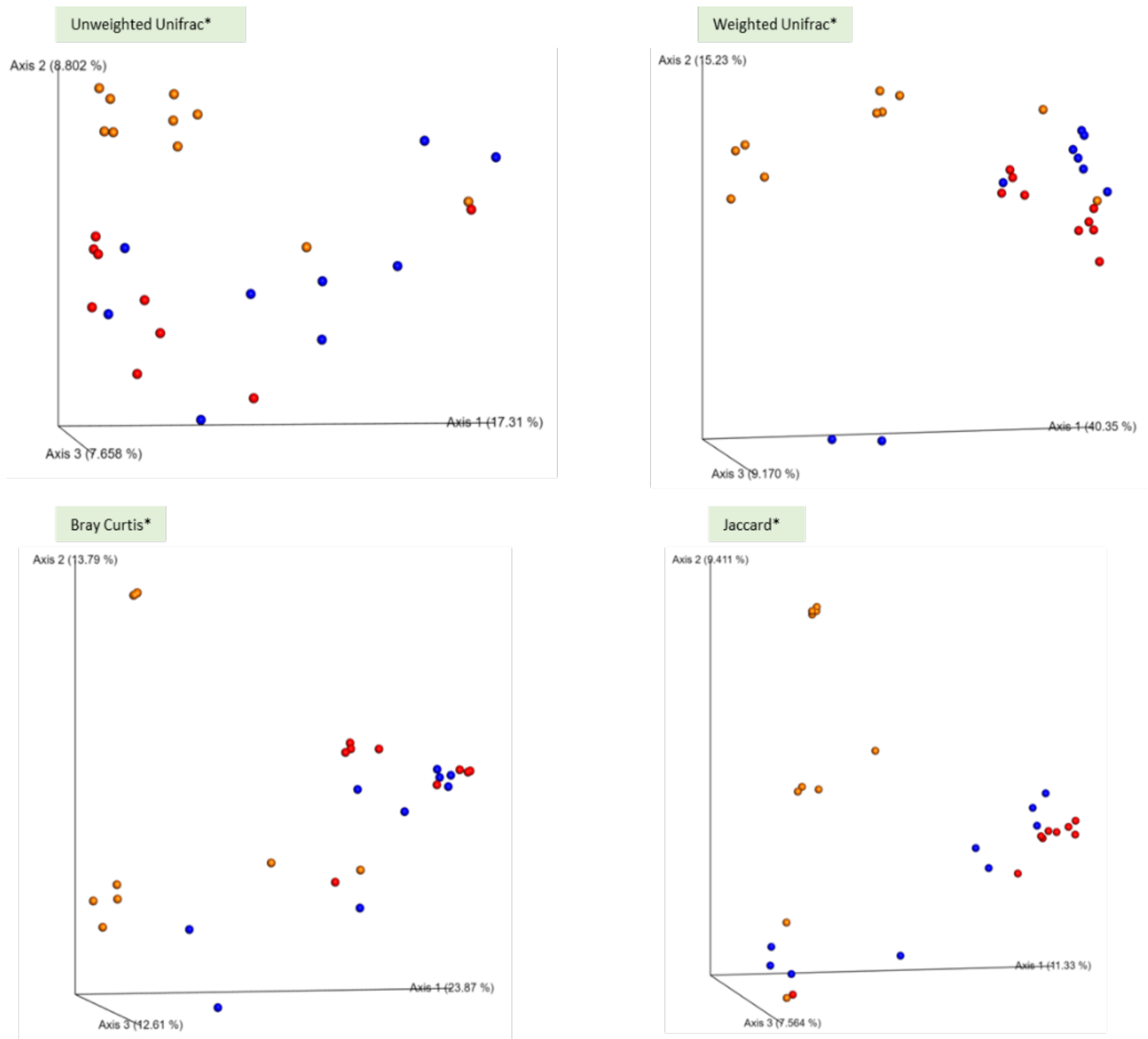


Figure 7. Changes in microbial communities according to location at which samples were collected: differences in bacterial and archaeal communities at each location (● GC02, ● GC06, ● GC09). Principal co-ordinates analysis (PCoA) on the basis of the Unweighted Unifrac, Weighted Unifrac, Bray Curtis and Jaccard measures of dissimilarity is shown. Diversity measures found to be significantly different on the basis of location are indicated with an asterisk and highlighted in green.

Table 25. Significantly different experimental factors (metadata categories) from analysis of the total dataset (28 samples, including pooled and individual samples) showing the over-riding effects of time (Collection day) and location from which samples were collected (Location). Data was either grouped to reduce the number of categories examined or analysed as actual data (numerical values treated as categories). Results presented include four measures of beta-diversity (Unweighted and Weighted Unifrac, Bray-curtis and Jaccard), including P values generated from PERMANOVA analysis.

			Beta diversity measures P values			
Grouped or actual data	**Metadata Category***	**No. of categories**	**Unweighted Unifrac**	**Weighted Unifrac**	**Bray-curtis**	**Jaccard**
Grouped	Collection day*	5	0.001	0.045	0.001	0.001
Grouped	Location	3	0.004	0.001	0.001	0.001
Grouped	Location.collection.day	12	0.001	0.001	0.001	0.001
Grouped	Rain	2	0.013	0.031	0.003	0.001
Grouped	Salinity	3	0.049	0.253	0.002	0.001
Grouped	Sample type	2	0.218	0.623	0.974	0.983
Grouped	Sample type.day	7	0.003	0.267	0.011	0.001
Grouped	Sample type.day.location	17	0.001	0.001	0.001	0.001
Grouped	Temperature Maximum	3	0.041	0.304	0.117	0.028
Grouped	Total Cyanobacteria	2	0.036	0.088	0.001	0.001
Grouped	Total Diatoms	3	0.026	0.008	0.001	0.001
Grouped	Total Dinoflagellates	4	0.001	0.001	0.001	0.001
Grouped	Total Zooplankton	3	0.021	0.022	0.004	0.001
Grouped	Water turbidity	3	0.001	0.005	0.002	0.001
Grouped	Water temperature	3	0.007	0.037	0.014	0.003
Grouped	Wind speed	3	0.005	0.03	0.001	0.001
Actual	Temperature Maximum	5	0.002	0.038	0.001	0.001
Actual	Rain	4	0.001	0.022	0.001	0.001
Actual	Wind speed	5	0.001	0.046	0.001	0.001
Actual	Water Temperature	12	0.001	0.001	0.001	0.001
Actual	Turbidity	4	0.009	0.016	0.001	0.001
Actual	Salinity	12	0.001	0.001	0.001	0.001
Actual	Total Zooplankton	12	0.001	0.001	0.001	0.001
Actual	Total Diatoms	12	0.001	0.001	0.001	0.001
Actual	Total Dinoflagellates	12	0.001	0.001	0.001	0.001
Actual	Total Cyanobacteria	10	0.001	0.001	0.001	0.001

* Grouped metadata categories: Collection day (samples collected on days 2, 4, 6, 18 and 19); Location (GC02, GC06 and GC09); Other categories applied to environmental and biological parameters are listed in Table 1.

3.3.2 Differences in microbial populations according to Sample Collection Day

Sub-division of the dataset enabled a more accurate assessment of the differences occurring between the samples used in the study i.e. pooled samples of microbial DNA or individual samples of microbial DNA. On each of the two collection days studied in further detail (days 2 and 4), there were no significant differences in the microbial communities occurring between the pooled and individual samples obtained for specific locations (Table 6, Table 7, Figure 8). This analysis therefore validated the approach whereby pooled DNA samples were used as an alternative to DNA extracted from individual samples, with both the pooled and individual samples providing a similar assessment of the bacterial and archaeal populations.

In the analysis of the microbial community present in samples collected on the same day, the sample collection location had the biggest effect on the populations with separation of sample by location visualised using PCoA analysis with each of four diversity measures tested showing similar results (Figure 12and Figure 13). PERMANOVA analysis also verified that in samples collected on the same day, the sampling location was strongly significant (P <0.05) (Table 6 and Table 7) across all four Beta diversity measures (Unweighted UniFrac, Weighted UniFrac, Bray-Curtis, Jaccard) demonstrating that each location had microbial communities different to the other two locations.

Examining the microbial communities at the taxonomic level of genus using Venn analysis found that in water samples collected on a single occasion (day 2) there were 112 genera present across all three sample locations (Figure 15). Location GC09 had the most diverse and unique community structure with 28% of the genera present found only at that location and only 58% and 69% of its genera also present at Locations GC02 and GC06 respectively. The community present at Location

Table 26. Significantly different experimental factors (metadata categories) from analysis of Day 2 showing the over-riding effects of the locations from which the samples were collected (Location). Data was either grouped to reduce the number of categories examined or analysed as actual data (numerical values treated as categories). Results presented include four measures of beta-diversity (Unweighted and Weighted Unifrac, Bray-curtis and Jaccard), including P values generated from PERMANOVA analysis.

			Beta diversity measures P values			
Grouped or actual data	**Metadata Category***	**No. of categories**	**Unweighted Unifrac**	**Weighted Unifrac**	**Bray-curtis**	**Jaccard**
Grouped	Sample type	2	0.997	0.977	0.93	0.892
Grouped	Location	3	0.007	0.001	0.001	0.001
Grouped	Turbidity	2	0.098	0.068	0.074	0.023
Grouped	Location.Turbidity	3	0.004	0.001	0.001	0.001
Grouped	Total Zooplankton	2	0.096	0.066	0.075	0.028
Grouped	Location.Total Zooplankton	3	0.003	0.001	0.002	0.001
Grouped	Total Diatoms	3	0.003	0.001	0.001	0.002
Grouped	Location.Total Diatoms	3	0.002	0.001	0.001	0.001
Grouped	Total Dinoflagellates	2	0.102	0.001	0.058	0.033
Grouped	Location.Total.Dinoflagellates	3	0.002	0.001	0.001	0.001
Actual	Salinity	3	0.005	0.001	0.001	0.001
Actual	Turbidity	2	0.088	0.072	0.061	0.027
Actual	Water temperature	3	0.004	0.001	0.001	0.001
Actual	Total Zooplankton	3	0.007	0.001	0.001	0.001
Actual	Total Diatoms	3	0.007	0.002	0.001	0.001
Actual	Total Dinoflagellates	3	0.003	0.001	0.001	0.001
Actual	Total Cyanobacteria	2	0.086	0.063	0.061	0.03

* Metadata categories applied to environmental and biological parameters are listed in Table 1.

Table 27. Significantly different experimental factors (metadata categories) from analysis of Day 4 showing the over-riding effects of the locations from which the samples were collected (Location). Data was either grouped to reduce the number of categories examined or analysed as actual data (numerical values treated as categories). Results presented include four measures of beta-diversity (Unweighted and Weighted Unifrac, Bray-curtis and Jaccard), including P values generated from PERMANOVA analysis.

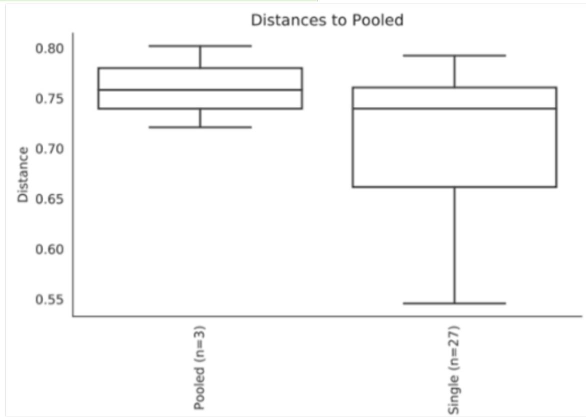
			Beta diversity measures P values			
Grouped or actual data	**Metadata Category***	**No. of categories**	**Unweighted Unifrac**	**Weighted Unifrac**	**Bray-curtis**	**Jaccard**
Grouped	Sample type	2	0.913	0.725	0.695	0.847
Grouped	Location	3	0.002	0.001	0.002	0.002
Grouped	Turbidity	2	0.004	0.01	0.005	0.01
Grouped	Location.Turbidity	3	0.001	0.001	0.003	0.001
Grouped	Total Diatoms	2	0.008	0.001	0.012	0.009
Grouped	Location.Total Diatoms	3	0.002	0.002	0.001	0.002
Grouped	Total Dinoflagellates	3	0.001	0.002	0.001	0.001
Grouped	Location.Total.Dinoflagellates	3	0.001	0.003	0.001	0.001
Actual	Salinity	3	0.004	0.001	0.001	0.002
Actual	Turbidity	3	0.007	0.014	0.004	0.011
Actual	Water temperature	3	0.002	0.002	0.001	0.001
Actual	Total Zooplankton	3	0.001	0.001	0.002	0.003
Actual	Total Diatoms	3	0.001	0.002	0.001	0.002
Actual	Total Dinoflagellates	3	0.001	0.001	0.001	0.001
Actual	Total Cyanobacteria	3	0.001	0.002	0.002	0.001

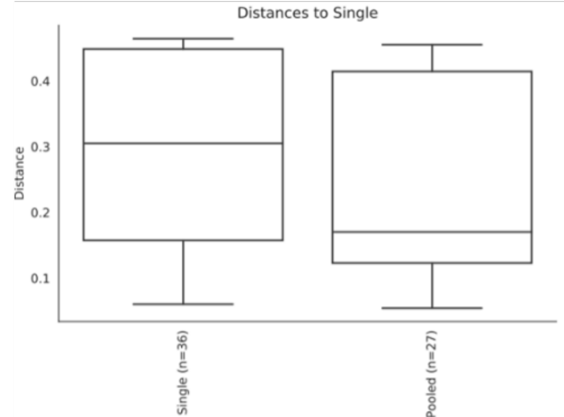
* Metadata categories applied to environmental and biological parameters are listed in Table 1.

GC02 was most similar to the GC06 location with 91% of GC02 genera also found in the GC06 community and only 75% of its genera also present in the GC09 community. Location GC02 had 6.5% of its genera unique to its location. The GC06 location samples, however, had slightly more genera in common with location GC09 (88%) and 78% of the genera present in its community were also found at location GC02. GC06 had 5.8% of the genera present unique to its location

Differential abundance analysis of bacterial and archaeal populations present on sample collection days 2 and 4 indicated some taxa varied significantly according to the location from which the water sample was sourced. In all of the collection day 2 samples, the phyla Bacteroides and Cyanobacteria were the most highly abundant populations in the water samples (Figure 9). However, some phyla varied according to location with the phyla Euryarchaeota and Marinimcrobia more abundant at location GC02 than the other locations; the phyla Cyanobacteria and Margulisbacteria more abundant at location GC06 and the phyla Bacteroidetes, Patescibacteria, Firmicutes and Epsilonbacteraeota more abundant in water samples collected from location GC09 (Figure 9). Examination of populations at the class level of taxonomy in samples collected on day 2, Marinimicrobia were more abundant at GC06 compared to other locations while Campylobacteria, Saccharimonadia and Gracillibacteria were enriched at GC09 (Figure 10). These differences in abundance at the different sample locations did not continue across the sampling days with differential abundance analysis in samples collected on day 4 revealing that Marinimicrobia was now more abundant at location GC02 (Figure 11). A full listing of the five most abundant taxonomic groups is detailed in Supplement 1, Table A.

Unweighted Unifrac Collection Day 2





Weighted Unifrac Collection Day 2

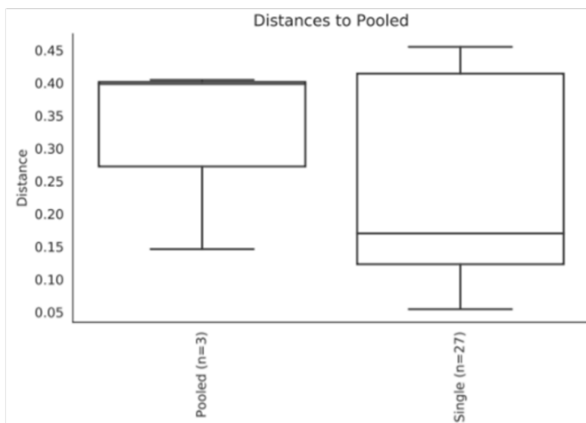


Figure 8. Statistical comparison of overall microbial communities present in individual (single) and pooled DNA samples from Collection Day 2. Boxplots showing distribution of the dataset, with the PERMANOVA pseudo-F p-value being > 0.05 for both the weighted and unweighted Unifrac measures of beta-diversity.

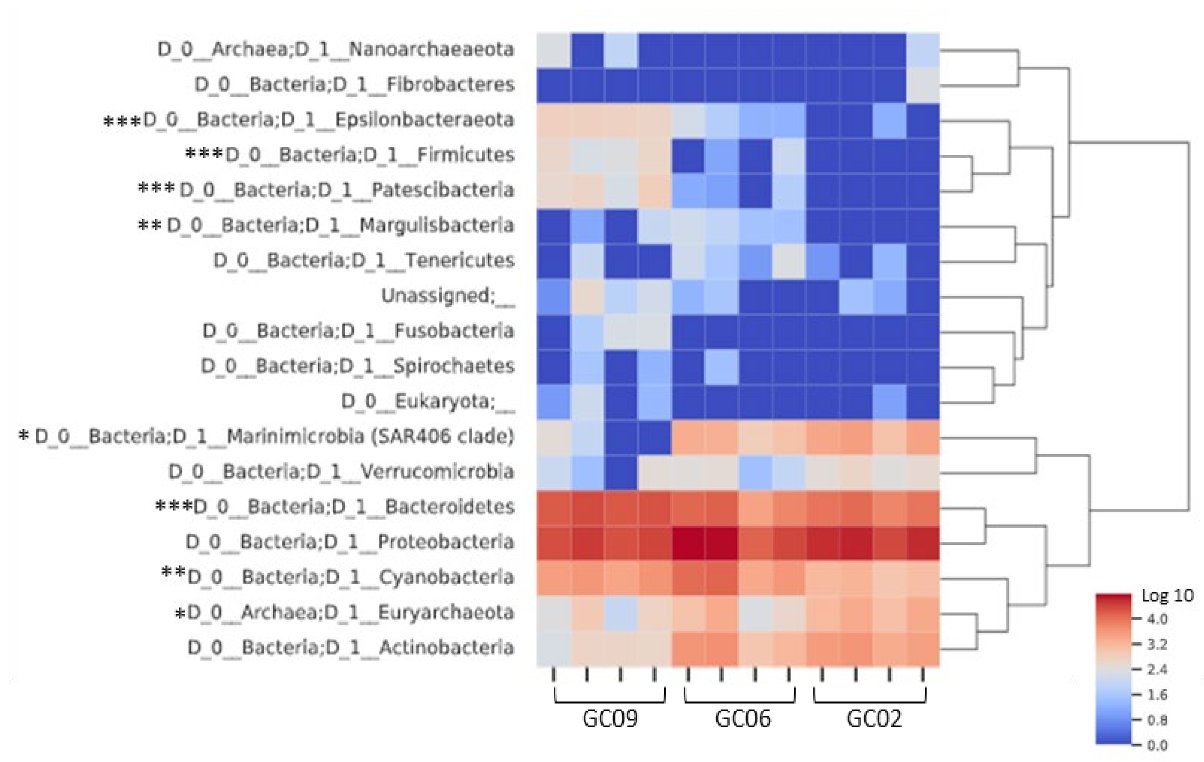


Figure 9. Heatmap of highly abundant bacteria and archaeal phyla identified in water collected on Collection Day 2, from three different locations (GC09, GC06 and GC02). Phyla which were identified as being significantly different between each location using differential abundance analysis (ancom) are marked with a superscript (significantly more abundant at GC02; ** significantly more abundant at GC06; *** significantly more abundant at GC09). Scale bar coloured according to a measure of relative abundance, Feature frequency (log_{10}).*

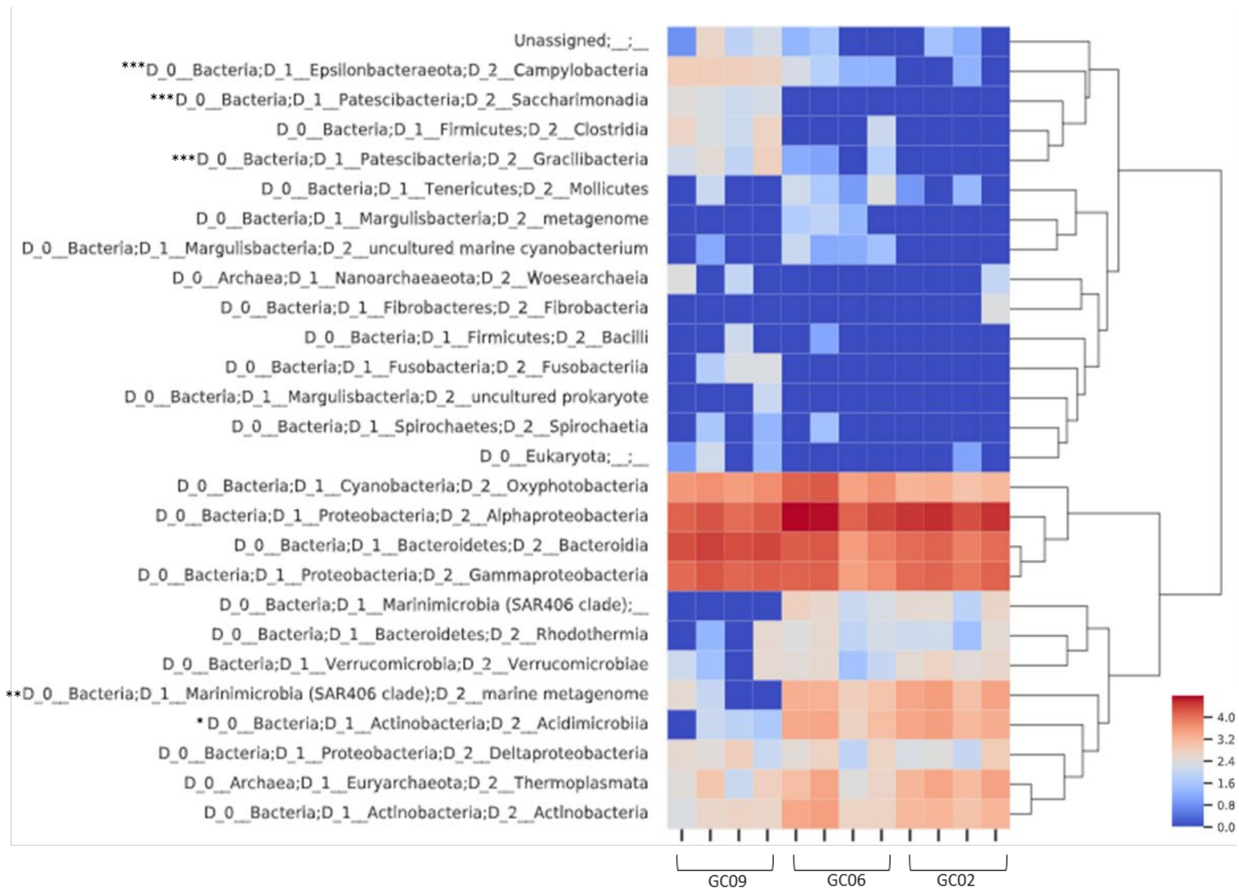


Figure 10. Heatmap of highly abundant bacteria and archaeal classes identified in water collected on Collection day 2, from three different locations (GC09, GC06 and GC02). Classes which were identified as being significantly different between each location using differential abundance analysis (ancom) are marked with a superscript (significantly more abundant at GC02; ** significantly more abundant at GC06; *** significantly more abundant at GC09). Scale bar coloured according to a measure of relative abundance, Feature frequency (log_{10}).*

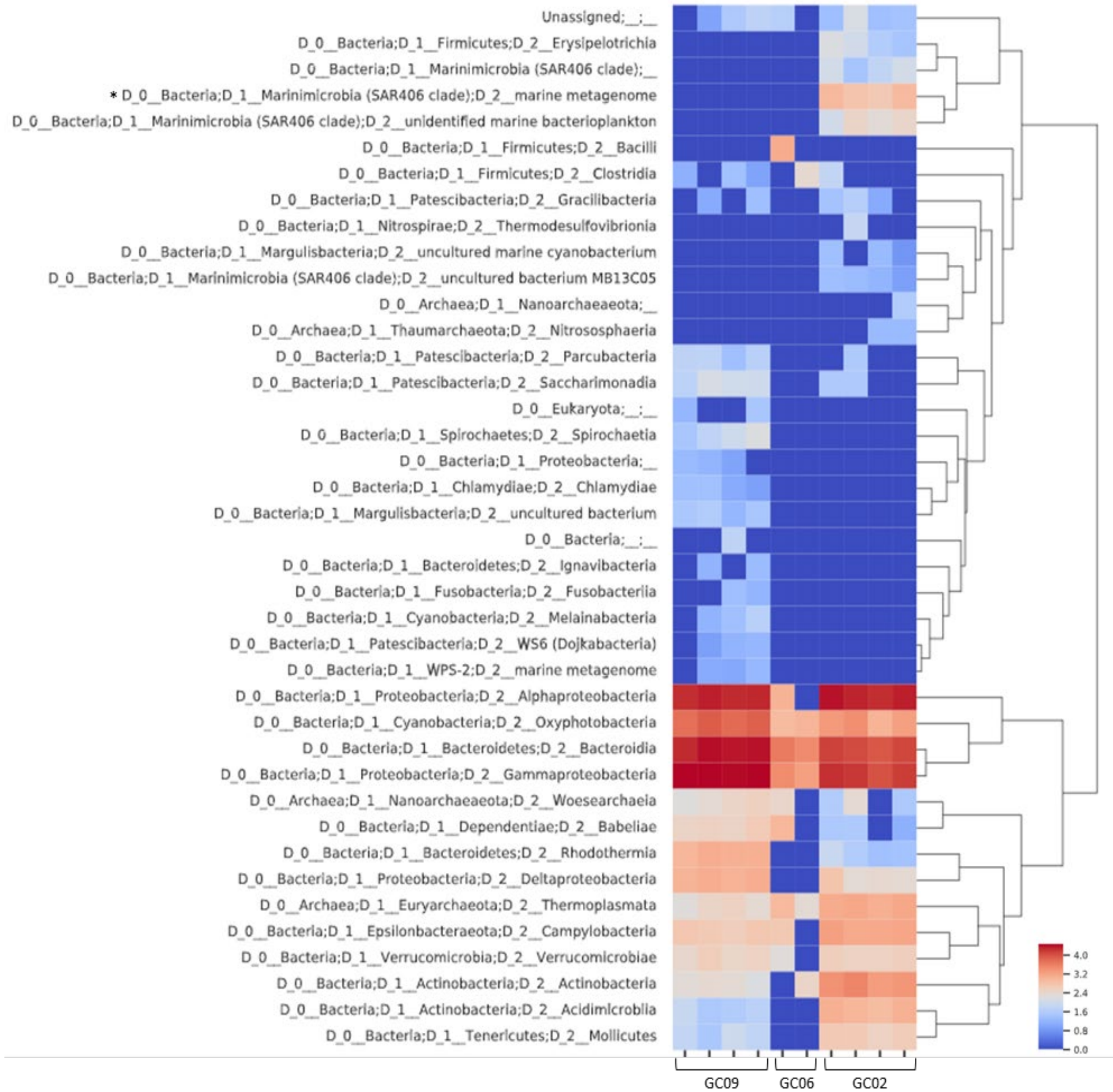


Figure 11. Heatmap of highly abundant bacteria and archaeal classes identified in water collected on Collection day 4, from three different locations (GC09, GC06 and GC02). Classes which were identified as being significantly different between each location using differential abundance analysis (ancom) are marked with a superscript (significantly more abundant at GC02). Scale bar coloured according to a measure of relative abundance, Feature frequency (log_{10}).*

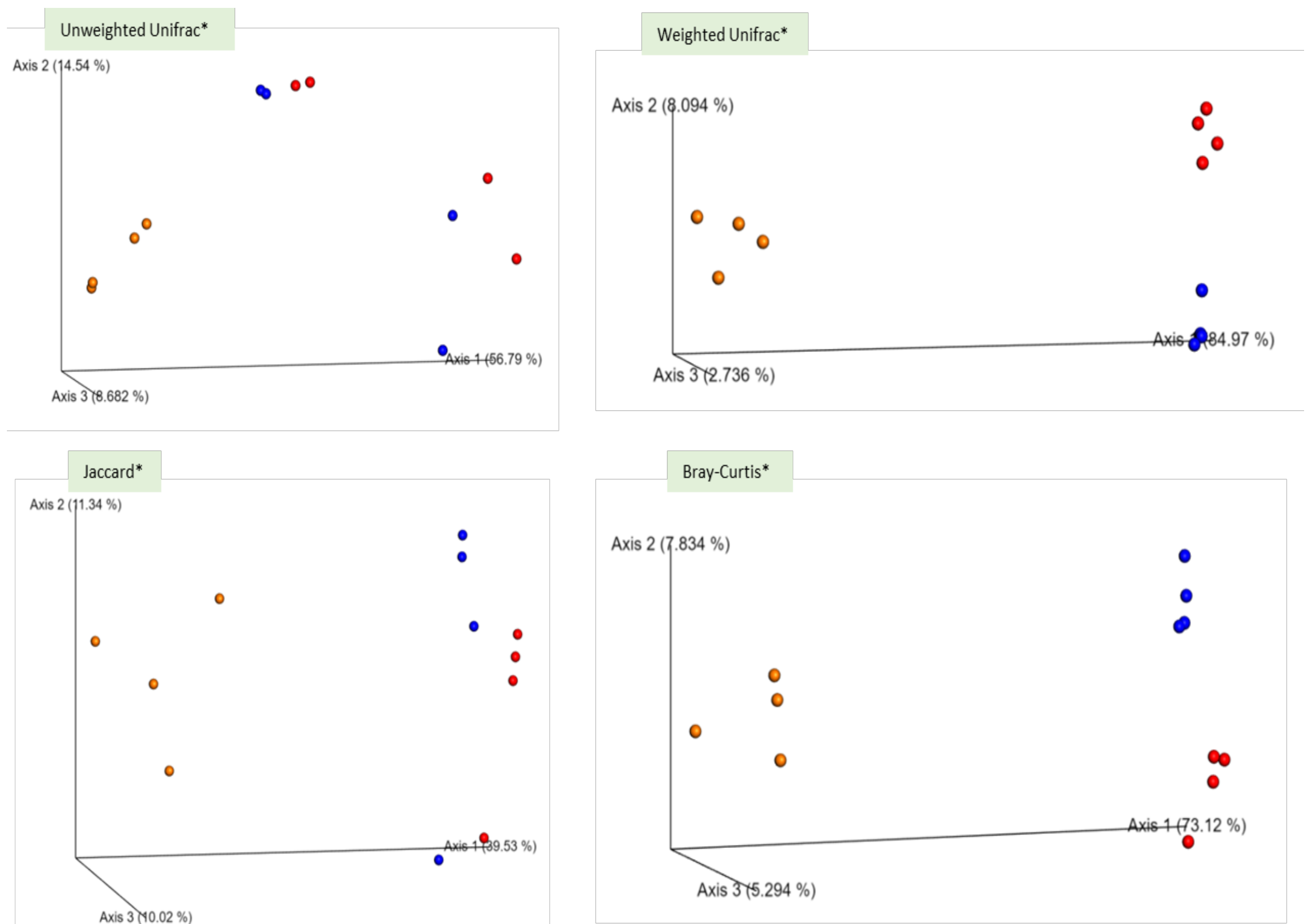


Figure 12. Effect of sample collection location on collection Day 2 on the highly abundant bacterial and archaeal communities (● GC02, ● GC06, ● GC09). Principal co-ordinates analysis (PCoA) on the basis of the Unweighted Unifrac, Weighted Unifrac, Bray Curtis and Jaccard measures of dissimilarity is shown. Diversity measures found to be significantly different on the basis of location are indicated with an asterisk and highlighted in green.

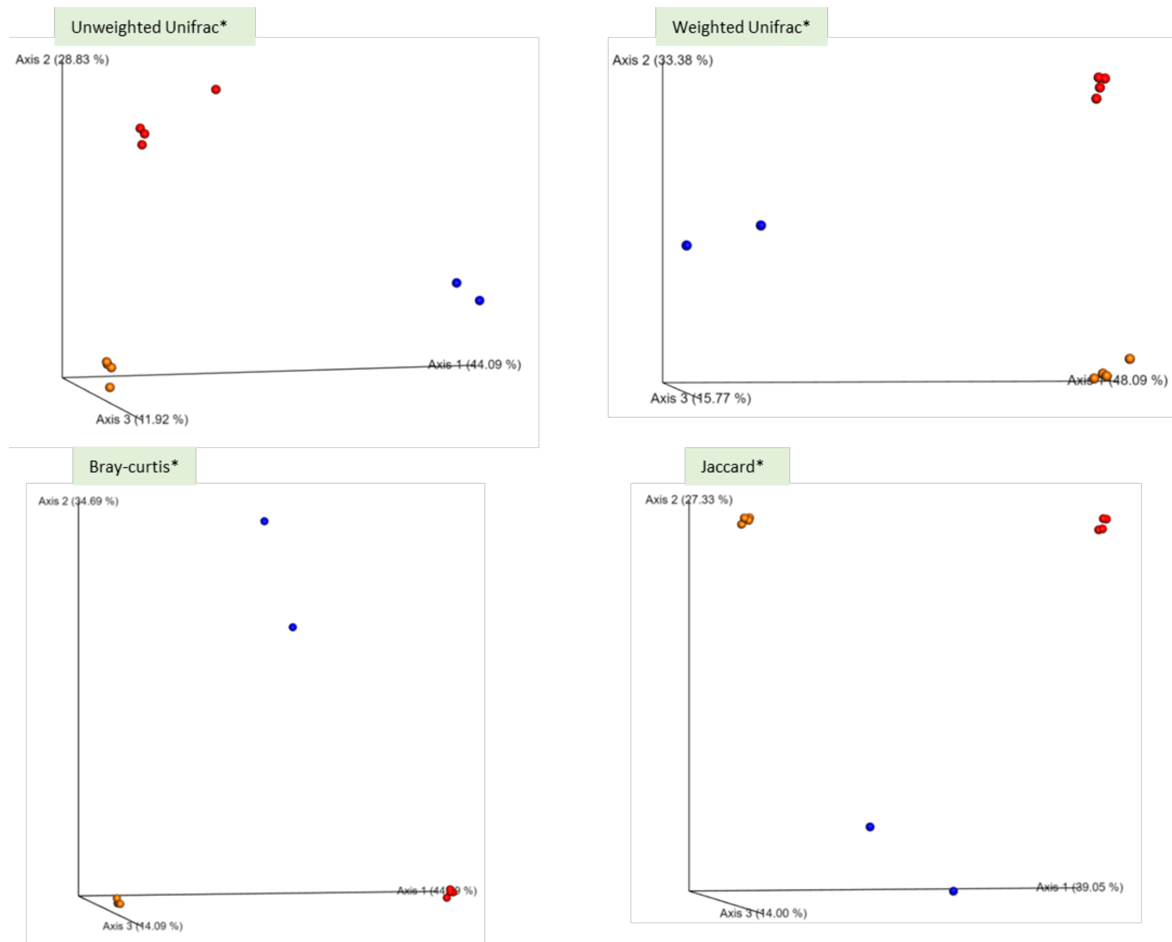


Figure 13. Effect of sample collection location on collection day 4 on the highly abundant bacterial and archaeal communities (● GC02, ● GC06, ● GC09). Principal co-ordinates analysis (PCoA) on the basis of the Unweighted Unifrac, Weighted Unifrac, Bray Curtis and Jaccard measures of dissimilarity is shown. Diversity measures found to be significantly different on the basis of location are indicated with an asterisk and highlighted in green.

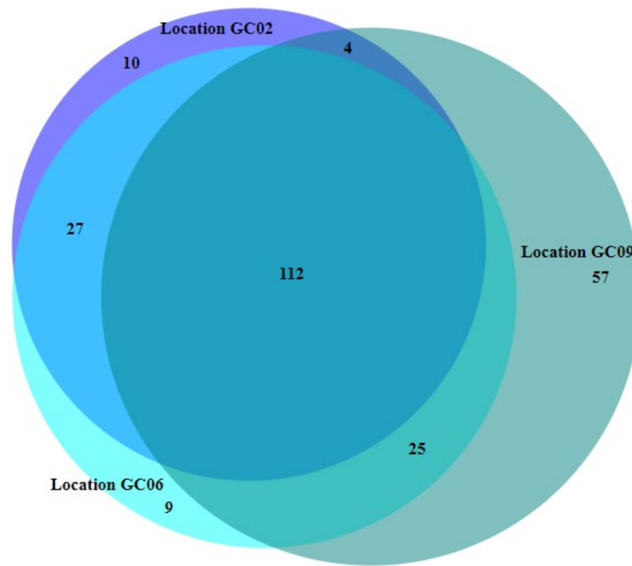


Figure 14. Effect of sample collection location on Collection day 2 only. The proportions of highly abundant bacterial and archaeal communities are compared using Venn diagrams, showing the number of Features classified to genus level either shared between respective locations, or found to be unique to each location.

3.3.3 Differences in microbial populations according to the sample location

When the dataset was subdivided into the three locations from which water samples were collected (GC02, GC06 and GC09), there was a significant effect of the day on which samples were collected for each location, with significant differences ($P < 0.05$) occurring in the microbial communities present across all four Beta diversity measures tested (Tables 8 - 10). While the water sample could not be analysed from for each sample collection time, at every location, clear differences could be seen in the proportions of respective bacterial and archaeal populations present (Figures 15 - 17) with significantly different, differentially abundant populations identified for each location.

At location GC02, the pre-filter pond where drum filters supply the reservoir (Figure 1), all samples collected were dominated by bacterial populations classified within the classes Alphaproteobacteria, Bacteroidia and Gammaproteobacteria (Figure 15). Bacteria from the phylum Marinimicrobia clade SAR406 and Archaea classified within the class Thermoplasmata, were found at higher abundances on collection day 2, than in samples collected on days 4 and 18. Similarly, in samples collected on day 4, bacteria classified within the classes Actinobacteria, Erysipelitrichia, Campylobcateria and the uncultured bacterial class MB13C05 (Marinimicrobia) were found to be present in high abundance. Venn analysis at genus level of taxonomy showed the microbial community present in location GC02 water samples collected on day 4 had the highest number of unique genera (Figure 18). It also shows that across the three sample collection days there were only 7.4% of the genera found all samples. In addition, samples collected on days 2 and 4 had the most genera in common, when compared to the sample collected on day 18 (Figure 18) which had the lowest number of genera present in the sample with 33 genera present.

The dataset for location GC06 was the most complete, with samples for five different days (collection days 2, 4, 6, 18 and 19) being used for microbial community analysis. This location occurred on farm one, at the Rachael reservoir outlet, at the pipe entry into the distribution channel from the reservoir (Figure 1). The day on which samples were collected at this location, was again found to have a significant impact on the microbial communities present (Table 9). All samples collected from location GC06 however, were dominated by bacterial populations classified within the classes Bacteroidia and Gammaproteobacteria, Oxyphotobacteria and Alphaproteobacteria (Figure 16). Similarly to samples collected from location GC02, at location GC06 bacteria from the phylum Marinimicrobia clade SAR406, were more abundant in samples from collection day 2. Bacteria classified within the class Rhodothermia were also significantly more abundant at this time. None of the bacterial classes identified from samples collected on day 4 were found to be differentially abundant (Ancom analysis) when compared to the bacterial classes identified in the other collection sample days. A single sample collected on day 6 was found to have increased abundance of bacteria which could not be classified according to current classifications available in the SILVA database. Bacterial populations classified within the class Fibrobacteria were found in relatively low concentrations in samples collected later in the experiment (collection days 18 and 19). Bacteria classified within the classes Acidimicrobiia and Deltaproteobacteria were also found to be more abundant in the collection day 19 sample and were absent in the two samples from collection day 4.

When genus level classifications and Venn analysis was done on the data for four samples, representing collection days 2, 6, 18 and 19 collected at location GC06, day 2 had the most diverse community with 107 genera identified and 27 unique genera representing 16.6% of the total number of genera identified for this location (Figure 18). At this location collection day 6 had the lowest diversity with only 35 different genera present on this day. Venn analysis also showed that bacterial genera present changing across the sample collection days with only 14% of genera present in all four collection day samples (Figure 18).

At the end of the southern water distribution channel adjacent to the pond inlet, (location GC09, Figure 1), samples from four different days (collection days 2, 4, 6 and 19) were used for microbial

population analysis. The bacterial populations identified in samples from this location at all collection days were dominated by bacteria classified within the classes Bacteriodia, Alphaproteobacteria, Gammaproteobacteria and Oxyphotobacteria. Collection day 2 had higher abundances of bacteria classified in classes Bacteroidia, Saccharimonadia, Campylobacteria, and Gracillibacteria compared to the samples from other collection days (Figure 16). Bacterial communities in the samples from collection day 4 had several differentially abundant bacterial classes, including Oxyphotobacteria, Alphaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Babeliae, Chlamydiae and an uncultured bacteria from the phylum Margulisbacteria. Only one bacterial class, Anaerolineae, was more abundant in the sample from collection day 6, and in the sample collected on day 19 no microbial populations were found to be differentially abundant when compared to the bacterial populations identified in samples collected on days 2, 4 and 6. A full listing of the five most abundant taxonomic groups is detailed in Supplement 1, Table A.1.

When genus level classifications for four samples collected at location GC06, representing samples taken at collection days 2, 4, 6 and 19, were compared using Venn analysis (Figure 18), day 4 was found have the most diverse community with 172 genera detected and unique genera (81) representing 33% of the total number of genera identified for this location. Only 28 different genera were identified in the water sample collected from day 19 which also was found to contain the lowest number of unique genera (6), suggesting that although there was a significant effect of time occurring for location GC09, there was not as much variability in the microbial populations found at this location between the start and the very end of the period and only 6 genera (2.5%) were found in samples from all four collection days.

Specific genera of interest, such as *Vibrio*, were found in water samples collected throughout the experiment, however they were at relatively low abundance within the overall bacterial populations identified (the maximum relative abundance was 1.98% in a sample collected at location GC09 on sample collection Day 2). *Vibrio* populations were detected only in water samples collected from locations GC02 and GC09, on the sample collection times 2 and 4. *Vibrio* populations were not detected at location GC06, on any of the sample collection times.

Table 28. Significantly different experimental factors (metadata categories) from analysis of samples collected from Location GC02 on different days showing the over-riding effects of the day on which samples were collected. Data was either grouped to reduce the number of categories examined or analysed as actual data (numerical values treated as categories). Results presented include four measures of beta-diversity (Unweighted and Weighted Unifrac, Bray-curtis and Jaccard), including P values generated from PERMANOVA analysis.

Grouped or actual data	Metadata Category	Beta diversity measures P values				
		No. of categories	Unweighted Unifrac	Weighted Unifrac	Bray-curtis	Jaccard
Grouped	Collection day	3	0.003	0.003	0.001	0.002
Actual	Salinity	3	0.003	0.009	0.005	0.004
Actual	Rain	3	0.005	0.004	0.004	0.004
Actual	Temperature Maximum	3	0.006	0.004	0.002	0.003
Actual	Turbidity	3	0.003	0.006	0.004	0.002
Actual	Water temperature	3	0.005	0.004	0.003	0.004
Actual	Wind speed	3	0.003	0.007	0.002	0.01
Actual	Total Cyanobacteria	3	0.004	0.005	0.004	0.007
Actual	Total Diatoms	3	0.004	0.007	0.006	0.002
Actual	Total Dinoflagellates	3	0.003	0.005	0.006	0.006
Actual	Total Zooplankton	3	0.006	0.004	0.005	0.006

Table 29. Significantly different experimental factors (metadata categories) from analysis of samples collected from Location GC06 on different days showing the over-riding effects of the day on which samples were collected. Data was either grouped to reduce the number of categories examined or analysed as actual data (numerical values treated as categories). Results presented include four measures of beta-diversity (Unweighted and Weighted Unifrac, Bray-curtis and Jaccard), including P values generated from PERMANOVA analysis.

Grouped or actual data	Metadata Category*	Beta diversity measures P values				
		No. of categories	Unweighted Unifrac	Weighted Unifrac	Bray-curtis	Jaccard
Grouped	Collection day	5	0.038	0.02	0.002	0.004
Actual	Salinity	5	0.03	0.018	0.004	0.003
Actual	Rain	4	0.015	0.005	0.011	0.006
Actual	Temperature Maximum	5	0.029	0.028	0.008	0.006
Actual	Water temperature	5	0.044	0.036	0.011	0.007
Actual	Wind speed	5	0.036	0.025	0.008	0.003
Actual	Total Cyanobacteria	4	0.083	0.063	0.057	0.043
Actual	Total Diatoms	5	0.038	0.021	0.009	0.004
Actual	Total Dinoflagellates	5	0.039	0.031	0.007	0.008
Actual	Total Zooplankton	5	0.028	0.026	0.007	0.009

*All had very high turbidity >85, turbidity not tested by PERMANOVA

Table 30. Significantly different experimental factors (metadata categories) from analysis of samples collected from Location GC09 on different days showing the over-riding effects of the day on which samples were collected. Data was either grouped to reduce the number of categories examined or analysed as actual data (numerical values treated as categories). Results presented include four measures of beta-diversity (Unweighted and Weighted Unifrac, Bray-curtis and Jaccard), including P values generated from PERMANOVA analysis.

Grouped or actual data	Metadata Category*	Beta diversity measures P values				
		No. of categories	Unweighted Unifrac	Weighted Unifrac	Bray-curtis	Jaccard
Grouped	Collection day	4	0.003	0.001	0.001	0.001
Actual	Salinity	4	0.004	0.002	0.003	0.001
Actual	Rain	3	0.003	0.006	0.004	0.002
Actual	Temperature Maximum	4	0.002	0.002	0.002	0.002
Actual	Water temperature	4	0.001	0.001	0.001	0.002
Actual	Wind speed	4	0.003	0.002	0.002	0.001
Actual	Total Cyanobacteria	4	0.002	0.002	0.001	0.001
Actual	Total Diatoms	4	0.001	0.001	0.001	0.001
Actual	Total Dinoflagellates	4	0.001	0.002	0.001	0.001
Actual	Total Zooplankton	4	0.001	0.001	0.002	0.002

*All had very high turbidity >85, turbidity not tested by PERMANOVA

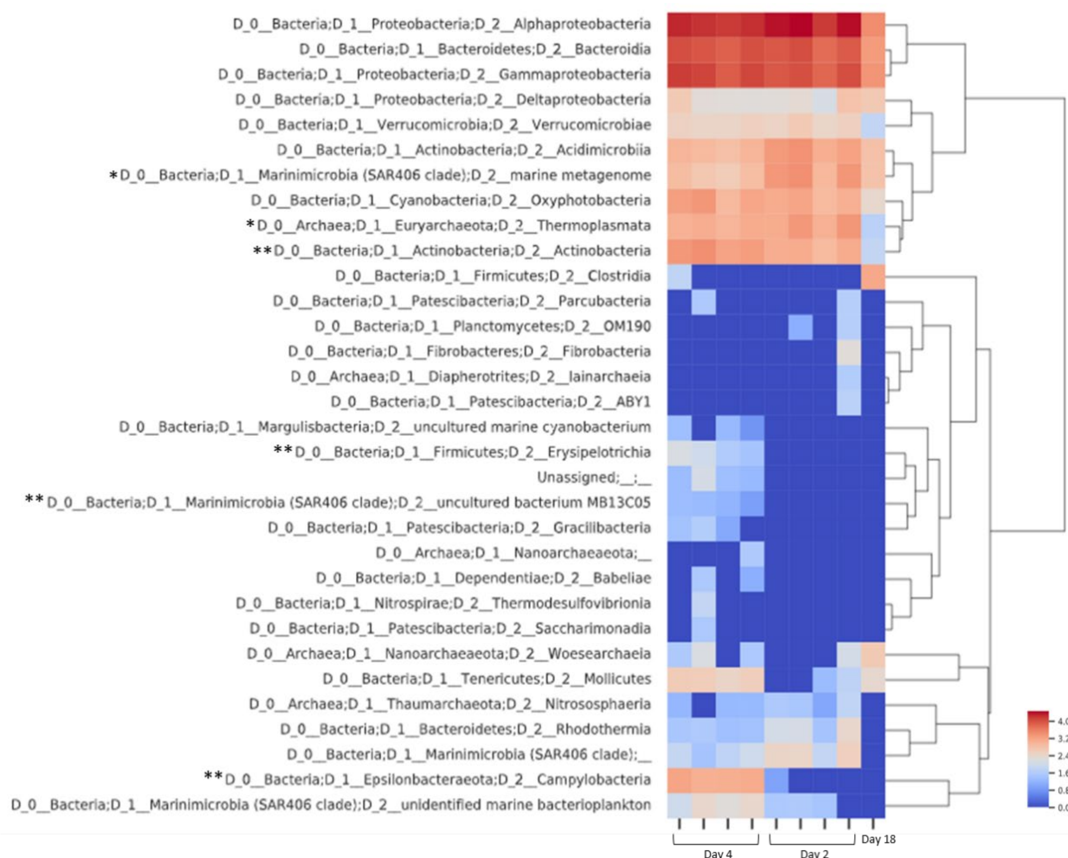


Figure 15. Heatmap of highly abundant bacteria and archaeal classes identified in water collected from location GC02, on three different days (collection days 2, 4 and 18). Classes which were identified as being significantly different between each collection day using differential abundance analysis (ancom) are marked with a superscript (most abundant on collection day 2; ** most abundant on collection day 4). Scale bar coloured according to a measure of relative abundance, Feature frequency (log$_{10}$).*

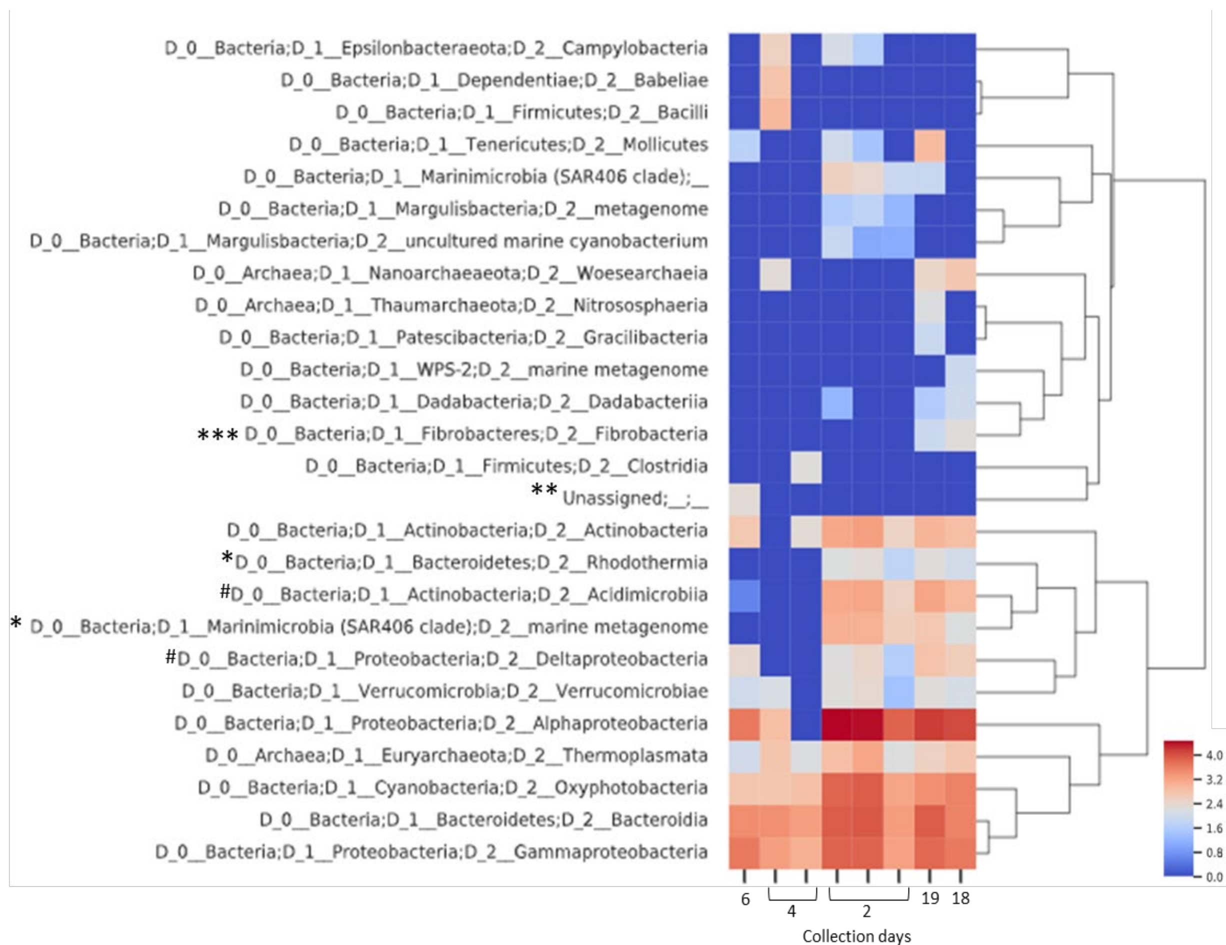


Figure 16. Heatmap of highly abundant bacteria and archaeal classes identified in water collected from location GC06, on five different days (collection days 2, 4, 6, 18 and 19). Classes which were identified as being significantly different between each collection day using differential abundance analysis (ancom) are marked with a superscript (most abundant on collection day 2; ** most abundant on collection day 6; and *** most abundant on collection day 18; #most abundant on collection day 19). Scale bar coloured according to a measure of relative abundance, Feature frequency (log_{10}).*

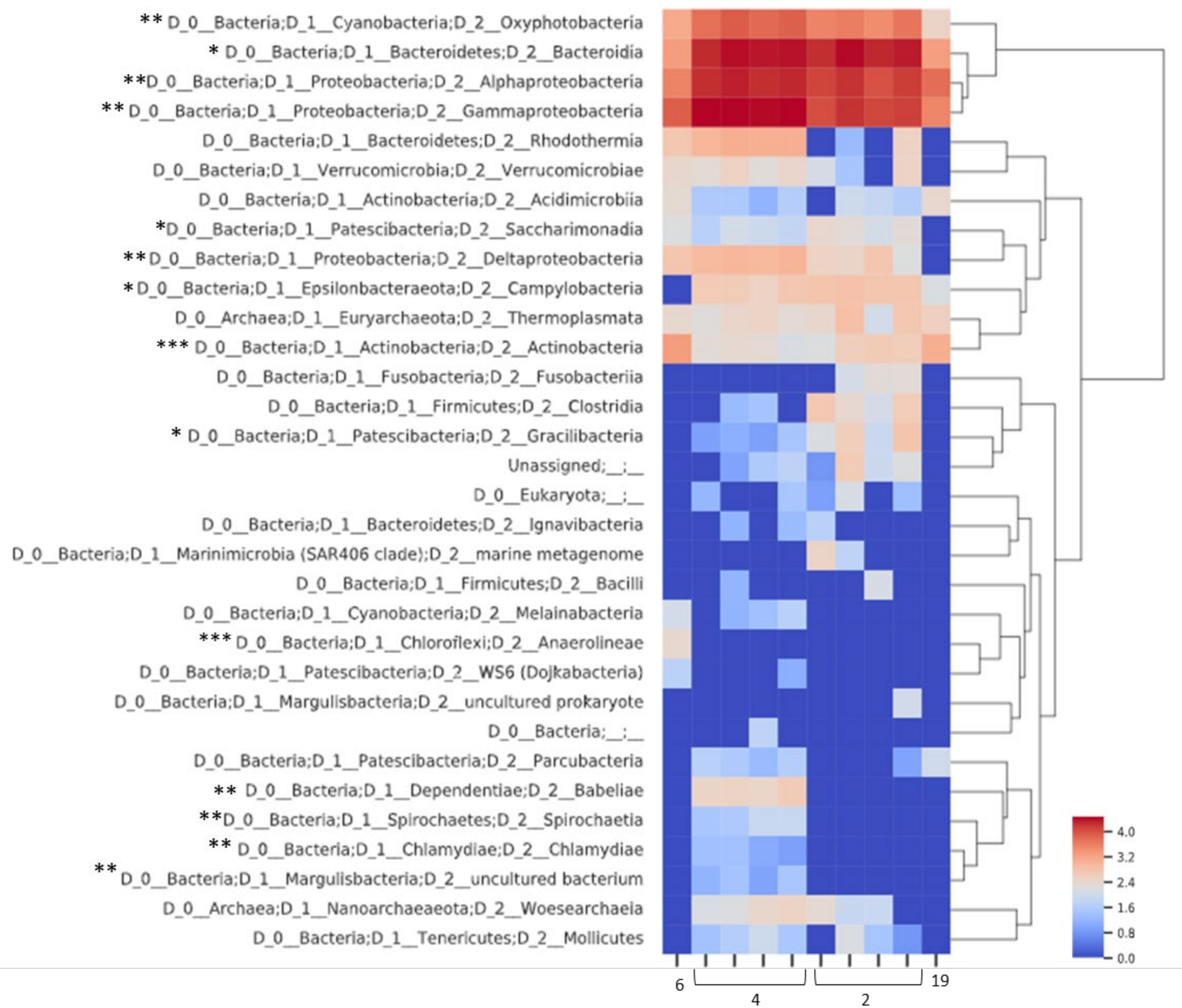
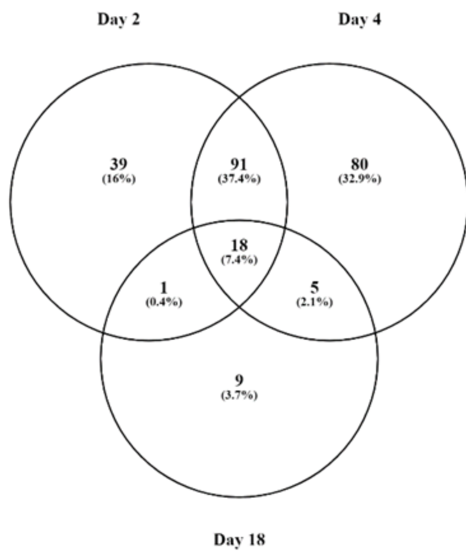
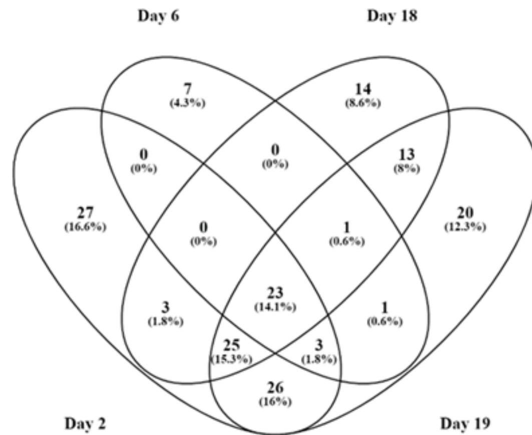


*Figure 17. Heatmap of highly abundant bacteria and archaeal classes identified in water collected from location GC09, on four different days (collection days 2, 4, 6 and 19). Classes which were identified as being significantly different between each collection day using differential abundance analysis (ancom) are marked with a superscript (*most abundant on collection day 2; ** most abundant on collection day 4; and *** most abundant on collection day 6). Scale bar coloured according to a measure of relative abundance, Feature frequency (log_{10}).*





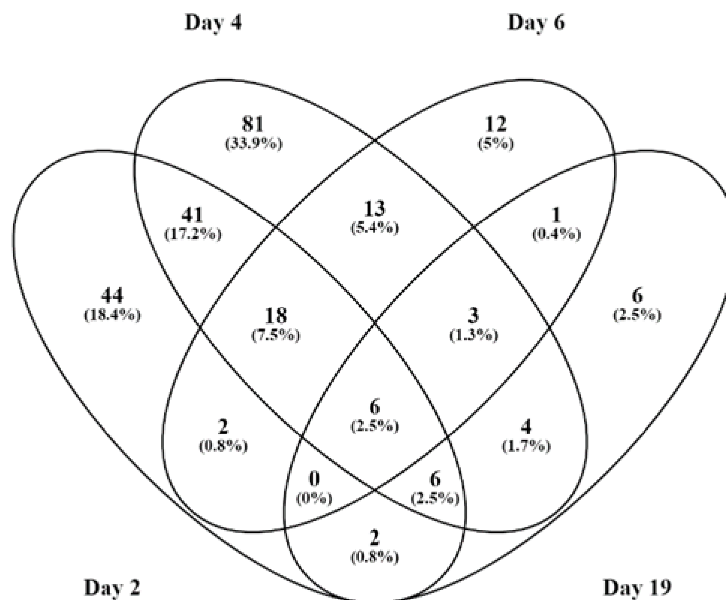


Figure 18. Comparison of numbers of microbial populations (Features) classified at genus level present at each of the sample collection locations (GC02, GC06 and GC09) from at least three different sample collection time-points (from either collection days 2, 4, 6, 18 or 19 dependant on where sample data was available). The proportions of microbial communities are compared using Venn diagrams, showing the number of Features classified to genus level (% of total number of Features identified at the location) either shared between respective locations, or found to be unique to each location.

4. Discussion

This microbiome diversity study highlighted some of the technical difficulties associated with extracting environmental DNA from marine water samples collected at distance from a laboratory. The sample collection method used, though simple, was one successfully implemented by another group working in a similar situation and initial testing had indicated it was adequate for the purpose. It is apparent however that the method did not account for the very high variability in microbial load that was experienced. Samples of the farm's water distribution system did not consistently provide sufficient DNA for valid analysis. Consequently this approach has been determined to be unsuitable for using in the aquaculture environments found in south east Queensland where there can be relatively low nutrient and microbial concentrations. Work is underway to test a range of field sample collection methods to optimise water collection volume, sample processing and DNA preparation steps for use in any aquaculture study. These methods will allow for an optimal concentration of microbial biomass to enable sufficient DNA to be extracted and employed in future microbiome diversity studies including bacterial and archaeal (16S rRNA gene), fungal (ITS2 region) and microeukaryotic (18S rRNA gene) PCR amplification and sequencing. This will permit greater characterisation of the microbiome diversity present within collected water samples.

Despite the technical difficulties experienced in the current study, the bacterial and archaeal populations were successfully determined for a total of 28 pooled and individual water samples, with days 2 and 4 collected from three farm locations GC02 (pre-filter pond), GC06 (Rachael reservoir outlet) and GC09 (Farm 2 channel end) (Figure 1) well represented within the microbiome sequence dataset. These three sample collection points encompassed the start, middle and end of the water distribution system within the farm and were therefore able to be used to describe the microbial populations present throughout the pond water supply system.

Because of the issues experienced with the sample collection and subsequent DNA extraction, care must be taken in the conclusions drawn from this study however, overall, the microbial populations within the farm water distribution system were found to be highly diverse. Statistical analysis of the alpha diversity analysis, which measured the microbial diversity within each sample, indicated this within sample diversity was not influenced by location and sampling time, or measured environmental (e.g. wind speed, rainfall, salinity, water temperature and turbidity) and biological (e.g. phytoplankton and zooplankton) factors. The water distribution system was maintaining a diverse microbial community across the farm which indicates the system was relatively healthy as the maintenance of a high level of microbial diversity, has been shown to contribute to the health of aquatic ecosystems (Zeglin et al., 2015; Comte et al., 2013). Previous studies have also indicated that marine microbial systems can maintain resilience and long-term stability in terms of their average community composition despite short-term variations in community composition sometimes occurring due to various contributing environmental factors such as changes in water current patterns and resulting phytoplankton blooms and feedback loops involved (Fuhrman et al., 2015; Lucas et al., 2015).

The majority of water samples collected in this study were found to contain microbial assemblages which are commonly associated with coastal marine ecosystems. Many of the microbial populations observed could not be taxonomically classified to a known genus, as marine bacteria are often unable to be isolated and cultivated. The analysis therefore focussed on the populations classified at higher taxonomic levels (phylum, class or family). The majority of the water samples (90% of samples examined) were dominated by the bacterial phyla Proteobacteria and Bacteroidetes. Within the phylum Protoeobacteria, the SAR11 clade within the class Gammaproteobacteria and members of the order Rhodobacteriales within the class Alphaproteobacteria were present in all samples. The SAR11 clade is one of the most abundant bacterial chemoheterotrophic groups found in marine environments, often representing 25-50 % of the total planktonic cells (Morris et al., 2002; Henson et al., 2018). These aerobic, free-living microorganisms cannot fix carbon and instead use organic

compounds, such as dissolved organic matter to satisfy their carbon requirements. The SAR11 clade are also thought to have a competitive advantage at low concentrations of dissolved organic matter (Buchan et al., 2014; Wilhelm et al., 2007). The other highly abundant taxon, Rhodobacterale that sits within the Class Alphaproteobactera, are known to form close associations with phytoplankton (Meyer et al., 2017). Previous studies have shown that inter-kingdom signalling can occur between the Alphaproteobacteria and phytoplankton, with the former able to recruit certain algae for symbiosis, and deter other types of microbes or algae, through the release of bioactives (Wagner-Döbler et al., 2004). A recent study found that when phytoplankton in rearing ponds recruited bacterioplankton belonging to Rhodobacterales there was a positive effect on shrimp health and growth in the rearing pond (Yang et al., 2020). The Alphaproteobacteria can even turn against their algal hosts when nutrients are limiting by inducing senescence through the release of algaecides (Meyer et al., 2017) and therefore may play a major role in modulating other microbial communities. Within the Phylum Bacteriodetes, the other major microbial communities identified in this study, were members of the families Cryomorphaceae and the NS5 group. The Cryomporphaceae are found in a variety of niches, known to be metabolically diverse, and are currently being taxonomically redefined (Bowman, 2020). They are found as the dominant taxa in productive ocean and coastal regions (Abell and Bowman, 2005; Fodelianakis et al., 2014; Campbell et al., 2015) and within the marine surface layer (Zäncker et al., 2018). The as yet uncultivated NS5 group is ubiquitously found in marine phytoplankton communities and their abundance is strongly associated with seasonal algal blooms (Diez-Vives et al., 2019, Krüger et al., 2019). The metagenome assembled genome (MAG) of members of the NS5 group has shown they have a small sized genomes and this genome streamlining is thought to be why they are resistant to isolation and cultivation. Analysis of the MAG of NS5 showed they have polysaccharide utilisation loci that allow them to degrade the polysaccharides found within marine-phytoplankton-derived organic matter (Krüger et al., 2019).

The composition of bacterial and archaeal communities of the farm water distribution system significantly changed according to the overriding effects of time and location. As water progressed through the distribution system the communities diverged, with the final location GC09, at the end of the supply channel network, possessing highest number of unique bacterial taxa (119 highly abundant genera) all the time points assessed. Despite this divergence in total microbial community profile, the most dominant (very high abundance) taxonomic groups showed some similarities across time and sampling location. In samples from the first sample collection point Location GC02 the pre-filter pond nearest to the Logan River supply pumps, as well as the groups seen across all samples, consistently had dominant populations taxa belonging to Alphaproteobacteria from the order Rhodospirillales - photosynthetic and sometimes chemo-organotrophic aquatic bacteria and family Rhodobacteraceae which comprise mainly aerobic photo- and chemoheterotrophs and are involved in sulfur and carbon biogeochemical cycling and symbiosis with aquatic micro- and macro-organisms (Pujalte et al., 2014). There were also members of Gammaproteobacteria within the family Halieaceae often found inhabiting marine environments, especially coastal areas and containing some members that are capable of aerobic photoheterotrophic growth (Spring et al., 2015). As well as the NS5 marine group in Bacteroidetes. The microbial community at GC02 contained members of the family Flavobacteriaceae which are thought to be important players in the “microbial loop” trophic model where micro-organisms consume dissolved organic matter and then they are consumed in turn by other organisms. They are also thought to be involved in breaking down large organic molecules (e.g., proteins, chitin, and other polysaccharides) thereby making them accessible to other microbes (Cottrell and Kirchman, 2000, Tully et al., 2014). The SAR406 clade which is ubiquitously distributed in marine environments and members from Actinobacteria within the Candidatus Actinomarina clade were also present in the GC02 community. Samples taken from the final collection point (Location GC09) had communities dominated at all collection time-points by Alphaproteobacteria (Rhodobacteraceae and SAR11 clade), Bacteroidetes (Cryomorphaceae and Flavobacteriaceae), Gammaproteobacteria (Litoricola, Halieaceae) and Betaproteobacteria (Methylophilaceae). The archaeal class Thermoplasmata (Marine Group II), was the most frequently

occurring Euryarchaeota being detected at all locations. This largely uncultivated taxonomic clade of archaea are often present in deep and surface ocean waters (Orellana et al., 2019) and were also found to prefer the conditions provided in the farm examined, particularly the final stages of the supply channel network.

However, at each location the microbial community composition differed in samples collected on different dates and this is not surprising as many studies have shown that the abundance of different species of bacteria, like the NX5 group are strongly correlated with events which supply organic matter for growth, like marine phytoplankton blooms (Lucas et al., 2016; Krüger et al., 2019). In this study the sampling commenced in spring and sample collection days 2 and 4 are two weeks apart but still show distinct differences in community composition.

Interestingly, most of the bacterial populations found to dominate in Location GC09 at most sample collection times, were those which have been found in other marine studies to be strongly associated with algal blooms, including the bacterial classes Flavobacteria, Alphaproteobacteria, Rhodobacteraceae, Gammaproteobacteria and most notably, the Gammaproteobacteria (Alteromonadales) (Buchan et al., 2014). It has been suggested that the metabolic abilities of taxa in these bacterial families are specialised for the successive decomposition of algal-derived organic matter (Teeling et al., 2012), enabling a ready response to the nutrient pulses that signify phytoplankton blooms (Buchan et al., 2014). Examination of the available on-farm metadata however, showed no evidence of an algal bloom occurring at this location at any time during the investigation.

A study of sea samples taken monthly from the same location over a three year period revealed a progression of microbial communities and community interactions over time and have shown that combinations of environmental variables in aquatic ecosystems are more predictive of the microbial community changes over time compared with single environmental variables (Steele et al., 2011). In the current study, consistent differences in the respective microbial communities from start to end of the water distribution system presumably reflect changes in the environmental conditions which occur as the water moves through this farming system. One such change is the large reduction in turbidity that occurs between the intake pond (GC02) and the second sampling point at the exit of the first reservoir (GC06).

Of relevance to the operators of the farm, the abundance of known and potential pathogens belonging to the *Vibrio* genus were very low in the communities within the farm water distribution system. This suggests that *Vibrio* were not selected for and enriched by the on-farm conditions and management practices employed in this aquaculture system. A recent study of the intestinal bacterial communities of animals (shrimp, crab and clam) from an aquaculture system indicated that the aquaculture environment was the main source of intestinal bacteria (Sun et al. 2020).

In summary, whilst the water sampling method employed in this study demonstrated a need for further optimisation to yield sufficient quantities of environmental DNA required for high throughput sequencing platforms, this is being addressed in a current project funded by DAF. The analyses of the microbiomes able to be done on samples across the aquaculture farm's water distribution system showed it was inhabited by highly diverse bacterial assemblages common to marine environments and the microbial community structure varied across spatial and temporal scales. The use of a cultivation-independent methodologies such as the bacterial and archaeal 16S rRNA gene amplicon analysis employed in this project, provides a comprehensive baseline of the microbial populations present on-farm and an indication of the "health" of an aquaculture system.

Microbial communities play a critical role in energy flow, biogeochemical cycling and animal health in all ecosystems, natural and constructed as prawn farms. It is important to comprehensively characterise baseline community structure and dynamics to provide context for production outcomes, and for instructing improvement to development of water management strategies. Similar methodologies to that employed here have the potential to be used to also investigate the community composition of micro-eukaryotic (includes microalgae and protozoans), fungal and viral communities to better understand the microbiome present in aquaculture systems.

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Microbiome Analysis Supplement

Table A. Listing of the five most highly abundant taxonomic groups (of all bacterial and archaeal Features identified in the overall sequence dataset), according to time of sampling and the location from which the samples were collected. (Taxonomic level key: D_0 = Kingdom; D_1 = Phylum; D_2 = Class; D_3 = Order; D_4 = Family; D_5 = Genus; D_6 = Species).

Location	Time
GC02	**Day 2**
	D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 Rhodospirillales;D_4 AEGEAN-169 marine group;
	D_0 Bacteria;D_1 Proteobacteria;D_2 Gammaproteobacteria;D_3 SAR86 clade; ;
	D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 Rhodobacterales;D_4 Rhodobacteraceae;D_5 HIMB11
	D_0 Bacteria;D_1 Bacteroidetes;D_2 Bacteroidia;D_3 Flavobacteriales;D_4 Flavobacteriaceae;D_5 NS5 marine group
	D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 SAR11 clade;D_4 Clade I;D_5 Clade Ia
	Day 4
	D_0 Bacteria;D_1 Proteobacteria;D_2 Gammaproteobacteria;D_3 Oceanospirillales;D_4 Nitrincolaceae;D_5 *Marinobacterium*
	D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 Rhodobacterales;D_4 Rhodobacteraceae;D_5 HIMB11
	D_0 Bacteria;D_1 Bacteroidetes;D_2 Bacteroidia;D_3 Flavobacteriales;D_4 Flavobacteriaceae;D_5 NS5 marine group
	D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 Rhodobacterales;D_4 Rhodobacteraceae;
	D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 SAR11 clade;D_4 Clade I;D_5 Clade Ia
	Day 6
	na*
	Day 18
	D_0 Bacteria;D_1 Proteobacteria;D_2 Gammaproteobacteria;D_3 Cellvibrionales;D_4 Halieaceae;D_5 OM60(NOR5) clade
	D_0 Bacteria;D_1 Marinimicrobia (SAR406 clade);D_2 marine metagenome;D_3 marine metagenome;D_4 marine metagenome;D_5 marine metagenome
	D_0 Bacteria;D_1 Bacteroidetes;D_2 Bacteroidia;D_3 Bacteroidales;D_4 Prevotellaceae;D_5 *Prevotella*
	D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 SAR11 clade;D_4 Clade I;D_5 Clade Ia
	D_0 Bacteria;D_1 Firmicutes;D_2 Clostridia;D_3 Clostridiales;D_4 Family XI;D_5 *Ezakiella*
	Day 19
	na
GC06	**Day 2**
	D_0 Bacteria;D_1 Bacteroidetes;D_2 Bacteroidia;D_3 Flavobacteriales;D_4 Flavobacteriaceae;D_5 NS5 marine group
	D_0 Bacteria;D_1 Cyanobacteria;D_2 Oxyphotobacteria;D_3 Chloroplast; ;
	D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 Rhodospirillales;D_4 AEGEAN-169 marine group;
	D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 Rhodobacterales;D_4 Rhodobacteraceae;D_5 HIMB11
	D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 SAR11 clade;D_4 Clade I;D_5 Clade Ia
	Day 4
	D_0 Bacteria;D_1 Bacteroidetes;D_2 Bacteroidia;D_3 Flavobacteriales;D_4 Crocinitomicaceae;D_5 *Fluviicola*
	D_0 Bacteria;D_1 Bacteroidetes;D_2 Bacteroidia;D_3 Flavobacteriales;D_4 Flavobacteriaceae;D_5 *Polaribacter* 4
	D_0 Bacteria;D_1 Bacteroidetes;D_2 Bacteroidia;D_3 Flavobacteriales;D_4 Cryomorphaceae;D_5 uncultured
	D_0 Bacteria;D_1 Cyanobacteria;D_2 Oxyphotobacteria;D_3 Chloroplast;D_4 Phalacroma mitra;D_5 *Phalacroma mitra*
	D_0 Bacteria;D_1 Proteobacteria;D_2 Gammaproteobacteria;D_3 SAR86 clade; ;
	Day 6
	D_0 Bacteria;D_1 Bacteroidetes;D_2 Bacteroidia;D_3 Flavobacteriales;D_4 Flavobacteriaceae;D_5 uncultured
	D_0 Bacteria;D_1 Proteobacteria;D_2 Gammaproteobacteria;D_3 Cellvibrionales;D_4 Halieaceae;D_5 OM60(NOR5) clade
	D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 Rhodospirillales;D_4 AEGEAN-169 marine group;

	D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 SAR11 clade;D_4 Clade I;D_5 Clade Ia D_0 Bacteria;D_1 Proteobacteria;D_2 Gammaproteobacteria;D_3 SAR86 clade; ; **Day 18** D_0 Bacteria;D_1 Proteobacteria;D_2 Gammaproteobacteria;D_3 SAR86 clade; ; D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 Rhodobacterales;D_4 Rhodobacteraceae;D_5 HIMB11 D_0 Bacteria;D_1 Cyanobacteria;D_2 Oxyphotobacteria;D_3 Chloroplast; ; D_0 Bacteria;D_1 Bacteroidetes;D_2 Bacteroidia;D_3 Flavobacteriales;D_4 Flavobacteriaceae;D_5 NS5 marine group D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 SAR11 clade;D_4 Clade I;D_5 Clade Ia **Day 19** D_0 Bacteria;D_1 Proteobacteria;D_2 Gammaproteobacteria;D_3 SAR86 clade; ; D_0 Bacteria;D_1 Cyanobacteria;D_2 Oxyphotobacteria;D_3 Chloroplast; ; D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 Rhodobacterales;D_4 Rhodobacteraceae;D_5 HIMB11 D_0 Bacteria;D_1 Bacteroidetes;D_2 Bacteroidia;D_3 Flavobacteriales;D_4 Cryomorphaceae;D_5 uncultured D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 SAR11 clade;D_4 Clade I;D_5 Clade Ia
GC09	**Day 2** D_0 Bacteria;D_1 Bacteroidetes;D_2 Bacteroidia;D_3 Flavobacteriales;D_4 Flavobacteriaceae;D_5 uncultured D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 Rhodobacterales;D_4 Rhodobacteraceae; D_0 Bacteria;D_1 Proteobacteria;D_2 Gammaproteobacteria;D_3 Alteromonadales;D_4 Alteromonadaceae;D_5 *Glaciecola* D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 Rhodobacterales;D_4 Rhodobacteraceae;D_5 HIMB11 D_0 Bacteria;D_1 Bacteroidetes;D_2 Bacteroidia;D_3 Flavobacteriales;D_4 Cryomorphaceae;D_5 uncultured **Day 4** D_0 Bacteria;D_1 Proteobacteria;D_2 Gammaproteobacteria;D_3 Cellvibrionales;D_4 Porticoccaceae;D_5 C1-B045 D_0 Bacteria;D_1 Bacteroidetes;D_2 Bacteroidia;D_3 Chitinophagales;D_4 uncultured;D_5 uncultured Bacteroidetes/Chlorobi group bacterium D_0 Bacteria;D_1 Cyanobacteria;D_2 Oxyphotobacteria;D_3 Chloroplast;D_4 Aureococcus anophagefferens;D_5 *Aureococcus anophagefferens* D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 Rhodobacterales;D_4 Rhodobacteraceae;D_5 HIMB11 D_0 Bacteria;D_1 Proteobacteria;D_2 Gammaproteobacteria;D_3 Oceanospirillales;D_4 Nitrincolaceae;D_5 *Marinobacterium* **Day 6** D_0 Bacteria;D_1 Actinobacteria;D_2 Actinobacteria;D_3 PeM15;D_4 uncultured Actinomycetales bacterium;D_5 uncultured Actinomycetales bacterium D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 SAR11 clade;D_4 Clade I;D_5 Clade Ia D_0 Bacteria;D_1 Cyanobacteria;D_2 Oxyphotobacteria;D_3 Chloroplast; ; D_0 Bacteria;D_1 Proteobacteria;D_2 Gammaproteobacteria;D_3 SAR86 clade; ; D_0 Bacteria;D_1 Proteobacteria;D_2 Gammaproteobacteria;D_3 Thiotrichales;D_4 Thiotrichaceae;D_5 uncultured **Day 18** na **Day 19** D_0 Bacteria;D_1 Actinobacteria;D_2 Actinobacteria;D_3 Propionibacteriales;D_4 Propionibacteriaceae;D_5 *Cutibacterium* D_0 Bacteria;D_1 Bacteroidetes;D_2 Bacteroidia;D_3 Flavobacteriales;D_4 Flavobacteriaceae;D_5 NS5 marine group D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 Rhodobacterales;D_4 Rhodobacteraceae;D_5 HIMB11 D_0 Bacteria;D_1 Proteobacteria;D_2 Betaproteobacteria;D_3 Methylophilales;D_4 Methylophilaceae;D_5 OM43 clade D_0 Bacteria;D_1 Proteobacteria;D_2 Alphaproteobacteria;D_3 SAR11 clade;D_4 Clade I;D_5 Clade Ia

*na = Sequence data not available.