MSc (by Research)

Does a 'natural environment' increase growth and reduce the mortality of *Litopenaeus vannamei* within a closed aquaculture system.

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Abstract

This investigation is into the ability to produce similar or increased levels of prawn growth and a reduction in mortality within two different enriched environments, compared to commercial standard of a bare environment. This is to address limitation issues with the sustainability and efficiency of commercially grown prawns in closed system aquaculture. The research is a response to a question proposed by a prawn aquaculture company (FloGro Fresh) as they would like to improve the sustainability and lower the costs of their current operation by optimising growth and reducing mortality rates. Two trials of differing enriched environments (Filter Brush and 'Natural') were carried out, with water quality analysis and husbandry checks carried out daily throughout both trials. The length and weight of individuals in the different treatments were measured at the end of each trial, to determine which environment had statistically significant effects on growth and mortality. Additionally, in trial 2 another tank size was introduced to analyse the effect of tank size on prawn growth and mortality, of each environment in addition to the previous tank size. Behaviour within trial 2 was recorded to assess the effect environment and tank size has on the behaviour of prawns within a closed system. This work demonstrates culturing *Litopenaeus vannamei* in added enriched material environments (of Filter Brush and 'Natural') can produce increased growth. The implications of this research are wide reaching within aquaculture because it introduces the possibility of creating a more productive environment, to optimise the husbandry process reducing the losses made in the rearing process, providing both economic and environmental benefits to the industry.

Keywords: Prawn husbandry, aquaculture, sustainability, aquaculture environment.

1. Introduction

1.1 General Species Information

The white leg prawn (*Litopenaeus vannamei*) is native to tropical Pacific waters, and inhabits year-round temperatures exceeding 20 ºC. Aquaculture of *L.*vannamei in varying intensities began in the 1970s; the species is suitable for farming as it is effective at using the existing natural productivity of ponds. In addition to this the cost of feed used in production is lower than other similar species used in aquaculture, such as king tiger prawn (*Penaeus monodon*) due to the protein requirement of *L.*vannamei being lower. Despite this lower protein requirement within five months, individuals reach marketable size and can weigh up to 25g (FAO, 2018; marinespecies, 2018).

1.1.1 Taxonomic Tree

Domain: Eukaryota
  Kingdom: Metazoa
    Phylum: Arthropoda
      Class: Malacostraca
        Order: Decapoda
          Family: Penaeidae
            Genus: Litopenaeus
              Species: *Litopenaeus vannamei*

Species described by Boone in 1931 (*Litopenaeus vannamei* (whiteleg shrimp), 2020).

1.2 Distribution, Habitat, Diet and Life Cycle

The habitat of *L.vannamei* is within the Gulf of Panama, ranging from Sonora, Mexico to Tumbles, Peru in the eastern Pacific (figure 1.1). Within this environment, water temperature ranges from 26-32°C and salinities of 2-45 (Holthuis *et al*., 1998). There has also been several introductions of the species: in the US, coasts around Florida and Texas, in Asia, Philippines (1980), Taiwan (1981), and China (1988), through aquaculture (Briggs *et al*., 2004; cabi, 2018).

Figure 1.1: Map of Central America and north- east South America. Distribution of *Litopenaeus Vannamei* shown in red (Species Distribution Map Viewer, 2020).

1.2.1 Habitat

Table 1.1 shows a variety of different water environments ranging in foremost salinity, from freshwater through brackish to marine, but also present varying habitats within those different water environments. Demonstrating the ability of *Litopenaeus vannamei* to inhabit various environments, with a range of food sources (*Litopenaeus vannamei* (whiteleg shrimp), Natural food sources (2020) Cabi.org.)

Table 1.1: Habitats of Litopenaeus vannamei, in varying waters, created by Author with information from (Litopenaeus vannamei (whiteleg shrimp), Natural food sources (2020) Cabi.org.)

Category	Habitat	Presence
Brackish	Inland saline areas	Present
	Estuaries	Principal habitat
	Lagoons	Principal habitat
Littoral	Coastal areas	Principal habitat
	Mangroves	Principal habitat
	Mud flats	Principal habitat
	Salt marshes	Principal habitat
Freshwater	Irrigation channels	Present
Marine	Inshore marine	Principal habitat
	Coral reefs	Present
	Benthic zone	Principal habitat

Within these environments there are variety of species that predate *Litopenaeus vannamei*, throughout their life stages, dragonflies, otters and various bird species: Egrets, Gulls, cormorants and terns (*Litopenaeus vannamei* (whiteleg shrimp), Natural food sources (2020) Cabi.org.)

1.2.2 Diet

The species has several metamorphic stages throughout its life, within the metamorphic stages individuals feed on a variety of different things. As the species develops through these stages, it is able to consume larger and more complex organisms, due to the species development and maturation of feeding appendages and digestive capabilities (Litopenaeus vannamei (whiteleg shrimp), Natural food sources (2020) Cabi.org; Ghosh *et al*., 1994). Table 1.2 and 1.3 show what the species eats within a natural environment and an animal husbandry environment.

Table 1.2: Diet through metamorphic stages within a natural environment, created by Author with information from (Litopenaeus vannamei (whiteleg shrimp), Natural food sources (2020) Cabi.org.)

Food Source	Life Stage
Aquatic And Benthic Phytoplankton e.g. Diatoms	Adult/Fry/Larval
Bacterial Flocs	Adult/Fry
Benthic Polychaetes	Adult/Fry
Detritus/Benthic Bacteria	Adult/Fry
Zooplankton e.g. Copepods	Adult/Fry

Table 1.3: Diet through metamorphic stages within aquaculture, created by Author with information from (Ghosh et al., 1994).

Stage	Feed
Protozoea and early Mysis	Phytoplankton
Mysis and early Post Larvae	Zooplankton, rotifers and Brine Shrimp
Post Larvae older than PL6	Bottom feeding starts, change in feeding appendages allows for particulate feed
Spawner	Feed on worms and squid

1.2.3 Life Cycle

Figure 1.2 shows the migration of peneaid prawns over their lifecycle, spawning in the sea and after several moult stages: naupli- protozoea- mysis, post larvae drift to coasts, inhabit estuaries and backwaters, and grow into juveniles. When they have reached a certain size at juvenile stage during their metamorphosis, they migrate back to the sea when spawning takes places (Ghosh *et al*., 1994).

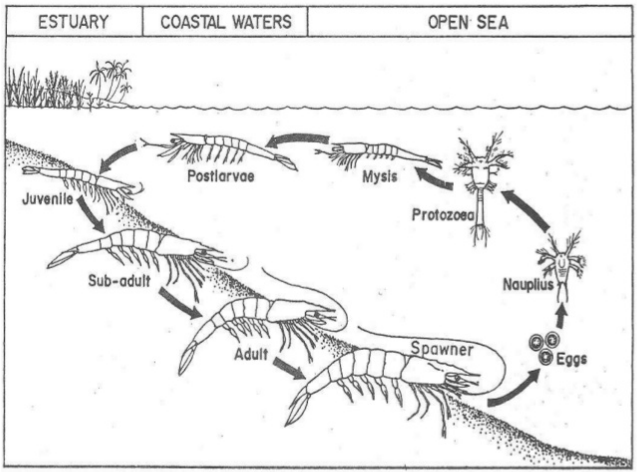


Figure 1.2: Life cycle of Penaeid Prawns, including movement between open and estuarine environments (Shrimpism, 2017).

1.3 Species Anatomy

1.3.1 External Anatomy

Figures 1.3 and 1.4 show the internal and external anatomy of a PL penaeid.

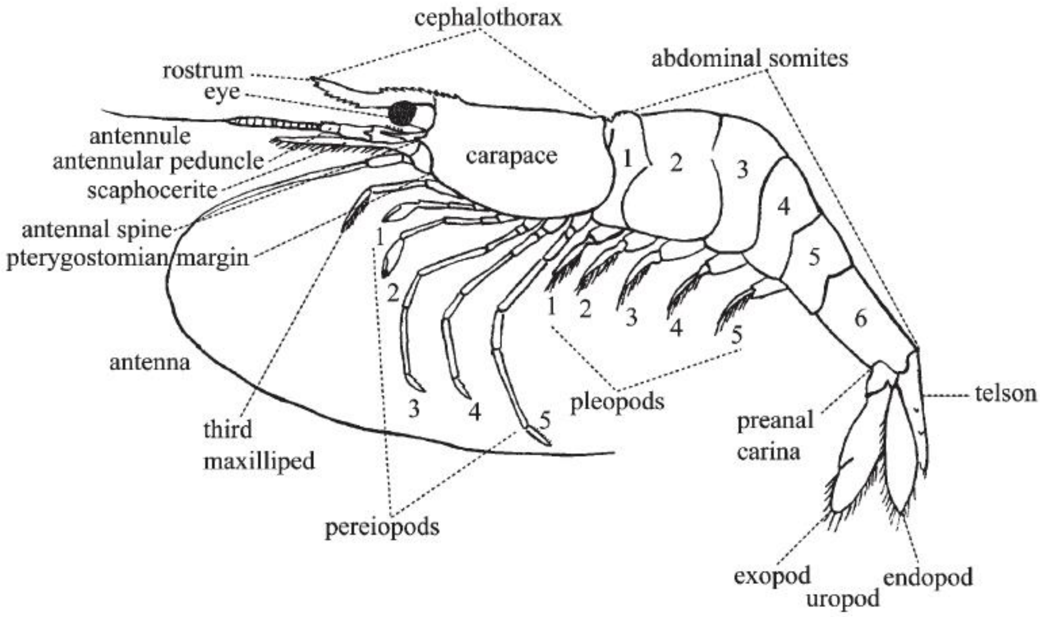


Figure 1.3: External Anatomy of a Penaeid Prawn (Shrimpism, 2017).

1.3.2 Internal Anatomy

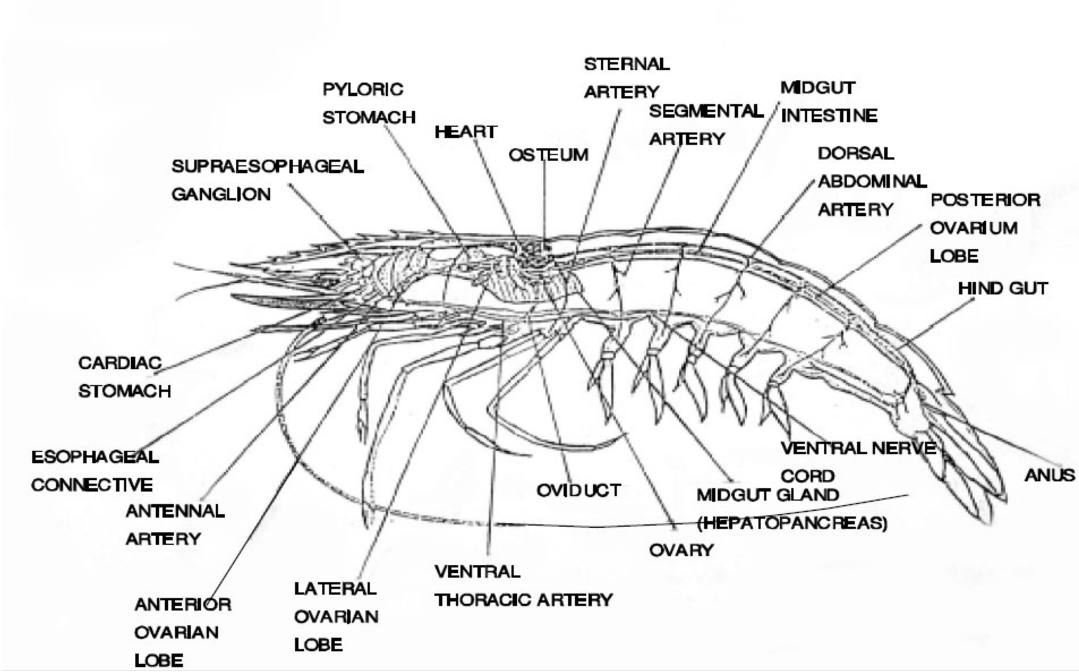


Figure 1.4: Internal Anatomy of a Penaeid Prawn (shrimpculture, 2017).

1.3.3 Metamorphic Stages

Nauplius is the first metamorphic stage (Figure 1.5). After hatching the body shape of naupli resemble a pear, with three sets of appendages, over a 2-day period naupli undergo six successive moult stages (N1(nauplii 1)-N6). Naupli swim actively but do not feed, they subsist on an internal yolk sac. Nauplius metamorphoses into protozoea. Protozoea consists of three stages (PZ1(protozoea 1)-PZ3) and lasts 3-4 days, distinct by a broad 'head' and a narrow forked 'tail'. PL1 (post larvae 1) has sessile eyes and frontal organs on the head. In PL2, the eyes become stalked and a rostrum appears. Within PL3, the abdominal segments develop dorsomedian spines and uropod buds form near the end of the forked tail. Additionally, the alimentary canal, mouth and feeding appendages form, enabling the larvae to filter feed unicellular algae. The larvae at this stage are attracted by light. The Mysis stage follows on from protozoea and comprises of three sub stages (M1(mysis 1)-M3), lasting 3-4 days. The latter two stages can be distinguished from the first stage by the development of pleopod buds (swimming leg rudiments). The second stage of Mysis sees the development of pleopod buds, this is further pronounced in third stage, as they undergo segmentation. The larvae retain the ability to filter algal cells, as the claws on the first three legs are not yet functional and are therefore unable to capture prey. The legs have only swimming setae and the mandibles are weak and within this stage individuals do not swim actively, they instead drift in the water column with their heads hanging down. The Post larvae stage follows the Mysis stage. This stage takes 25 days to complete and resembles the 3rd Mysis stage, except for several developments. Individuals develop setae on the pleopods, feeding appendages develop sharp cutting edges and claws become functional. The post larvae cease to be filter feeders and become capable of grasping large particular matter and zooplankton. The transition from post larval stage, PL25 to juvenile is gradual and not marked by metamorphic change. Juveniles migrate to the sea when they are ready to become breeders and experience gonad development, most males can experience this is brackish water, however females cannot attain maturity in such salinities, and therefore mature at sea in open water (Ghosh *et al*., 1994).

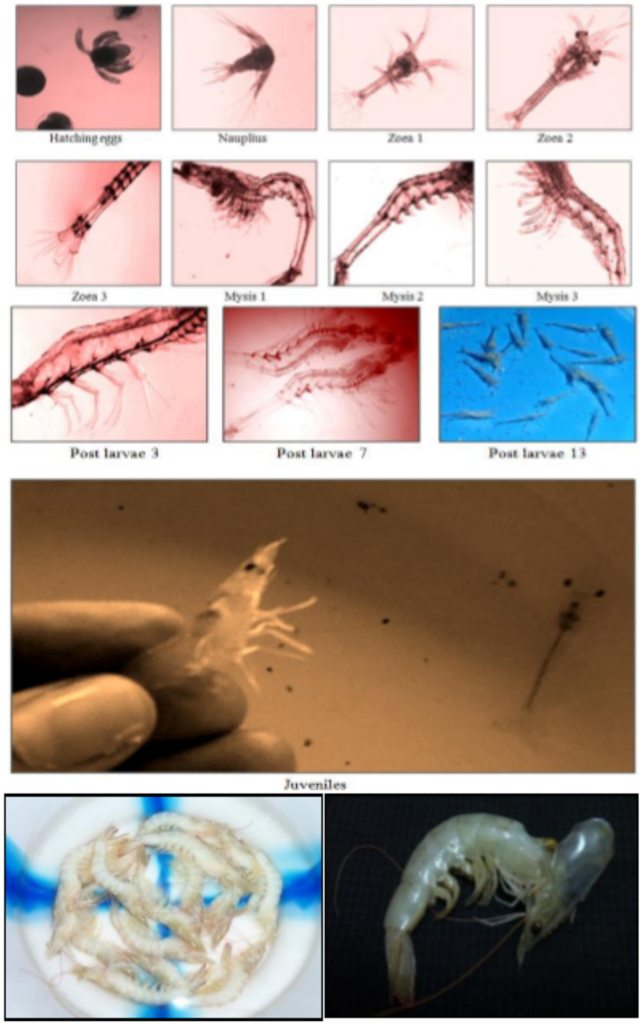


Figure 1.5: Photos showing the metamorphic stages of Penaeid Prawns, from Hatching eggs to Breading Adults (Chakraborty and Ghosh, 2013).

1.3.4 Male and Female Anatomy

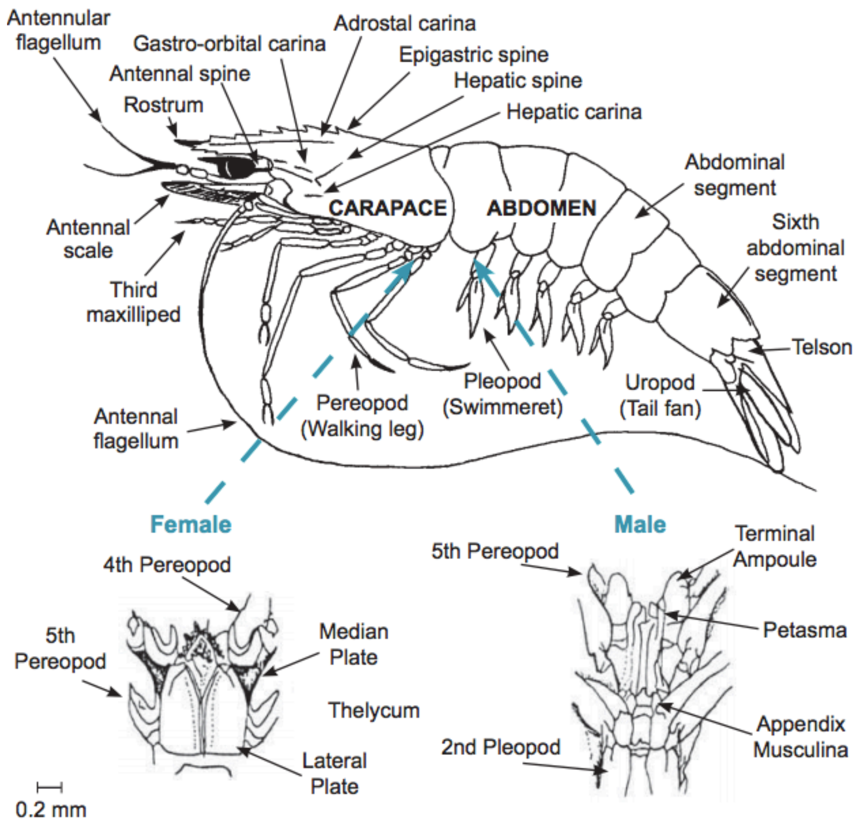


Figure 1.6: Male and Female morphology and body parts (modified from Motoh 1981 and SEAFDEC 1988, taken from Australian prawn farming manual, 2006).

The female has a sperm storing organ called thelycum (Figure 1.6), this is located on the ventral side of the cephalothorax, between the 4th and 5th walking legs. The ovary is located on the dorsal side of the prawn, in a spawner the ovary is dark green in colour and displays a lateral bulge in the first abdominal segment. Oviducts open at the base of the third pair of walking legs. Within males the petasma is the sex organ, this is located on the first pair of swimming legs on the first abdominal segment. The two sperm ducts from the male testis are located at the base of the 5th pair of walking legs. The terminal end of the sperm is enlarged forming the terminal ampule, where the spermatophore containing sperm is located. This is visible as a white mass at the end of the 5th pair of a walking leg (Ghosh *et al*., 1994).

1.4 Species Reproduction

Mating takes place after females moult, when the carapace is soft. The male spermatophore are transferred to the lycum within the female when mating, the spermatophore within the female can be seen as a whitish mass below the cuticle of the thelycum. During the time of impregnation, the ovary of females are immature, this means there is a lag in time between mating and spawning.

Spermatophores are retained for the inter-moult period and the sperm can then be used for fertilisation for successive spawning cycles (Ghosh *et al*., 1994).

1.5 Spawning

Spawning takes place during the night, the eggs are shed, and the females simultaneously releases sperm from the thelycum and fertilisation takes place in the sea water (Ghosh *et al*., 1994).

1.6 Purpose of Aquaculture

Firstly, *"aquaculture is the breeding, rearing, and harvesting of fish, shellfish, algae, and other organisms in all types of water environments."* (FAO, 2020). Aquaculture has taken place for many centuries as a method of farming fish for local consumption, these methods still take place today, providing areas that are landlocked, or do not have access to fishing with aquatic organisms. In many parts of the world, these traditional methods have remained unchanged; however, there has been developments in aquaculture and the technologies that surround it in the last 40 years, leading to its rapid use and employment around the world. By 2016, the aquaculture production of crustaceans have grown by 3% yearly since 2000. This is in part due to cost of aquatic products and their economy; weight for weight requiring less units of energy whilst also taking up less space than animal agriculture (FAO, 2020).

The demand for seafood and seafood products has increased and is set for further increases as world population rises and affluence increases. Aquaculture is seen as the answer to keep up with the increased demand for these products. In the European Union (EU) aquaculture is endorsed as an answer to food security issues, as a way to provide high quality healthy seafood, as well as creating employment opportunities alongside it (Gutiérrez *et al.*, 2020). Currently about 50% of seafood for human consumption is from aquaculture, this is set to rise 62% by 2030. Additionally, the world population is expected to rise to 10 billion (from 7 billion now) by 2050, this puts a greater demand on food production with the worlds animal protein requirement set to increase by 52%, sustainable approaches are sought after. Figure 1.7 demonstrates the protein and energy retention of a variety of reared animals for food production, with the conversion of edible meat i.e. product you get from feed given. As illustrated, food produced through aquaculture has a high protein and energy retention in comparison with the other animal protein sources, with the highest edible meat to feed given ratio. Multiple organisations see aquaculture as a way to provide consumers with the products they want with a reduced impact on the environment, through overfishing and stresses on natural resources. In addition the pressures that animal agriculture produces, is ever increasing, so providing an aquatic product without affecting wild stocks, allowing for those stocks particularly if they are endangered or threatened it can help these stocks to rebuild and replenish their populations. Whilst providing food for an ever increasing population with fewer impacts. (oceanservice, 2020; aquaculturealliance, 2020).

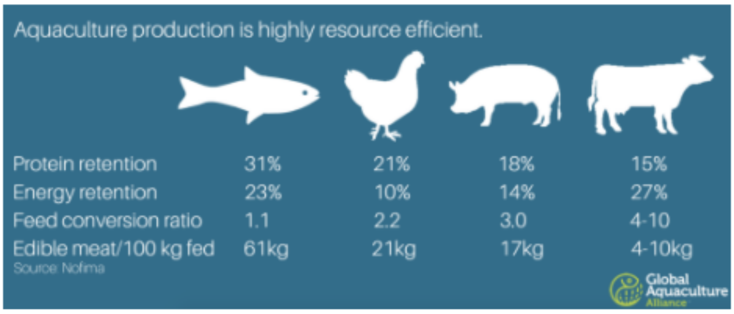


Figure 1.7: Protein and meat economy within a variety of animals (aquaculturealliance, 2020).

1.7 Commercial Aquaculture

The recent global development of aquaculture, means the industry supplies over 27% of the world's fish for by-product use and 50% of the world's fish for direct human consumption, Over the last 50 years, there has been a large increase in crustacean culture; in which over 30 prawn species are cultivated, with over 28% of prawns consumed worldwide being produced through aquaculture. Figure 1.8 charts the export value of a variety of species, prawns make up the largest value of a species as an aqua product. This results in large profits and employment opportunities within the aquaculture industry (Tacon, 2003; Advancing the Aquaculture Agenda, 2010; Agnalt *et al.*, 2011). A key challenge of the industry is the initial development of prawn aquaculture, this means that the wider effects of the industry are largely unknown, and therefore difficult to quantify. Prawn aquaculture is thought to pose issues and threats to sustainability through impacts on the health of world fish stocks, and can often create societal and environmental challenges for local communities; that include diverting much needed resources such as food and water. It is believed the most prominent worldwide effects are felt through using unsustainable fishing practices. Examples include collecting fish to make pellets for prawn aquaculture from often already exploited fish stocks, as some research suggests that over three quarters of the world's fish stocks are overexploited (Hertrampf *et al*, 2003; Botsford *et al*, 1997). The aim of sustainable aquaculture is to maximise benefits and minimise negatives on the environment; this needs to be done in a way that is sustainable, protecting the external environment for stakeholders that use it as well as those stakeholders protecting the environment for their own purposes. This is a key point that needs to be understood by the aquaculture industry because improper practices that negatively impact fish stocks, harm the industry and could lead to price of fish meal and oil as the main composite of feed being more expensive for the industry; without this understanding of the intrinsic link sustainable business growth is not viable (Frankic and Hershner, 2003).

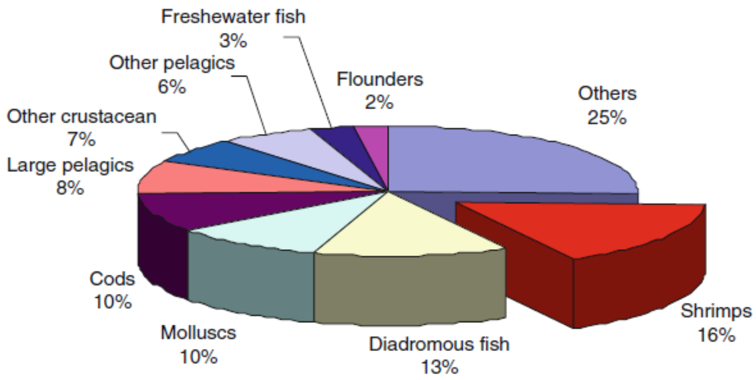


Figure 1.8: Export values of world aqua products by species in 2006 (Agnalt *et al.*, 2011).

1.7.1 Commercial Aquaculture of *Litopeneaus vannamei*

Increased human population and standard of living has supported the growth of aquaculture as an industry (Sookying *et al*., 2011). The majority, over 90%, of the prawn aquaculture in the western hemisphere involves one species, *Litopeneaus vannamei* (Araneda, *et al.*, 2008).

The culture of *L. vannamei* has expanded (figure 1.9) and its growing success and emerging preference over previously used species such as *Penaeus monodon,* around the world particularly in Asia where it is the most popular commercially, this emergence of culturing different penaeid species has a variety of explanations. The reasons for this change of species and growing preference within aquaculture are; because the production of *L. vannamei* has a variety of advantages compared to *P. monodon*, namely the lower production costs and higher productivity of the species as well its ability to be cultured in a wider range of environments (Agnalt *et al.*, 2011). These production advantages are due to *L. vannamei* having a higher availability of low cost broodstock that have been genetically selected to produce viral and pathogen free stocks, higher larval survival rate, greater tolerance to high stocking densities. *L. vannamei* is less carnivorous (FAO, 2020) and has lower protein requirements in its diet, 30% of their diet compared to a protein requirement of 45-50% within the diet of *P. monodon*, (Shiau, 1998). *L. vannamei* also exhibits better utilization of plant proteins in formulated feeds, faster growth rates (time taken to reach commercially viable size) (Cuzon *et al.*, 2004). This combination of lower protein requirements and better use of protein, as well as a reduced time period to reach commercially viable sizes, lends to reduced production costs due to the lower amount of feed required throughout the process. The lower amount of feed namely fish meal required reduces pressures on marine resources.

The species compared to other cultured penaeids has greater tolerances and adaptability to ranges in salinity e.g *Penaeus monodon* thought to have a salinity range of between 15 to 25, whereas *L. vannamei* can tolerate salinity ranges of between 1 to 50 (Jaffer *et al.,* 2020), larger tolerance to waste products (ammonia and nitrate) toxicity (Lin and Chen, 2003), and a lower susceptibility than *P. monodon* to viral pathogens. In addition to the physiological benefits of *L. vannamei* within aquaculture, the general supply of the broodstock is more stable than *P. monodon,* making it a more reliable species to some farmers, particularly if you do not breed and just culture juveniles (Agnalt *et al.*, 2011).

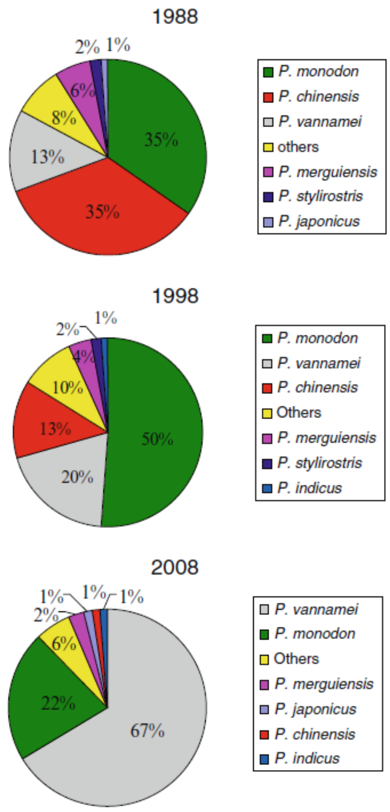


Figure 1.9: World consumption of penaeid prawns, 1988-2008 (Agnalt *et al.*, 2011).

However, despite these factors that are making *L. vannamei* the preferred species in Asia, a market remains for *P. monodon*, as it can grow larger and therefore is dominant in the 'jumbo sized' market, which is particularly profitable in the United States, as 'jumbo shrimp' are liked by its consumers. When the U.S. market does consume *L. vannamei*, it prefers individuals cultured in freshwater over those grown in brackish or saltwater. The increasing use of *L. vannamei* in penaeid culture could lead to a reduction in genetic diversity within the species due to domestication and breeding selection, the movement of individuals around the world, particularly within Asia, the largest market could lead to Asian specific viral and microbial diseases emerging and spreading within populations. *L. vannamei* is one of several cultured species that have established populations in alien habitats; this has an array of environmental and socioeconomic impacts (Agnalt *et al.*, 2011).

The growth of the production of *L. vannamei* within aquaculture and the rise of is preference as a species over *P. monodon* in Asia is charted within Figures 1.10 and 1.11. Figure 1.10 shows the rise of the production of *L. vannamei* in both aquaculture and in fisheries, with the aquaculture industry production taking over from the number caught in fisheries in 2007 (highlighted by a red circle (figure 1.10)). The growth of the use of *L. vannamei*, is further illustrated through Figure 1.11, showing the production of *P. monodon* and *L. vannamei* within Asia between 1988-2008. The figure demonstrates the rise of *L. vannamei* as the prevailing species within Asian aquaculture, the species production becomes more prevalent from 2004 (red circle (figure 1.11) onwards, perhaps due to the reasons mention above, outlining its suitability for a range of different culture methods making it more adaptable of the previously most widely used *P. monodon* (Agnalt *et al.*, 2011).

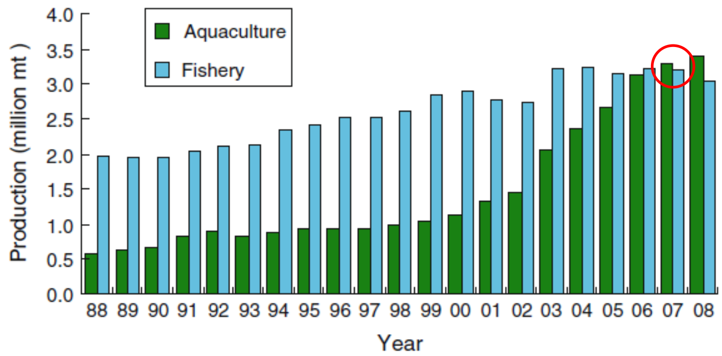


Figure 1.10: World prawn production of *L. vannamei*, 1988-2008 (Agnalt *et al.*, 2011). Red circle added by author.

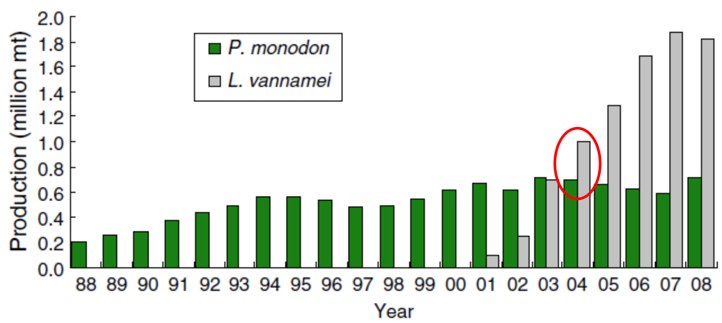


Figure 1.11: Prawn production of *L. vannamei* and *P. monodon* in Asia, 1988-2008 (Agnalt *et al.*, 2011). Red circle added by author.

Another possible explanation to the rise of the use of *L. vannamei* and its emerging preference is the greater tolerance and resistance to viruses and diseases the species exhibits (Briggs *et al*., 2004). These are critically important within aquaculture, as viruses spreading throughout cultured populations can be costly (Agnalt *et al.*, 2011). White Spot Syndrome Virus, (WSSV), a virus prevalent amongst cultured penaeid species that causes high levels of mortality, reduced food consumption and lethargy within populations (Mahy & Van Regenmortel, 2008; FAO, 2020). The emergence of WSSV in the early

1990s amongst prawn farms around the world almost brought the industry to collapse and left many farms bankrupt as the high mortality rates and the increased time taken to reach market size caused by the disease reduced the productivity of farms (Chamberlain, 2010). Flegel (2006) estimates the economic impact of WSSV to prawn aquaculture at US$10 billion, so a species resistance to WSSV is of great economic importance. Due the species ability to be cultured in a wide range of environments, including that of low salinity levels. *L. vannamei* can be produced in inland areas, reducing the potential of individuals being exposed to disease and creating environmental problems through releasing disease to native and local populations through being isolated from other species (Agnalt *et al.*, 2011). As well as WSSV, several other viruses can be costly to aquaculture facilities. Taura Syndrome (TS) causes an array of symptoms that can result in low mortality, as well as Infectious Hypodermal and Haematopoietic necrosis (IHHNV) causing mortality and reduced growth rates; however availability of *L. vannamei* SPF and SPR broodstock reduces the effect these other viruses can have on the industry (FAO, 2020).

1.7.1.1 Main Producer Countries

L. vannamei is cultured all over the world via different methods. According to the Food and Agricultural Organisation (FAO) the main producing countries are as follows: “China, Thailand, Indonesia, Brazil, Ecuador, Mexico, Venezuela, Honduras, Guatemala, Nicaragua, Belize, Vietnam, Malaysia, Tawian P.C., Pacific Islands, Peru, Colombia, Costa Rica, Panama, El Salvador, the United States of America, India, Philippines, Cambodia, Suriname, Saint Kitts, Jamaica, Cuba, Dominican Republic, Bahamas” (FAO, 2020).

1.7.2 Commercial Procedure

Figure 1.12 illustrates the most common commercial production cycle of the aquaculture of *L. vannamei.* The following section will discuss these practices in detail.

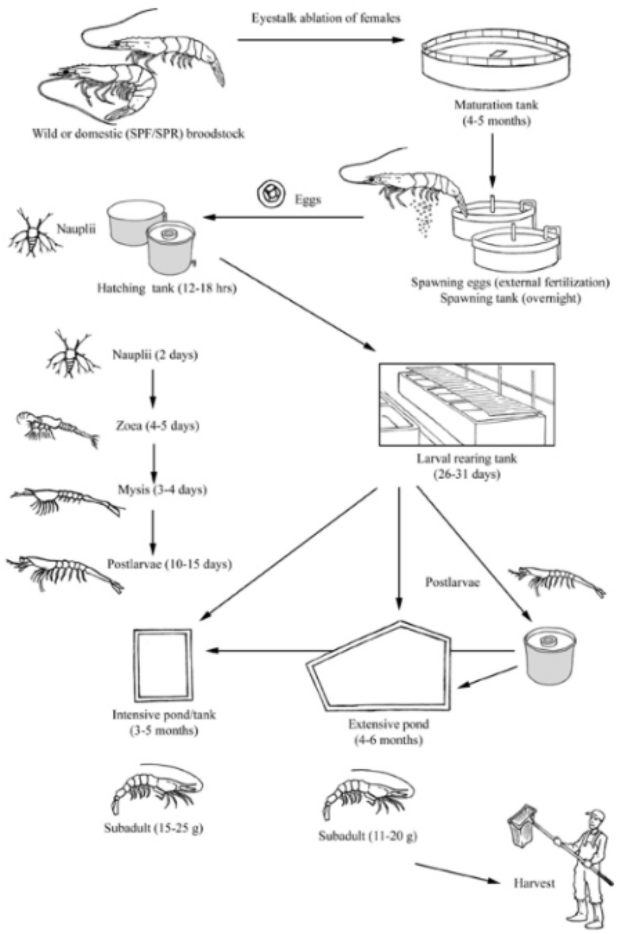


Figure 1.12: Production cycle of *L. vannamei* within aquaculture (FAO, 2020).

1.7.2.1 Broodstock

Broodstock are obtained in 3 different ways;

- Sea-caught, weighing over 40g and over 1 year old, as this is an indication that they are of spawning age.
- Cultured prawns that have been assigned for spawning and transferred on to maturation tanks.
- Specifically reared Specific Pathogen Free (SPF) and Specific Pathogen Resistant (SPR) stock.

Maturation tanks in dark rooms are supplied with filtered seawater and have a diet of fresh and specifically designed broodstock feeds. One eyestalk of each female is usually ablated. Unilateral eyestalk ablation is used in captivity to induce maturation. Once the carapace has hardened, 2-3 days after moulting, prawns are ablated, a technique used to mature individuals. A blade is passed through the middle of eyestalk and then the contents of eyestalk are squeezed out removing the optic ganglia and neurosecretory centres which produce ovary inhibiting hormones (Ghosh *et al*., 1994) leading to repeated maturation and spawning. Inducing moulting in commercial aquaculture takes 4-7 days and involves acclimatising the broad stock by aerating the tank and replacing 60% of the water daily. Once the prawns have recovered from transport stress, their moult is induced through reducing salinity levels to 4-5ppt for 2 days and then after moulting the salinity is returned to its normal level of 30-32ppt (Ghosh *et al*., 1994). Females reproduce most effectively between 8-10 months old, spawning success is dependent on the quality of the broodstock and water conditions, the better the quality of the broodstock and water condition higher spawning rates are achieved. Females either spawn collectively or in separate tanks to avoid the transmission of disease. Nauplii are collected aided by them being attracted to light, rinsed with seawater and then bathed in iodine and or formalin to disinfect them, before being rinsed again and placed into larval rearing tanks, for the next stage of the process (FAO, 2020).

1.7.2.1 Hatchery and Nursery

Nauplii are stored in tanks of varying degrees of sophistication, with preferably ‘U’ or ‘V’ shaped bottoms of volumes of up to 100m^3, made from a variety of materials e.g. concrete to plastics. The larvae stay in these tanks till PL10-12, or in more sophisticated systems harvested at PL4 and moved into flat bottomed raceways and reared until PL10-30. If good water quality is maintained survival rates are about 60% (FAO, 2020). In order to maintain good water conditions, water should be filtered or exchanged daily. At this developmental stage individuals can be fed on formulated feed or live food such as microalgae or *Artemia* as part of their diet. Throughout this process measures are put in place to varying degrees to reduce pathogen contamination. This is achieved by having adequate filtration and/ or chlorination of tanks and water feeding into the system. The nauplii or PL can also be disinfected, or be given anti or probiotics to inhibit or reduce pathogens (FAO, 2020; Ghosh *et al*., 1994).

Not all farming systems use separate nurseries, some facilities transport individuals at PL10 to grow out ponds and systems. However, some facilities use nurseries for 1-5 weeks, particularly in colder regions, PLs can be nursed to larger sizes (0.5g) in heated tanks before going into larger grow out systems (FAO, 2020).

1.7.2.1 Growing Techniques

Extensive

A method popular in Latin America, the grow out of *L. vannamei* is conducted in tidal areas where there is usually no aeration or it is limited. Ponds are up to 30ha and on average 1m deep. Individuals are fed on high protein formulated feeds daily, but feed mostly on naturally occurring food within the ponds. The ponds are usually stocked at low densities of 4-10 individuals per m^2 from hatcheries. Production yields of this method are low of up to 500kg/ha per crop and often enable 1 to 2 crops a year (FAO, 2020).

Semi-intensive

Also popular in Latin America, semi-intensive ponds of 1-5ha are usually stocked at a density of 10-30 hatchery produced post-larvae (PL) per m^2 in pond depths of 1m. A pump carrying out water exchange facilitates minimal aeration. A formulated feed is added 2-3 times a day; individuals also additionally feed on natural food available within the ponds. Semi-intensive methods often produce a yield of 500-200kg/ha/crop at 2 crops a year (FAO, 2020).

Intensive

This type of growing technique is most common in Asia and Latin America. This method can be located further inland away from tidal areas, the ponds can be more easily drained and prepared before re-stocking, reducing contamination. The species tolerance to low salinity allow for this move away from the sea to cheaper inland areas. These ponds are often smaller than the above methods at 0.1-1ha, but deeper at more than 1.5m in depth and stocked at a density of 60-300PL/m^2. Intensive grow out methods actively put in place measures to enhance and maintain water quality in order to increase productivity, e.g. ponds are often lined, and the water is heavily aerated to increase oxygenation and water circulation. Some systems add in 'bacterial floc' to maintain water quality through keeping ammonia and nitrates levels low. Individuals within these systems are fed 4-5 times a day. There is a greater focus on viruses and the damage they can cause within a population and the subsequent loss in productivity and income to the industry. Therefore some aquaculturists use Specific Pathogen Free (SPF) and Specific Pathogen Resistant (SPR) stock and carry out biosecurity checks to reduce the effect an outbreak might have. This method of production can produce an up to 20,000kg/ha/crop, with 2-3 crops within a year (FAO, 2020).

Super-intensive

There is growing research into the productivity of super-intensive farming methods, the majority of this research is being carried out in the United States of America. In which *L. vannamei* is being grown in enclosed raceway systems; systems that have an enclosed area for husbandry and another area for water purification, in greenhouses, with only water exchange through replacement of losses through evaporation. These systems are stocked with SPF post larvae and fed multiple time a day, sometimes hourly on a food belt system. As the systems are enclosed they are bio secure, and have a lower ecological footprint as waste is minimal due to the ability of the system to recycle waste products. This is a more eco-friendly, cost efficient method of producing high quality prawns for consumption. The raceways are stocked with 300-450PL/m^2 for a growth period of 3-5 months producing an up 68,000kg/ha/crop yield (FAO, 2020).

1.7.2.1 Harvesting and Processing

The harvesting technique used is dependant to the grow out method used. Individuals in extensive and semi-intensive systems can be harvested through draining the pond or pumping water out, or directly pumping the prawns out into a system where the water can drain out. A similar process may be used in intensive ponds or through using seine nets to corral individuals to one area where they then can be removed through hand netting. In Asia partial harvesting is common after 3 months. Super intensive systems harvest with scoop nets when individuals are required (FAO, 2020).

The processing of prawns is carried out by teams specialised in the harvesting and handling of individuals in order to maintain quality and preserve profit (higher quality, higher the price). Prawns are then placed in an ice bath (0-4°C) to immediately kill them, before they are washed and weighed for further processing. During this process, sodium metabisulophate is added to prevent melanosis; which reduces the shelf life of the individuals (FAO, 2020; Gonçalves & de Oliveira, 2016). Prawns are then transported in icy-water insulated containers for further processing or to market. For export, processing plants further process them by size, and often freeze them for further national or international travel (FAO, 2020).

1.7.3 Issues with Commercial Procedure

Current practices around the aquaculture of *L. vannamei* and its expansion have created prompted discussion over the procedures in use and their effect on the environment leading to questions surrounding the industries sustainability. The main issues that arise in some of the aquaculture procedures used are:

- Production in tidal areas using mangrove ecosystems for ponds.
- The use of an area for ponds for a minimal amount of time before moving to a new location.

- Farming in inland ponds leading to the salinisation of agricultural land and groundwater stores, through saltwater leaking.
- Pond effluents polluting coastal and inland waters.
- Destruction of marine ecosystems through the over and inefficient use of fishmeal.
- Accidental introduction of non-native species into alien waters, and possible pathogens they could be carrying.
- Resource conflicts.
- Discharges from farms causing pollution issues in pond growing areas.

The aquaculture industry is driven by consumer concerns and is trying to reduce and mitigate these impacts. The improvements in aquaculture technology help to alleviate some of these concerns, as the movement of the industry to inland closed intensive farming systems lessens these impacts (FAO, 2020).

1.8 Sustainable Aquaculture

The growth in aquaculture industry alongside the following concerns about the current state of fish stocks, has led to greater interest in developing more sustainable feeds and procedures. This is in part due to consumers becoming more interested in where their food comes from and wanting their food to be as environmentally friendly as possible. One way of achieving a more sustainable food product is through gains in efficiency within the aquaculture process between hatcheries and the grow out process; increasing the food conversion efficiency through an increased survival in ponds, reducing the grow-out period, and increasing the amount of crops per year, all have produced a more cohesive efficient process increasing the sustainability of prawn aquaculture (Arnold *et al*., 2006). Additionally, the aquaculture producers of *L.*vannamei were once consigned to the prawns' native Pacific countries however the intensive aquaculture of the species has expanded into Europe, and therefore more types of feed and new procedures need to be considered in order to keep up with the growing number of nations producing prawns through aquaculture (Wickens, 2008). Many organisations like FloGro Fresh have built sustainability into their business model. FloGro Fresh ensure all their fishmeal is traceable and complies with the International Fishmeal and Fish Oil Associations Global Standard and the Certification Programme for the Responsible Supply of Fishmeal and Fish Oil (IFFO RS), guaranteeing none of their fishmeal ingredients are from unsustainable sources, this is an avenue that many companies are beginning to explore (flogrosystems, 2018; iffo, 2018).

Another area from which a sustainable solution may come from is microalgae. Algae are thought to be a good solution for several reasons: as they inhabit aquatic environments, and therefore if algae have sufficient access to light and CO_2 they will grow and will provide a continual source of food for the husbandry of aquatic animals (Rosenberg *et al,* 2008). Currently, annual production of algal biomass is 5 million kg/yr, one fifth of this is used to feed fish and shellfish within aquaculture systems (Muller-Feuga, 2004). Within prawn farming microalgae are necessary at the beginning of the process as they are required for larval development (Jamali, *et al,* 2015), and in some systems algal blooms are encouraged, because they produce favourable conditions for prawn growth (Rosenberry, 1991). Due to the high costs of algae and the labour required to keep blooms under control thereby avoiding the interruption the nutrient balance of a system, it is unlikely that algae will be a viable partial feed replacement in the near future. Algae similarly to other alternative feeds to fishmeal needs to be investigated and fine-tuned, before it could become a viable option as a sole or supplementary feed for prawn aquaculture (Hemaiswarya *et al,* 2010).

1.8.1 Flogro Fresh Systems

FloGro Fresh, a prawn aquaculture company in Lincolnshire, UK sustainably produces inland prawns within a contained closed ecosystem, and delivers them within 24hrs. This simulates the physical and biological processes within aquatic ecosystems that oxygenate water and remove potentially harmful waste. The closed looped system, aims to provide an alternative to imported prawns, reducing air miles and the carbon footprint, as well as the damage caused to the environment that harvesting wild farming prawns can cause. The contained ecosystem uses natural filtration, probiotic bacteria and seaweed to ensure good water quality; a fully traceable sustainable feed endorsed by the International Fishmeal and Fish Oil Associations Global Standard and Certification Programme for the Responsible Supply of Fishmeal and Fish Oil (IFFO RS); as well as running on renewable energy sources (solar and wind) to reduce the carbon footprint of the system (Flogrosystems, 2018). Due to the company's interest in sustainable production and the reduction of its carbon footprint, FloGro Fresh systems want to breed their own prawns humanly, reducing the need of importing larvae from the USA. A humane focus to breeding is crucial with fitting in with the company's ethos, but is also essential to enable sales to more welfare conscious consumers, a study by Yin *et al*. (2020), found consumers valued prawns with organic labels, traceable information over those that did not, they were also willing to pay more for these products than others (Yin *et al*., 2020). Furthermore, FloGro Fresh wish to investigate and explore other avenues of sustainability, which has initiated and informed this research study.

1.9 Ethical Statement

As this experimentation for this research involves a live species: *Litopenaeus vannamei*. Handling this species requires care, especially when removing the prawns from the tanks for measuring, this needs to be done as quickly as possible to minimise the stress the species is under. Tanks will be kept at conditions optimal to life to reduce stress and harm to the species. This study was given ethical approval by the University of Hull (Ethics number 'U113 Prawn', dated 18/09/2019).

1.10 Similar Work

One the challenges facing the food industry as a whole is sustainable production. To achieve sustainable aquaculture the effect of introducing shelters, varying environments and artificial substrates into culture environments of several prawn species have been investigated. However, there are no published studies on the effect of shelters on *Litopenaeus vannamei,* in regard to their growth, mortality and behaviours, but this section will discuss the effect of shelters on freshwater and marine prawn species. A study by Murthy *et al* (2012) looked at the effect of creating shelter through placing pipes, tyres and plants into tanks, with the aim of reducing aggression and cannibalism, and therefore increasing survival and growth rates within the widely cultured Freshwater Prawn- *Macrobrachium rosenbergii* (Murthy *et al.,* 2012). Similarly with this species Ra'anan and Cohen (1984) investigated the effect of juvenile individuals being reared communally and in isolation to size distribution, the study found a greater size difference in the individuals reared communally, suggesting that *Macrobrachium rosenbergii* size variance is less effected by genetic differences in growth potential but growth is more effected by the interactions that take place within a cohort, relating to the effect aggression and cannibalism plays within a system (Ra'anan and Cohen, 1984). Another study that looked at differences in environment by Hermawan and Nirmala studied the effect of different tree branches (Mangrove, Coconut and Bamboo) on the growth and survival rate of Tiger Shrimp, *Penaeus monodon*, the shelter created through the branches increased survival rates compared to a controlled branchless environment, however the study found there was no significant difference in growth of the Tiger Shrimp (Hermawan and Nirmala, 2011).

The effect of different textured tank bottoms in laboratory conditions have also been studied to investigate the effect substrate has on production and what substrate is most suitable for maximum prawn growth and survival, and for this there has been studies on *Litopenaeus vannamei*. Moss and Moss (2204) found there was an effect in the weight of post-larval Pacific white leg shrimp *Litopenaeus vannamei* when reared in tanks with an artificial substrate, individuals in substrate lined tanks had a higher final growth weight than those without, it is thought the substrate provided a greater surface area for individuals to graze on as well as serving as refuge from other individuals (Moss and Moss, 2004). Schveitzer *et al* (2013)found cohorts of *Litopenaeus vannamei* grown in a system with an artificial substrate had greater survival rates than those without a substrate, regardless of stocking density, due to the positive effect the artificial substrate had on reducing the stress levels of individuals by increasing the surface area of the system (Schveitzer *et al.*, 2013). Otoshi *et al* (2006), found artificial substrate provided refuge for recently moulted individuals of *Litopenaeus vannamei*, which during this stage are vulnerable to cannibalism, leading to greater growth and survival rates amongst individuals raised in tanks with substrate (Otoshi *et al*., 2006).

1.11 Aims and Objectives of the Study

Aim:

- Does a ‘natural environment’ increase growth and reduce the mortality of *Litopenaeus vannamei* within a closed aquaculture system.

Objectives:

- Undertake a Literature review to better understand the current research related to the study.
- Create an experimental tank system with varying environments: ‘Natural’, ‘Filter Brush’ and ‘Control’ (Trial 1 and Trial 2) to see the effects of varying environments.
- Create an experimental tank system with varying Tank Sizes: ‘Large’ and ‘Small’ (Trial 2) to see the effect of tank size.
- Record water quality parameters throughout the trials (Trial 1 and Trial 2) to ensure good husbandry.
- Measure growth (weight and length) at the end of the trials (Trial 1 and Trial 2) to measure the difference in growth between the environments.
- Observe mortality in the varying environments (Trial 1 and Trial 2) to see if environment impacted mortality.
- Observe mortality in the varying Tank Sizes (Trial 2) to see if Tank Size impacted mortality.
- Observe behaviour in the varying environments (Trial 1 and Trial 2) to see if environment impacted behaviour.
- Observe behaviour in the varying Tank Sizes (Trial 2) to see if Tank Size impacted behaviour.

Hypotheses:

- There will be a difference in the weight, condition factor, and mortality of the individuals from differing treatments (each individual tank).
- There will be a difference in the weight, condition factor, mortality, and behaviour of the individuals from differing environments.
- There will be a difference in the weight, condition factor, mortality, and behaviour of individuals in different sized tanks.

2. Methodology

In order, to test the Hypotheses the following methodology was developed.

2.1 Experimental Design

2.1.1 General Design

In order to investigate the effect of shelter environments as requested by the Aquaculture farm (Flogro Fresh) providing the individuals the following design was created. The farm had filter brushes (long cylinders made of plastic bristles) and wanted to investigate the effect this shelter would have on growth and mortality, this became the first environment. The second environment was the control environment (this had no added material in it), as a control is needed as a “reference against which the results of an experimental manipulation can be compared” (Ruxton and Colegran, 2003). In order to look at the effect of shelter environments a third environment was created –‘Natural’(fake plants and a gravel bottom), to investigate the effect of a high amount of shelter (figure 2.1).

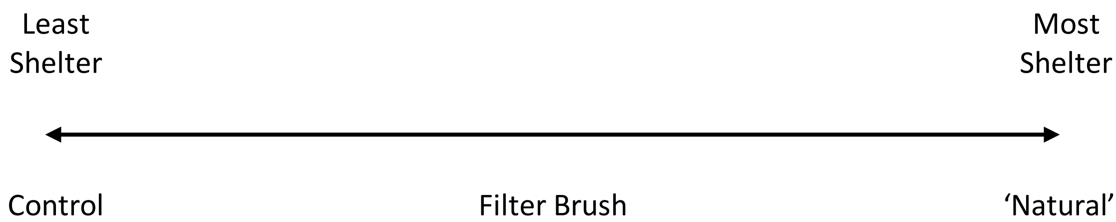


Figure 2.1: A scale of amount of shelter the environments have.

The tank systems used in the experiment are similar to that used in aquarists shops to display fish. Two bank systems were used over the course of the experimentation. These bank systems were housed in a controlled environment research laboratory. The control room was kept at a constant temperature of 10ºC by a cooling unit, excess humidity was extracted by a dehumidifier and the room lighting was on a 12hr on/ 12hr off cycle.

The bank systems used are made up of three levels of tanks used to house aquatic organisms and a lower tank, known as a sump for the filtration of waste water (figure 2.2). The diagram in Figure 2.2 details the set-up of one bank system, two identical bank systems were used, water is pumped (green square) from the sump up the system and separates off at each level and flows into the tanks at those levels simultaneously through inflow taps (blue square). As the system is constantly pumping water through the inflow taps, outflow drains (red circle) in the tanks at each level return the water to the sump, where the water flows through the filtration media in the sump to be pumped back through the system. Each bank is a closed cycle housing 500l. The bank systems contain a varying number of glass tanks at each level; level 3 contains one large tank, level 2: 8 small experimentation tanks, level 1: 3 medium sized tanks.

The two systems have a middle row (level 2) of smaller experiment tanks, these smaller tanks have holes within the dividing glass pieces to allow for the flow and return of new and waste water. These holes have a plastic disc within them to allow for the flow of water but to reduce the movement of animals between them. These 8 middle experiment tanks (level 2) have an inlet of water flowing directly into one of the tanks (blue circle), so water inflow tank will not be used to avoid and minimise the effect water flowing directly into one tanks might have. The 3 environments will double up to create replicates, in 6 of the 8 tanks in each bank system creating a balanced experiment; an experiment designed with "equal numbers of experimental units in each environment group" (Ruxton and Colegran, 2003), in this case a replicate in each bank system, effectively creating 4 units of each of the 3 environments ('Natural', Filter Brush, Control) across the two bank systems over the experiment. The environments were randomly allocated to each experiment tank through the roll of a dice, to avoid any bias on tank placement (figure 2.3).

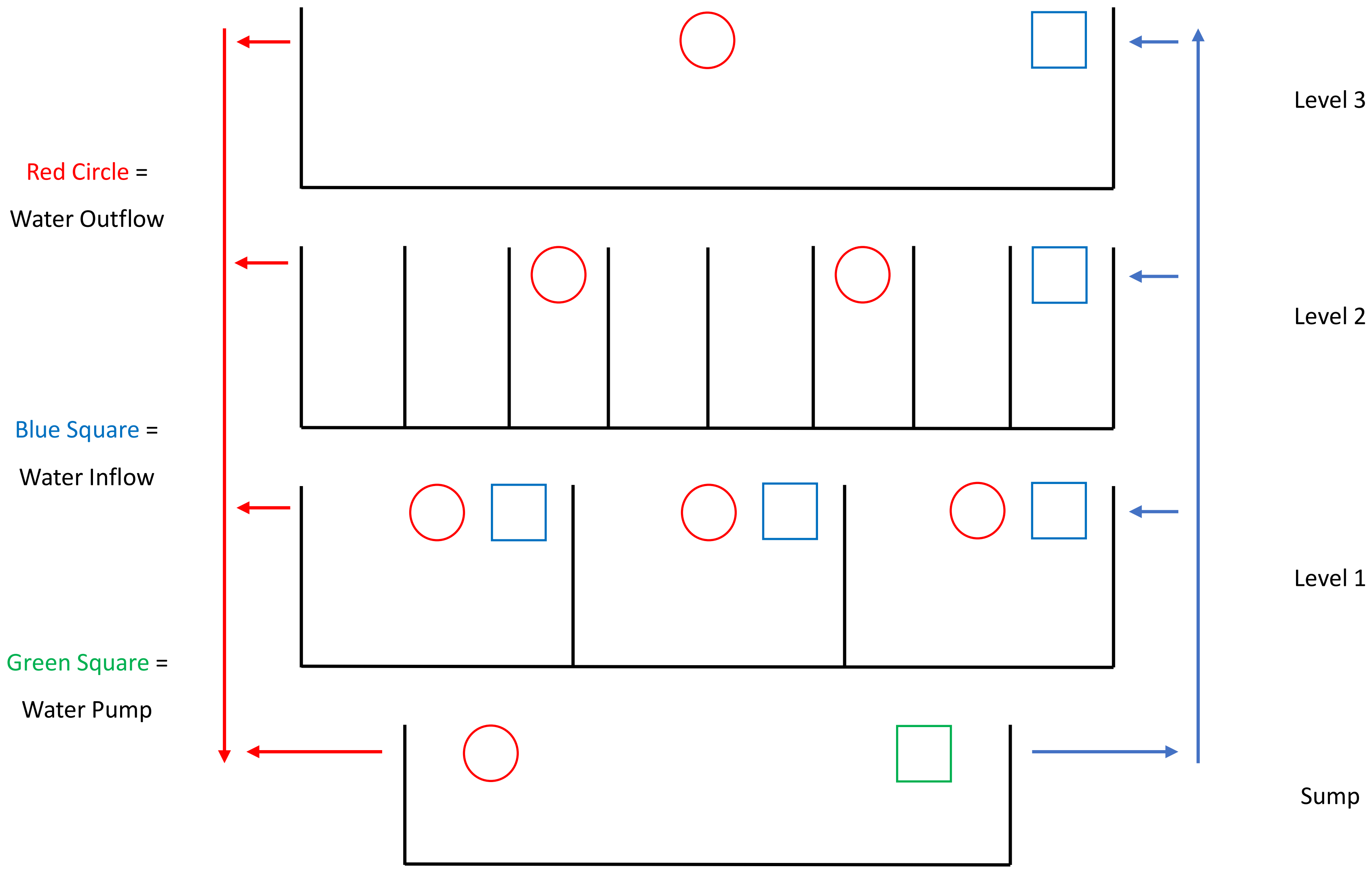


Figure 2.2: Diagram of the bank system used in both trials, detailing the enclosed system and how water is recycled and circulated throughout.

2.1.2 Changes and Additions for Trial 2

To investigate the effect of larger tanks has on growth and mortality within shelter environments, a second trial was undertaken (Trial 2), due to the restrictions of the bank system, i.e. the banks only having 3 medium sized tanks and one large tank each (figure 2.2). The medium sized tanks at level 1 displayed in Figure 2.2, were used, as there are only 3 medium sized tanks in each bank system replicates of the larger tanks were unavailable. The environments were randomly allocated to the tanks in the same way as the first trial, through the roll of dice (figures 2.4 and 2.5).

2.1.3 Tank Environment Set up

2.1.3.1 Trial 1

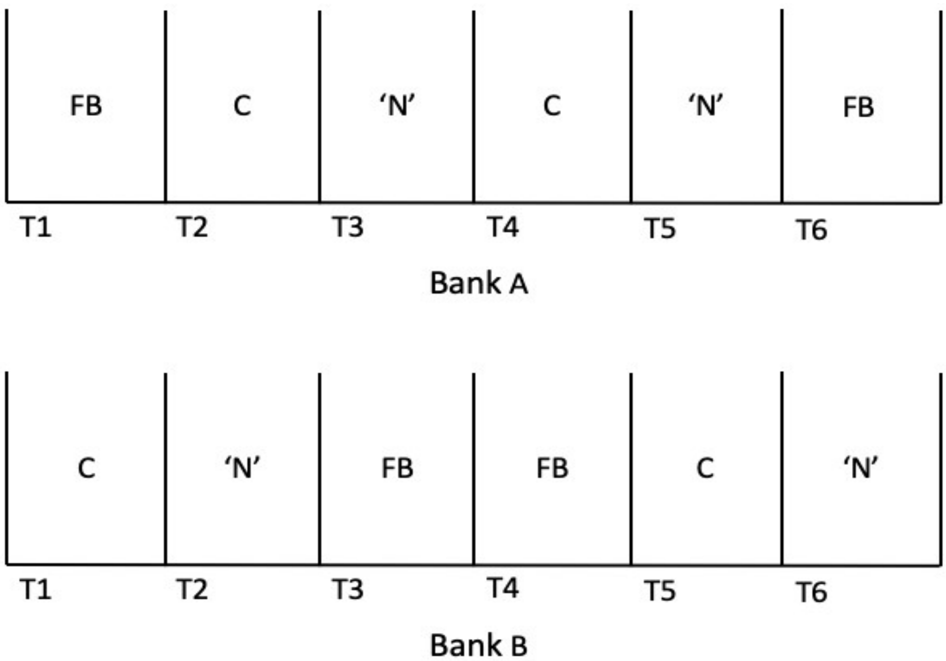


Figure 2.3: Tank Environment set up for Trial 1 across Bank A and B. 'N'- 'Natural', FB- Filter Brush, C- Control.

2.1.3.2 Trial 2

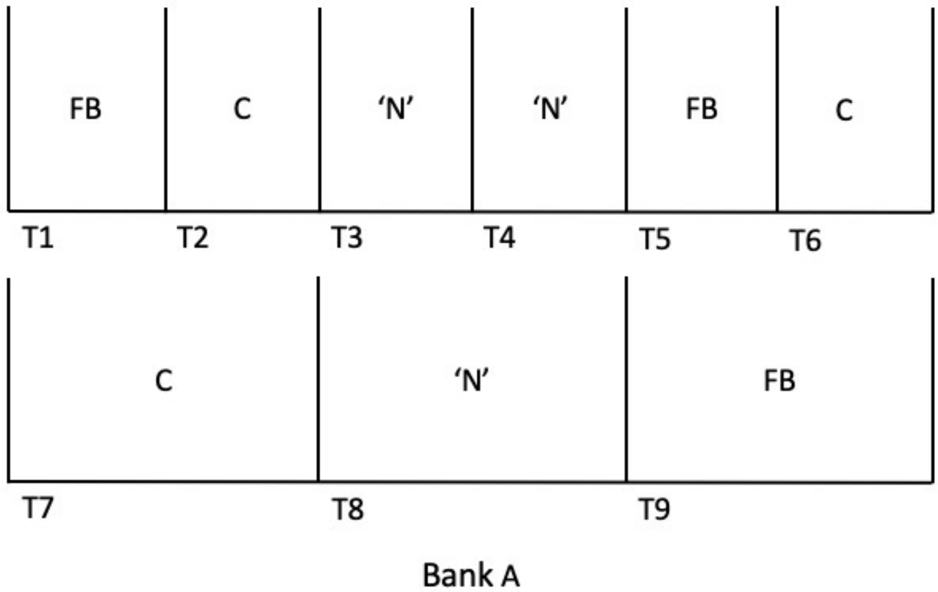


Figure 2.4: Tank Environment set up for Trial 2 across Bank A and B. 'N'- 'Natural', FB- Filter Brush, C- Control.

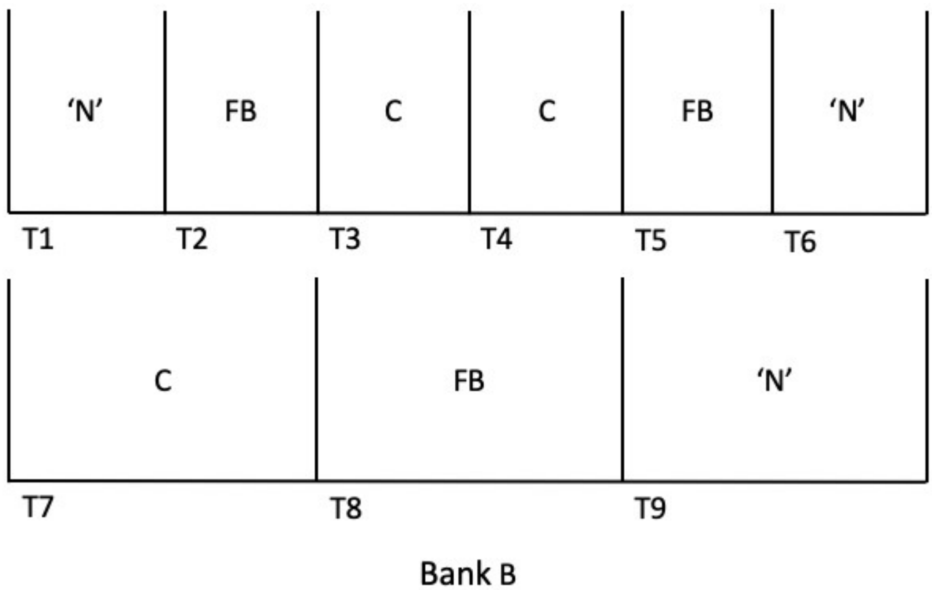


Figure 2.5: Tank Environment set up for Trial 2 across Bank A and B. 'N'- 'Natural', FB- Filter Brush, C- Control.

2.2 General Husbandry

2.2.1 System Design

Each bank (figure 2.2) contains 500 litres of water, the system is made up of a large tank at the top (level 3), that was not used, 8 middle experimental tanks (level 2), 6 of which were used for both trials, 3 larger tanks (level 1) at the bottom, used in Trial 2 and a sump at ground level (figure 2.6 and figure 2.7). Figure 2.7 shows the sump with the pump on the right hand side which pumps water throughout the system, the right image of figure 2.6 shows the pipework at the back of each bank systems, which transports the water being pumped up from the sumps into each row of tanks, these rows then have an overflow hole with pipework attached which run back into the left hand side of the sump, so the water can run through the filtration media, to filter the water and be pumped back up throughout the system, creating a cyclic closed system.

Figure 2.6: Bank systems, Left image – front of the bank with differing environments set up. Right image – back of the systems, showing the plumbing of the banks.

Figure 2.7: Sump at the bottom of the bank systems, containing filtration media. Outflow return from the tanks on the left, pump on the right hand side, filtration media in the middle for bacterial flock to adhere to.

2.2.2 System Set Up for Experimentation

Wherever possible the same procedures were put in place, to achieve as similar conditions to those at the aquaculture farm. Both bank systems had 500l added, a mix of saltwater at 35 and freshwater was used to create a salinity between 20-22, this was measured using a refractometer and an air pump was in place to de-chlorinate water and oxygenate water to generate healthy O_2 levels, as the seawater had sat stagnant in the supply tank. Sumps at the bottom of the systems were seeded with the bacterial flock used at the prawn aquaculture company, FloGro Fresh, to adhere to the filtration media in the sumps and filter the water as it runs through the system. Water heaters were used to keep the systems running between 26-29ºC, several comprehensive water analyses (see *2.2.3 Regular Checks*) were carried out to show whether the water quality was acceptable for the introduction of the prawns, avoiding the adverse effects bad water quality has on individuals, death being the most unwanted.

Following the randomised experimental design, the materials for the environments were added, following the allocated tanks shown in figures 2.3 to 2.5. The Control environment tanks had nothing added. The Filter Brush environment tanks had a filter brush added to each tank, as per the request of the company FloGro Fresh, the larger tanks for Trial 2 had the brushes the FloGro Fresh wanted added, as the larger tanks could accommodate this size (30cm in length), however these were too large for the smaller tanks, so smaller bottle brushes were added to the smaller tanks for both trials, as filter brushes (12cm in length). The 'Natural' environment tanks were created by adding aquarium grade gravel, ensuring it would be safe for aquatic life at a depth of 1cm around the tank. To create the shelter for the 'Natural' tanks artificial aquarium safe plants (26cm in height) were added, to ensure no adverse health effects. In the smaller tanks across both the trials 3 'plants' were put in each, for the larger tanks used in trial 2, 9 ‘plants’ were added to achieve a similar density. Figure 2.8 displays photos of each environment.

The tanks were equilibrated to required experimental conditions before prawns were placed into their environment.

Control Filter Brush ‘Natural’

Figure 2.8: Images of each tank environment, Control, Filter Brush and ‘Natural’.

Individuals were acclimated by slowly dripping tank water into the bags they came in from Flogro Fresh, over a period of several hours (figure 2.9), before they were added into each tank, 20 individuals at a time. The same process was used for all individuals across both trials, for both Small and Large Tank Sizes.

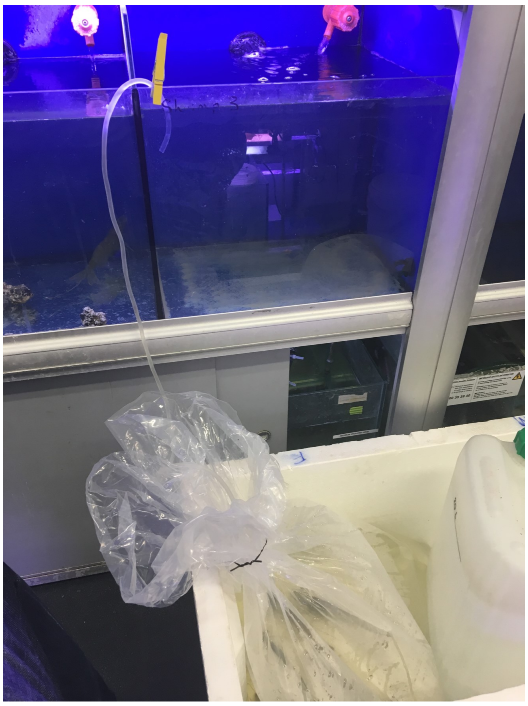


Figure 2.9: Acclimation process before adding individuals.

2.2.3 Regular Checks

Daily water quality checks were carried out in order to ensure the health of the prawns. The water quality of both banks was measured, readings of salinity, temperature (ºC), pH, ammonia, nitrite, nitrate levels (all in ppm) were recorded. An API MARINE 'Saltwater Master Test Kit', was used to measure the levels of: pH, ammonia, nitrite and nitrate (apifishcare, 2021). This water analysis was undertaken for 10 days up to the start of the trials, to ensure water parameters were in order (recorded on paper in aquarium checklists figures 7.1 and 7.2 (*7. Appendix*), translated into figures 2.10 to 2.15).

If the salinity was out of the range of 20-22 either more saltwater or more freshwater was added to maintain optimum conditions. Temperature was regulated through the use of water heaters these were added or removed if the temperature diverged from 26-29 ºC. Daily water tests on the nitrogen within the banks ensured ammonia, nitrite and nitrate levels did not reach harmful levels which would inhibit growth and cause death and may lead to further issues with algal blooms. The levels of these compounds were reduced by clearing out waste every other day, which gave the prawns the opportunity to consume all the food, but simultaneously preventing nitrate and ammonia levels from increasing. In addition to this measure both banks underwent a weekly water change, when the 500l systems were drained of 150l of water and replaced with a new mix of saltwater and de-chlorinated freshwater, to improve the water chemistry and ensure its correct salinity. Moreover, every two days 20l dechlorinated freshwater was added to the banks, to replace the water lost through evaporation, necessary to ensure salinity levels did not rise above 22, but additionally to ensure the pumps within the system did not run dry, ensuring the constant pumping and flow of water throughout the system.

The following figures (Figure 2.10 – Figure 2.15) detail the acclimatisation of the parameters before the start of the two trials and the parameters during the trial. The spikes shown in Figures 2.10 to 2.15 have a variety of different causes. The drop in temperature in Trial 2 (figure 2.10) was because a heater failed, the heater was then removed and another was added, to maintain the required temperature. Salinity fluctuated up and down throughout the trials because of evaporation, but remained within appropriate levels (figure 2.11). pH increased in trial 2 for an unknown reason, however this increase was not toxic, so no further actions were taken (figure 2.12). Rises in ammonia, nitrate, and nitrite were caused by excess waste or dead individuals, when this occurred waste was removed and water changes were undertaken in order to bring levels back down to as close to 0 as possible, as indicated by water quality tests, as high levels of these compounds are toxic and can lead to death (figures 2.13 to 2.15).

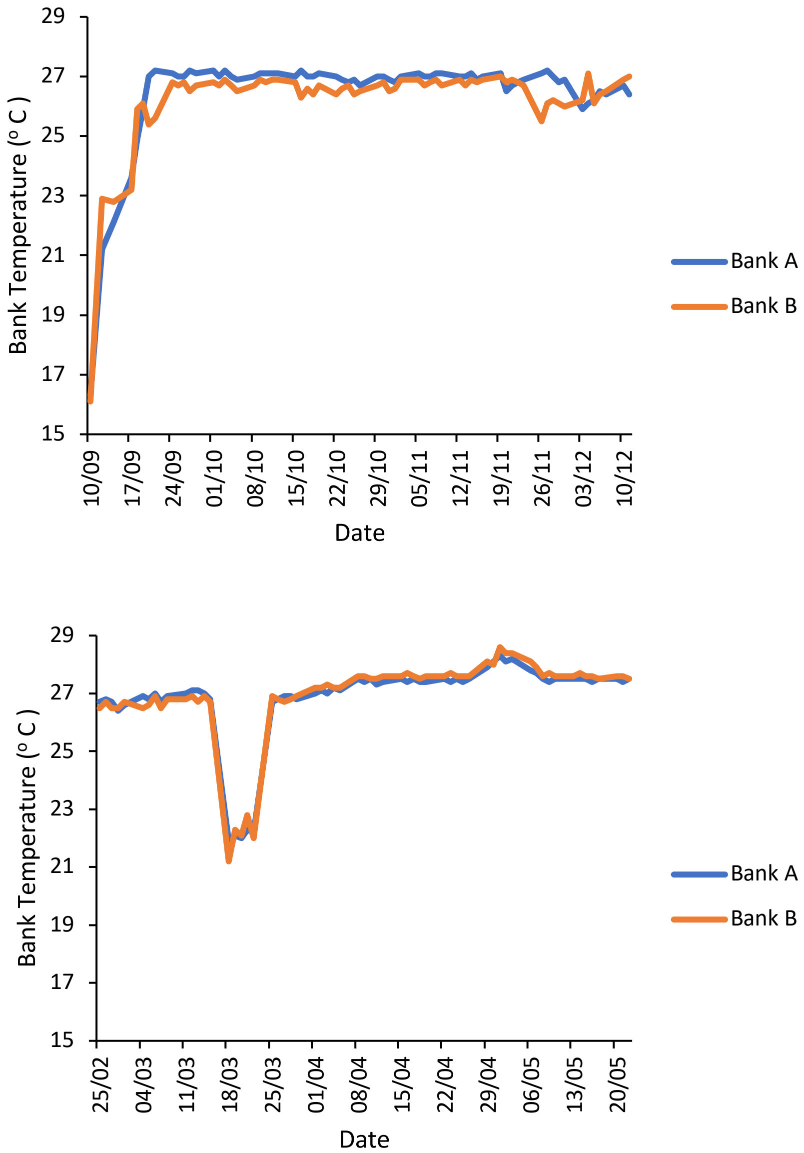


Figure 2.10: Bank temperature across the trials. Above Trial 1, Below Trial 2.

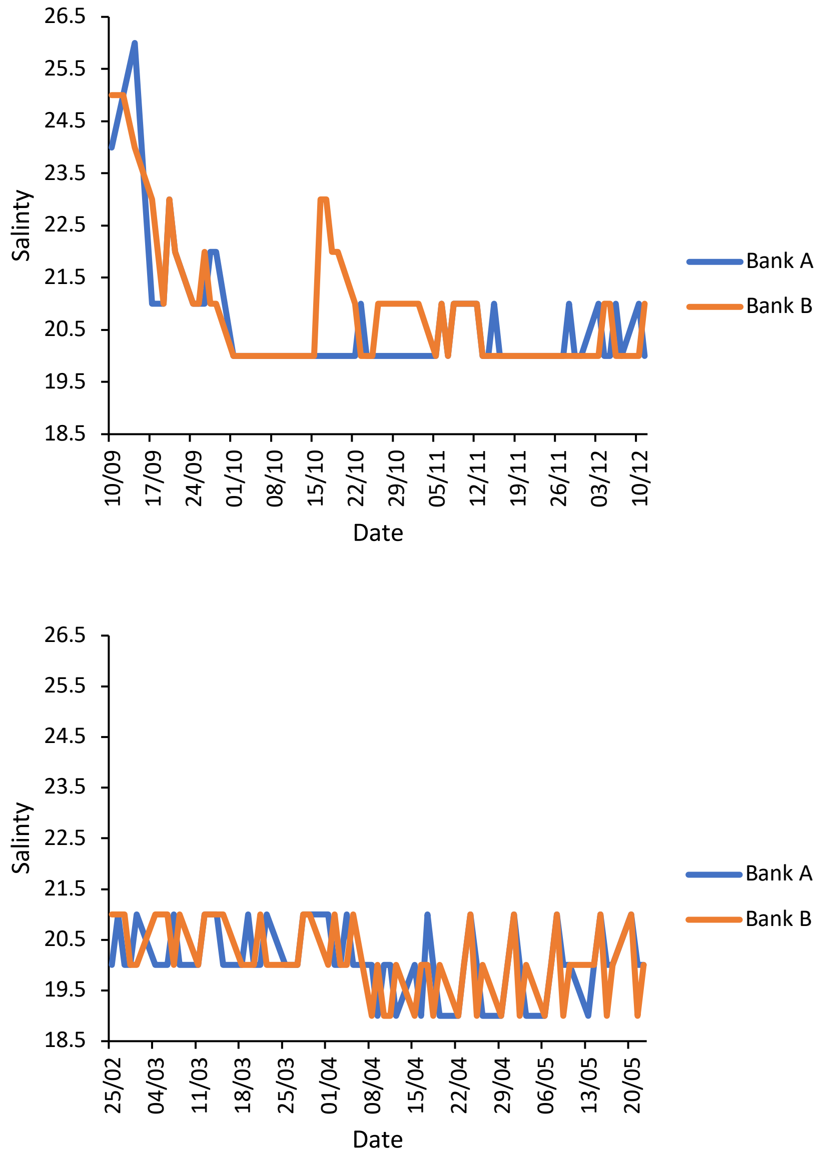


Figure 2.11: Salinity across the trials. Above Trial 1, Below Trial 2.

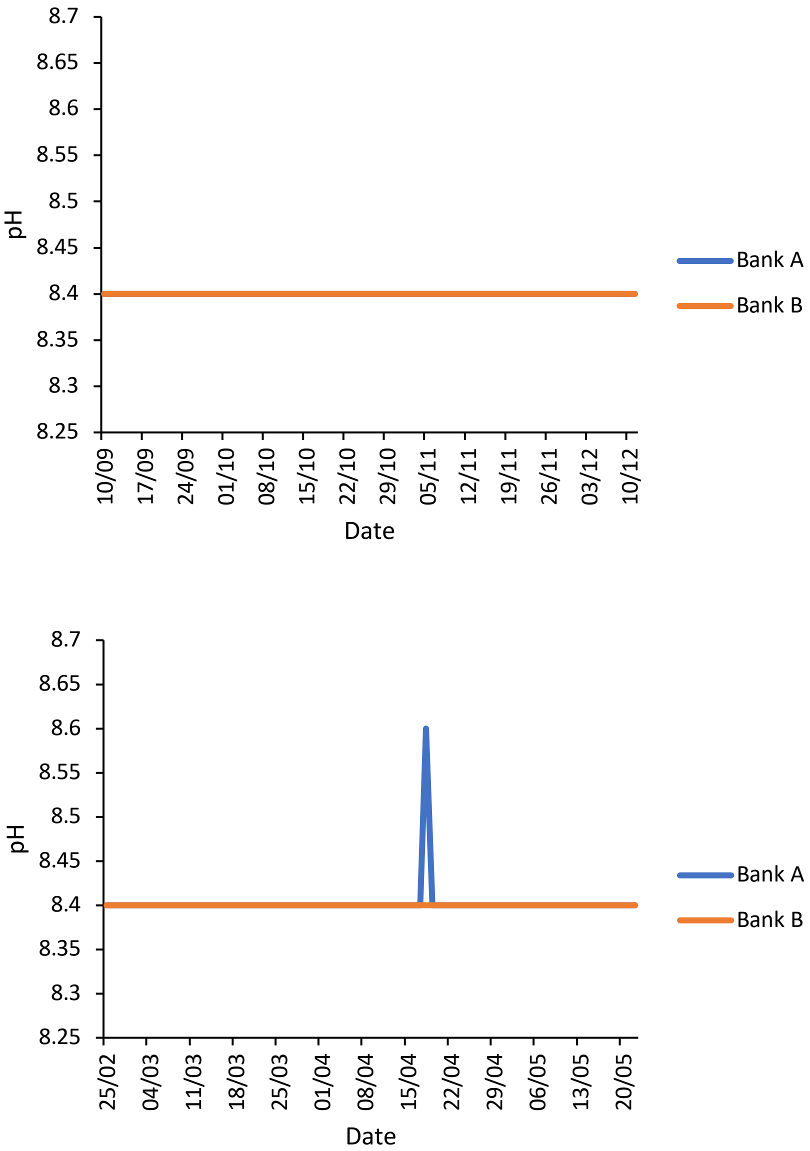


Figure 2.12: pH across the trials. Above Trial 1, Below Trial 2.

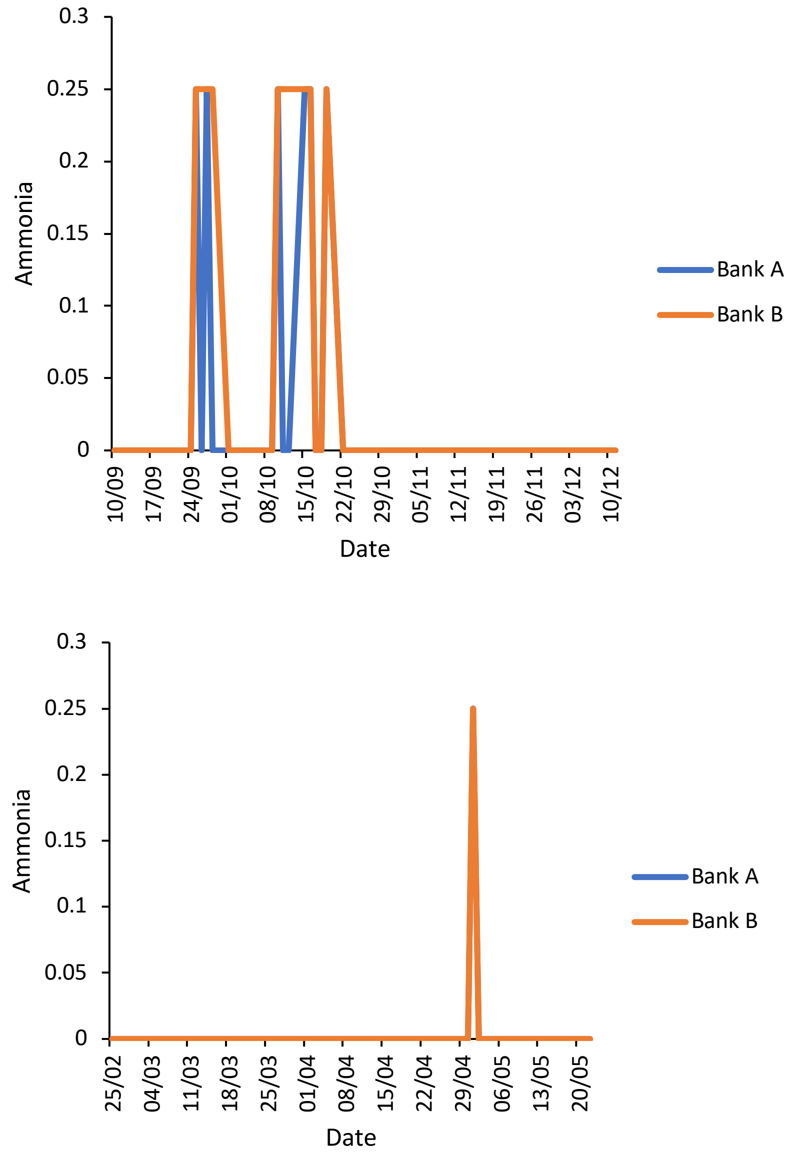


Figure 2.13: Ammonia (ppm) across the trials. Above Trial 1, Below Trial 2.

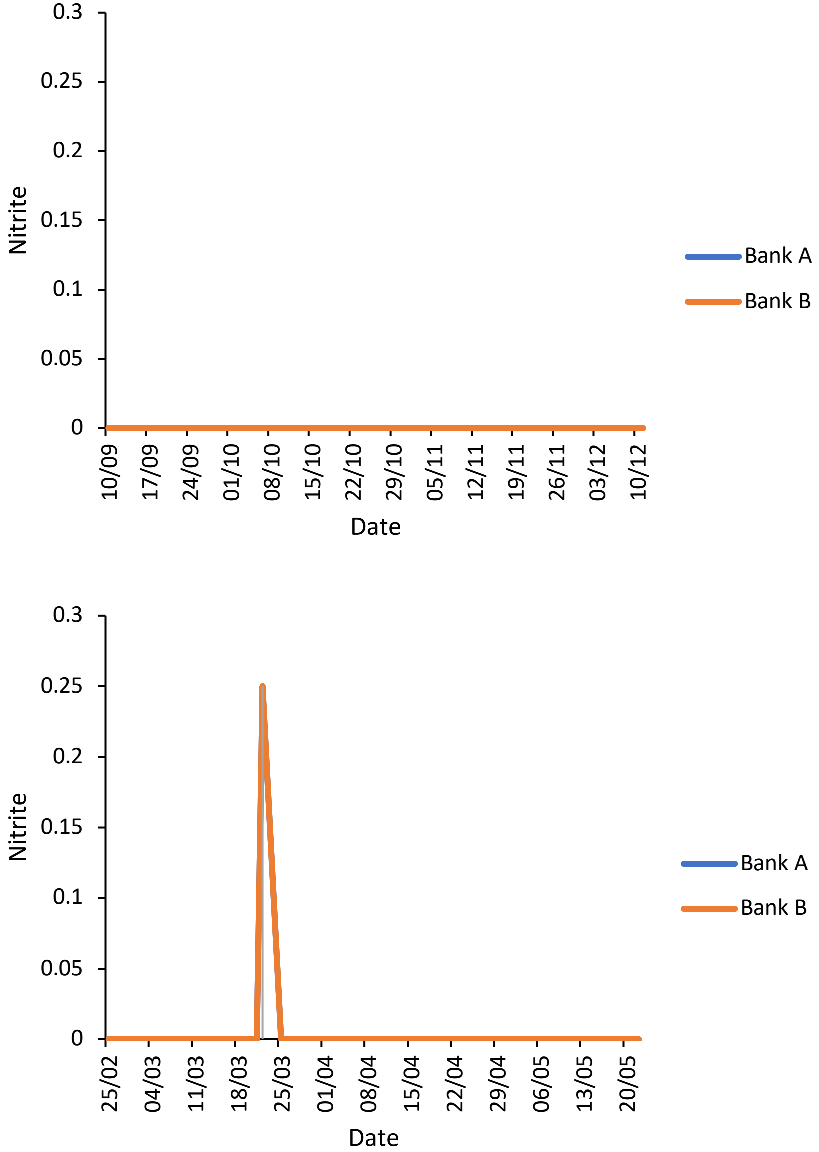


Figure 2.14: Nitrite (ppm) across the trials. Above Trial 1, Below Trial 2.

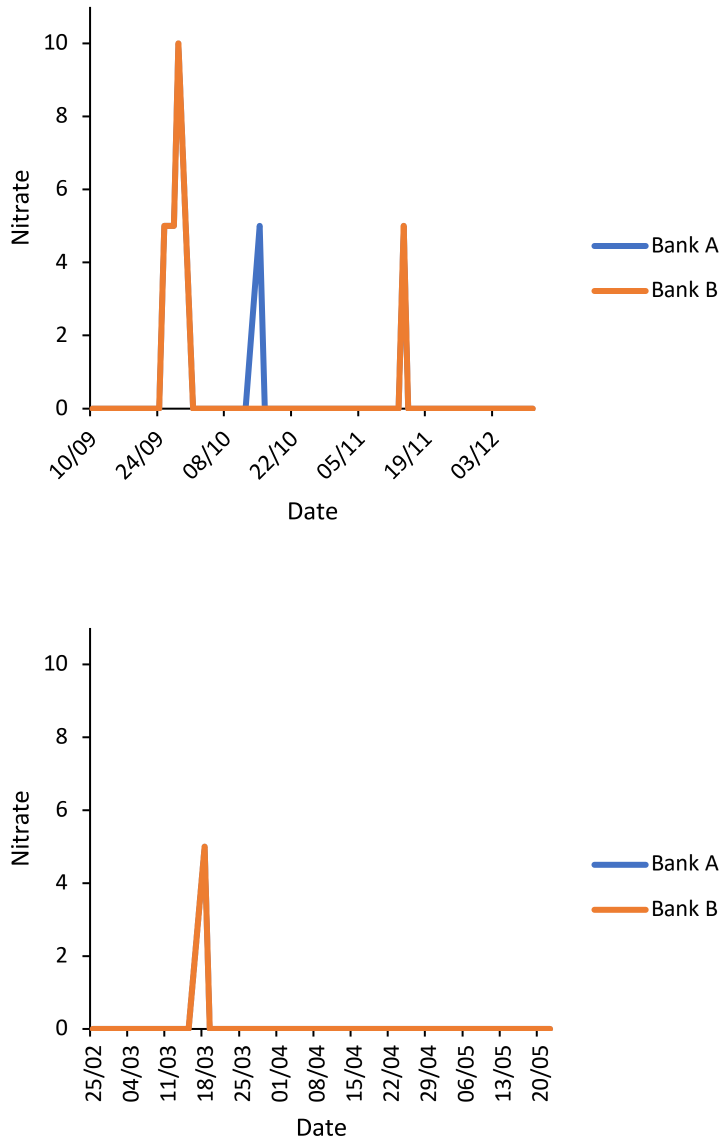


Figure 2.15: Nitrate (ppm) across the trials. Above Trial 1, Below Trial 2.

2.2.3.1 Changes and additions for Trial 2

During Trial 1 differences in behaviours across tanks were observed, leading to an additional behaviour study being designed and undertaken within the second trial. There were 4 behaviours being noticed and it was decided to record these behaviours daily to see if there was particular overriding behaviour in certain environments or tank sizes. As one of these behaviours was eating, it was decided that behaviour would be noted Before and After feeding. The categories for behaviour are defined as:

1. **Sitting on the bottom of the tank-** remaining on the bottom of the tank motionless.
2. **Swimming-** movement within the water.
3. **Interacting with added material-** Sitting on, hiding within or eating food within the Filter brushes or added artificial plant material. Sitting on bottom of the added substrate in the 'Natural' tank not included but walking on the substrate is.
4. **Eating-** either eating the food added, algae or shed exoskeletons or dead individuals.

2.3 Obtaining Prawn measurements at the end of the trial

Measurements at the end of both trials were obtained in the same way. As the prawns used in the experiment are tropical and inhabit waters of 26-29 °C the most humane way to kill them is using a cold-water ice bath which stuns them (figure 2.16) and within a minute they will have been euthanised. The individual length (mm) from the rostrum to their telson and weight (g) (figure 2.17) were measured and the prawns were photographed in their experimental group tanks. Individuals were patted down with a paper towel before weighing, to remove additional water weight on the animal.

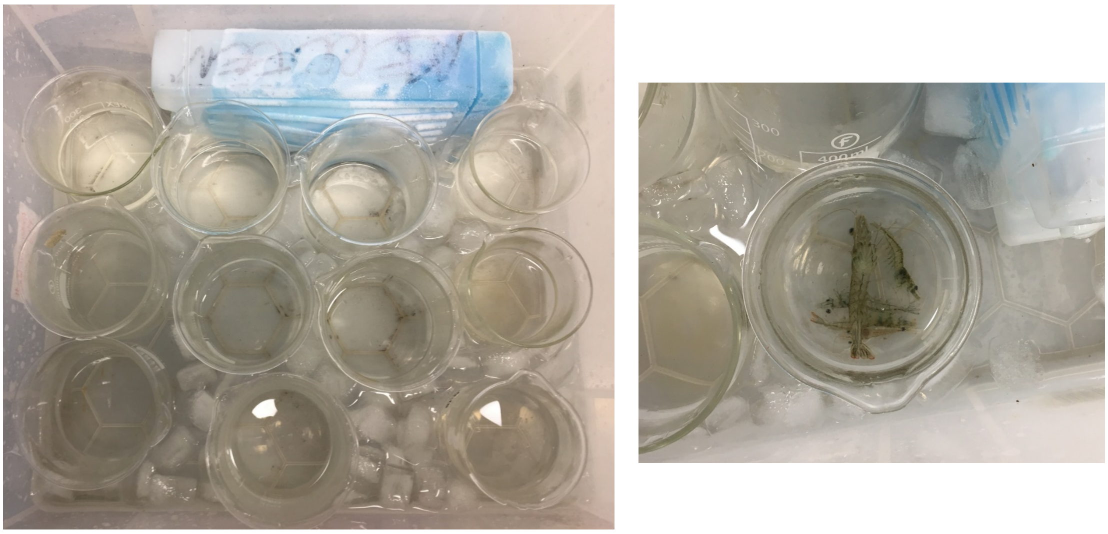


Figure 2.16: Ice bath to euthanise prawns, right shows a zoomed in image of the ice bath with prawns in a container.

Figure 2.17: (Left) Photo of a prawn at the end of Trial 2, red arrow running rostrum to telson, showing where the length is measured from. (Right) Image of a prawn being weighed on a balance.

2.4 Analysing the Results

2.4.1 Statistical Analysis

IBM SPSS Statistics version 24 was used to analyse the effect of environment and tank size on the weight and condition factor of individuals. In order to test the hypothesis a Kruskal-Wallis test within the programme was undertaken. A Kruskal-Wallis test is a non-parametric test, which determines statistically significant differences. This test was used as it compares multiple independent samples of different sample sizes whilst also not assuming normal distribution (statistics laerd, 2021).

Condition Factor is a combined measurement of weight and length that gives a measure of 'fatness' and the health of an individual. Its use is particularly relevant to this study and aquaculture in general as the industry wants to produce the 'fat' and healthy individuals that are desired by consumers.

$$K=100(W/L^3)$$

This study will use Fulton's condition factor- K, a common method of measuring health in fish. Where W is weight in grams, L is length in centimetres, and is times by 100 to bring K closer to a value above 1 (Nash *et. al*, 2006).

2.4.2 Mortality

Mortality is displayed through graphs. These graphs display the mortality of prawns by treatment (each tank), environment and tank size. They demonstrate mortality by showing the remaining number of individuals in each treatment, environment and tank size.

2.4.3 Behavioural Analysis

Graphs display the occurrence of various behaviours before and after feeding across treatment, environment and tank size (noted on paper figures 7.3 and 7.4 (*7.Appendix*)).

2.4.4 Visual Observation

Photographs and notes were taken throughout the trials to record behaviours and incidences that cannot be graphed or represented in a numerical form.

3. Results

All of the different treatments resulted in weight increases and condition factor growth regardless of environment or tank size over both of the trials, demonstrated through figures 3.1 to 3.4. Figures 3.1 and 3.3 display the growth of prawns in each tank, divided by bank within trial 1. Figures 3.2 and 3.4, show images at either end of the trial, visualising the growth within trial 2.

Figures 3.5 and 3.6 show the Environment tank set up for both the trials. Figure 3.5 shows the set up over both Banks in trial 1 and the number of prawns that survived in each individual tank and the number of 'Escapees' found throughout the system. Similarly Figure 3.6 shows the Environment tank set up for Trial 2, with the number of prawns that survived in each individual tank and the number of 'Escapees' found throughout the system, but with the addition for Trial 2 of the Large tanks. The Environment tank set up will be referred to throughout this section and subsequent sections.

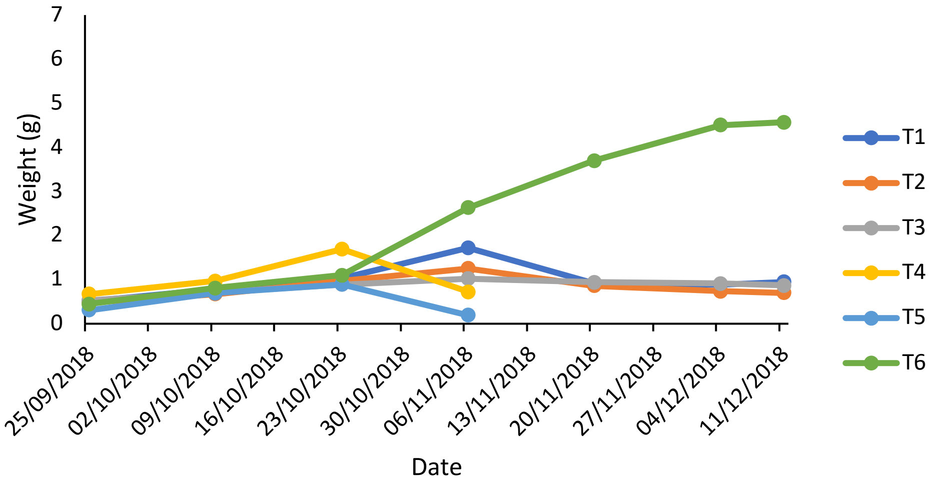


Figure 3.1: Weight growth in tanks over time for Bank A during Trial 1. 'T'- Individual tanks within the system.

Figure 3.2: A photo of a dead prawn from the beginning of Trial 2, 8mm long.

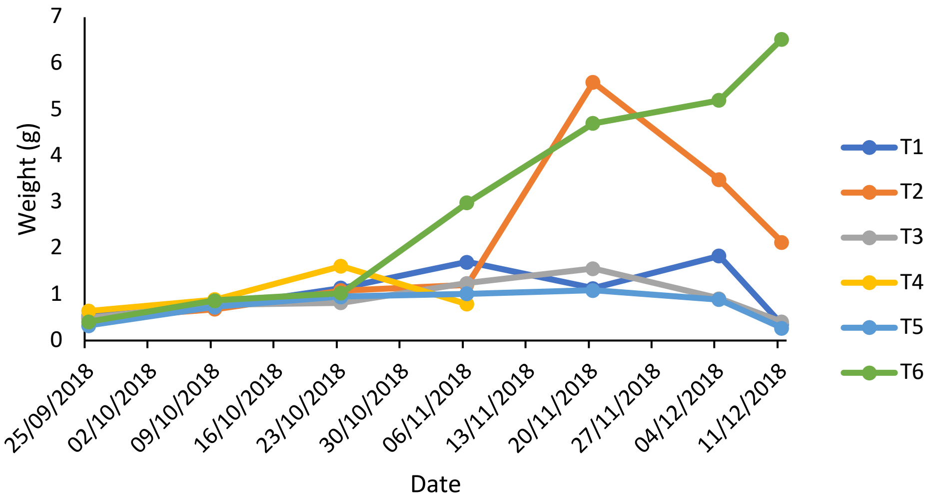


Figure 3.3: Weight growth in tanks over time for Bank B during Trial 1. 'T'- Individual tanks within the system.

Figure 3.4: Photo of a prawn at the end of Trial 2, callipers stating its length at 82.40mm.

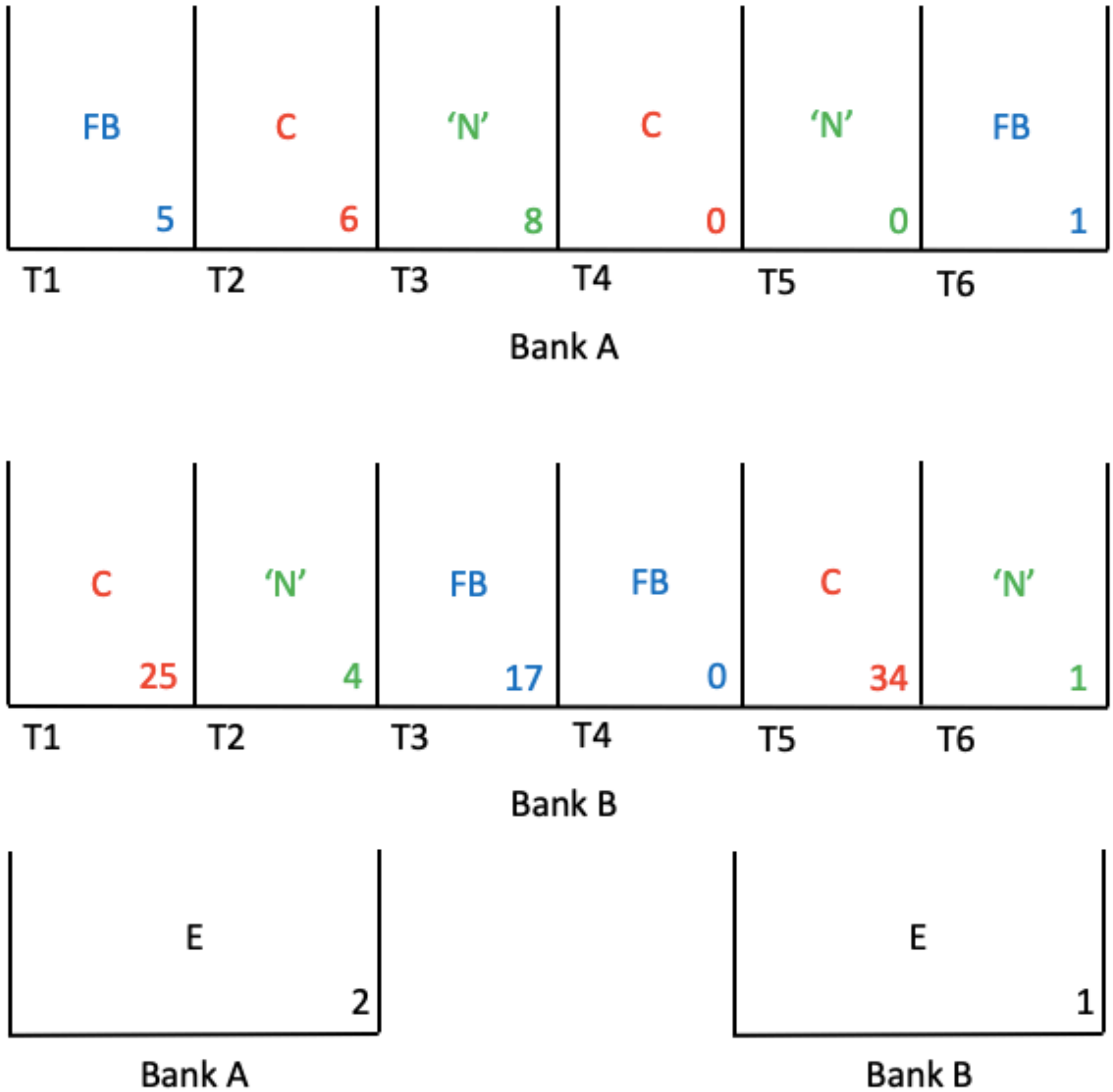


Figure 3.5: Tank Environment set up for Trial 1 across Bank A and B, with the remaining prawns in each tank in the bottom right of each treatment. 'N'- 'Natural', FB- Filter Brush, C- Control and E- 'Escapees'.

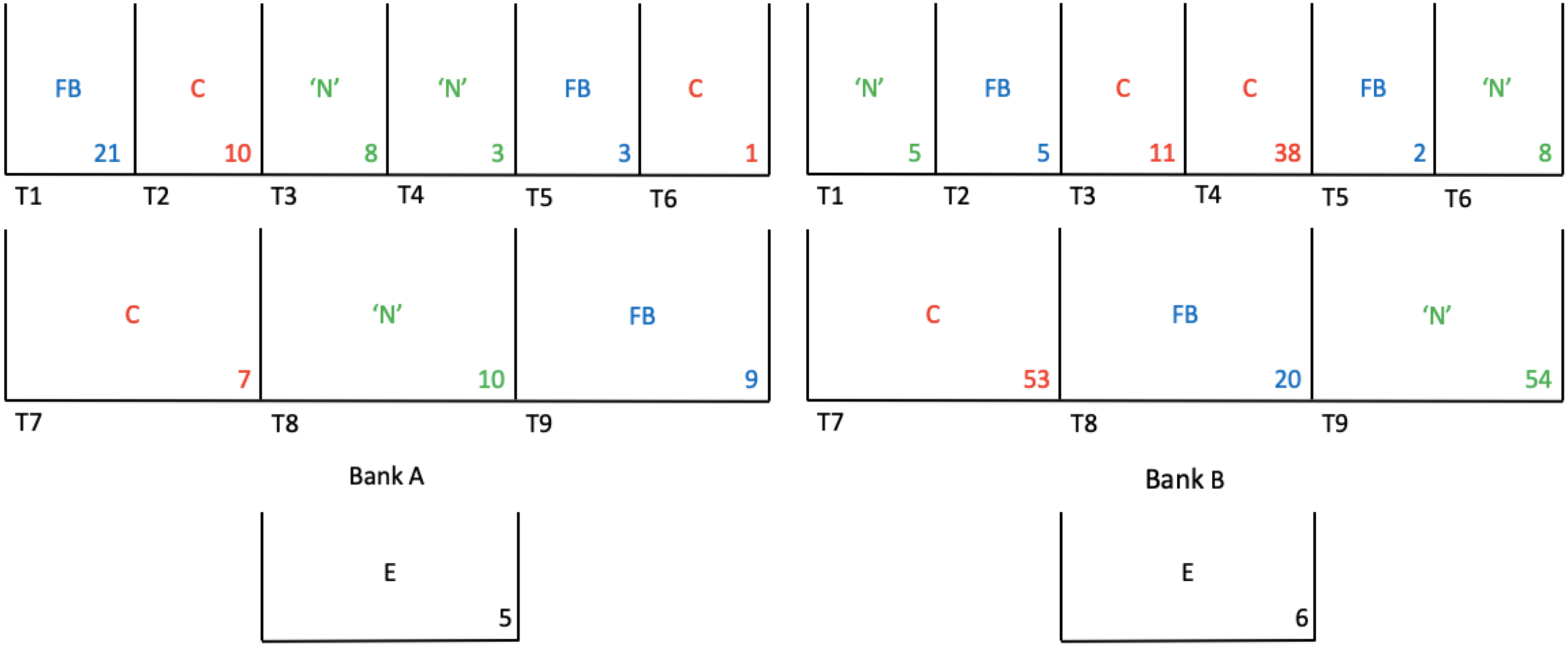


Figure 3.6: Tank Environment set up for Trial 2 across Bank A and B, with the remaining prawns in each tank in the bottom right of each treatment. 'N'- 'Natural', FB- Filter Brush, C- Control and E- 'Escapees'.

3.1 Statistical Analysis

3.1.1 Weight Analysis

3.1.1.1 Weight Against Treatment

A Kruskal-Wallis test of the comparison of weight of individuals against their treatment (each individual tank) produced a test summary of a significance level of 0.0001, which is below the level of 0.05, the null hypothesis: 'There will be no difference in the weight of individuals from differing treatments' can be rejected, showing there is a difference in the weight of individuals from differing treatments. This test tested 375 individuals across 25 treatments (figure 3.7). As this null hypothesis can be rejected, it can be inferred that the tanks played a difference in the outcome of the weight of individuals across treatments allowing for a test on the effect of environment against the weight of individuals.

Independent-Samples Kruskal-Wallis Test Summary

Total N	375
Test Statistic	247.215[a]
Degree Of Freedom	24
Asymptotic Sig.(2-sided test)	.000

a. The test statistic is adjusted for ties.

Figure 3.7: Test summary of total number of individuals and number of treatments (Deg. Of Freedom, N-1).

A box and whiskers of the weights across treatments demonstrates the differences in weights that exist between them, weights ranging between 0 and 3 grams (figure 3.8). A simplified version of the box and whiskers can be seen in the common under bar chart (table 3.2), homogenous subsets of similar overlapping means, showing 8 common groups, with treatment 'EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4' (table 3.1) having the lowest mean weight of '0.0774g' and treatment 'Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2' having the highest mean weight of '2.1325g'. A common under bar chart of homogenous subsets used instead of a pairwise comparison chart (table 3.2) as the former is a more succinct manner of displaying a larger amount of data, however a pairwise comparison of the weight of all treatments compared can be found in the appendix (Table 7.1).

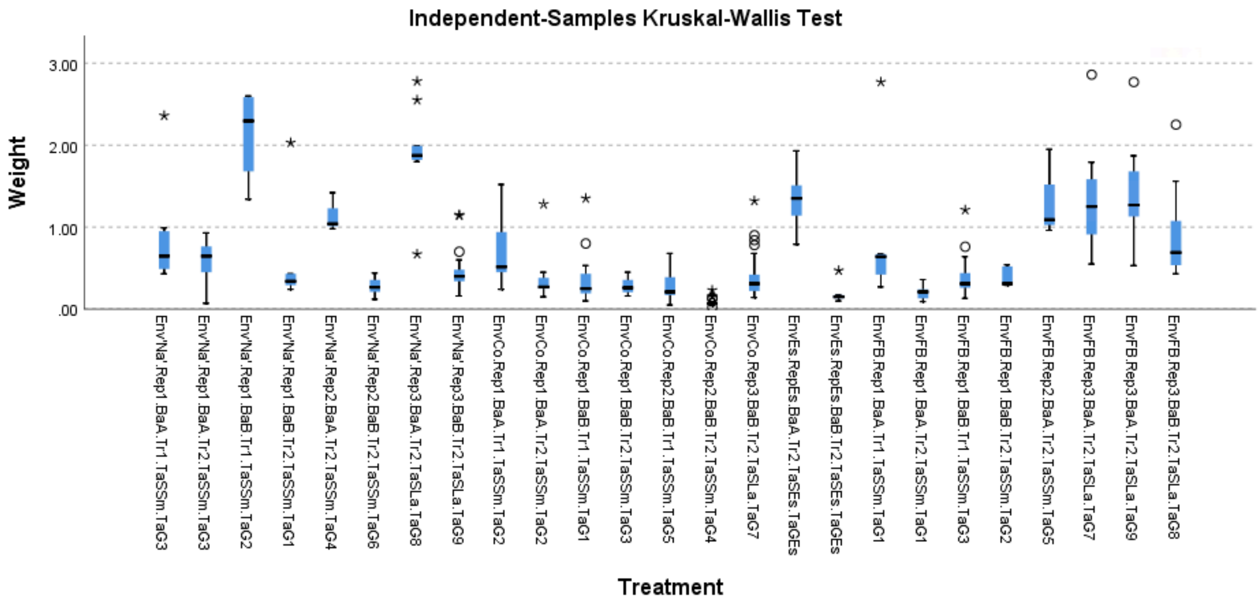


Figure 3.8: Box and whiskers graph, Weight (g) charted for each treatment.

Tank Descriptors	Category
Env = Environment	'Na'= 'Natural', Co = Control, FB = Filter Brush
Rep = Replicate	1, 2, 3, Es = 'Escapees'
Ba = Bank System	BaA = Bank A, BaB = Bank B
Tr = Trial	1,2
TaS = Tank Size	La = Large, Sm = Small, Es = 'Escapees'
TaG = Tank Group	1,2,3,4,5,6,7,8,9, Es = 'Escapees'

Table 3.1: Key to Abbreviations

			Subset							
Treatment	**No.**	**Means**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4	38	0.0774								
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs	6	0.1950								
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1	21	0.2033								
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5	34	0.2735								
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6	8	0.2775								
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3	11	0.2855								
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1	25	0.3452								
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	53	0.3636								
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2	10	0.3900								
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	5	0.3920								
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3	17	0.4047								
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	54	0.4313								
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	8	0.5900								
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	5	0.6660								
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	6	0.6967								
EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	20	0.8670								
Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	8	0.8700								
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	5	0.9540								
Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	3	1.1467								
EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	3	1.3333								
EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	5	1.3440								
EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	7	1.3786								
EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	9	1.4367								
Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	10	1.9130								
Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	4	2.1325								
Significance			0.069	0.280	0.059	0.067	0.122	0.097	0.081	1.000

Table 3.2: Common under bar chart of homogenous subsets of treatment weights, with number of individuals, treatment means and significance. Pairwise comparison in 7. Appendix, Table 7.1.

3.1.1.2 Weight Against Environment

A Kruskal-Wallis test of the comparison of weight data against environment (‘Natural’, Filter brush, Control and Escapees) produced a test summary of a significance level of 0.0001, which is below the level of 0.05, the null hypothesis: ‘There will be no difference in the weight of individuals from different environments’ can be rejected, showing there is a difference in the weight of individuals from differing environments. This test tested 375 individuals across 4 environments (figure 3.9).

Independent-Samples Kruskal-Wallis Test Summary

Total N	375
Test Statistic	67.936[a]
Degree Of Freedom	3
Asymptotic Sig.(2-sided test)	.000

a. The test statistic is adjusted for ties.

Figure 3.9: Test summary of total number of individuals and number of environments (Deg. Of Freedom, N-1).

A box and whiskers of the weights across environments demonstrates the differences in weights that exist between them, weights ranging between 0.00 and 3.00 grams, with the ‘Escapees’ having the highest mean weight and ‘Control’ having the lowest mean weight; indicating that in these trials, adding material to a tank does not produce disadvantageous effects in terms of low weights (figure 3.10). In order to establish significance difference between differing environments a pairwise comparison of weights across environments was carried out. Figure 3.11, shows a pairwise comparison displaying adjusted significances, as the significances of ‘Control-Filter Brush’, ‘Control-‘Natural’’ are below 0.05, a significant difference can be assumed between these environments, showing the greatest difference in weight between these environments. Outliers are considered in the discussion (*4. Discussion*).

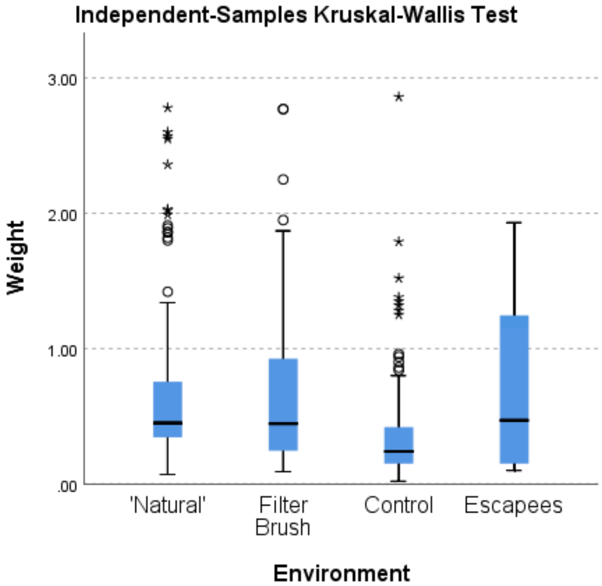


Figure 3.10: Box and whiskers graph, Weight (g) charted for each environment.

Pairwise Comparisons of Environment

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
Control-Escapees	-61.876	33.641	-1.839	.066	.395
Control-Filter Brush	80.310	14.515	5.533	.000	.000
Control-'Natural'	101.833	13.465	7.563	.000	.000
Escapees-Filter Brush	18.434	34.853	.529	.597	1.000
Escapees-'Natural'	39.957	34.429	1.161	.246	1.000
Filter Brush-'Natural'	21.524	16.257	1.324	.186	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Figure 3.11: Kruskal-Wallis test pairwise comparison of weight in differing environments.

3.1.1.3 Weight against Tank Size

A Kruskal-Wallis test of the comparison of weight data against tank size (Large, Small and Escapees) produced a test summary of a significance level of 0.0001, which is below the significance level of 0.05, the null hypothesis: 'There will be no difference in the weight of individuals from different tank sizes' can be rejected, showing there is a difference in the weight of individuals from differing environments. This test tested 375 individuals across 3 tank sizes (figure 3.12).

Independent-Samples Kruskal-Wallis Test Summary

Total N	375
Test Statistic	52.730[a]
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	.000

a. The test statistic is adjusted for ties.

Figure 3.12: Test summary of total number of individuals and number of differing tank size (Deg. Of Freedom, N-1).

A box and whiskers of the weights across tank size demonstrates the differences in weights that exist between them, weights ranging between 0.00 and 3.00 grams, with the 'Escapees' having the highest mean weight and 'Small' having the lowest mean weight (figure 3.13). In order to establish significance difference between different tank sizes a pairwise comparison of weights across tank size was carried out. Figure 3.14, shows a pairwise comparison displaying adjusted significances, as the significances of 'Small-Large' is below 0.05, a significant difference can be assumed between these tank sizes, showing the greatest difference in weight between these tank sizes.

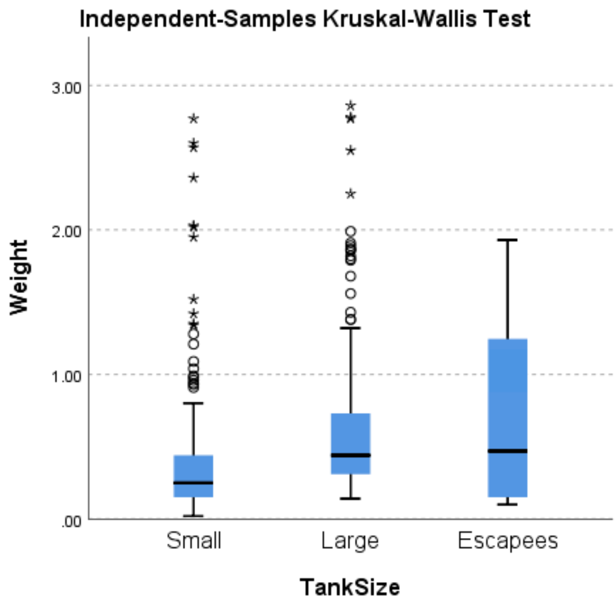


Figure 3.13: Box and whiskers graph, Weight (g) charted for each Tank Size.

Pairwise Comparisons of TankSize

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
Small-Escapees	-51.296	33.519	-1.530	.126	.378
Small-Large	-83.380	11.509	-7.245	.000	.000
Escapees-Large	32.083	33.833	.948	.343	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Figure 3.14: Kruskal-Wallis test pairwise comparison of weight in differing Tank sizes.

3.1.2 Condition Factor Analysis

3.1.2.1 Condition Factor Against Treatment

A Kruskal-Wallis test of the comparison of condition factor of individuals against their treatment (each individual tank) produced a test summary of a significance level of 0.0001, which is below the level of 0.05, the null hypothesis: ‘There will be no difference in the condition factor of individuals from differing treatments’ can be rejected, showing there is a difference in the condition factor of individuals from differing treatments. This test tested 375 individuals across 25 treatments (figure 3.15). As this null hypothesis can be rejected, it can be assumed the tanks played a difference in the outcome of the condition factor of individuals across treatments allowing for a test on the effect of environment against the condition factor of individuals.

Independent-Samples Kruskal-Wallis Test Summary

Total N	375
Test Statistic	253.068[a]
Degree Of Freedom	24
Asymptotic Sig.(2-sided test)	.000

a. The test statistic is adjusted for ties.

Figure 3.15: Test summary of total number of individuals and number of differing treatments (Deg. Of Freedom, N-1).

A box and whiskers of the weights across treatments demonstrates the differences in weights that exist between them, weights ranging between 0.00 and 15.00 ($K=100(W/L^3)$) (figure 3.16). A simplified version of the box and whiskers can be seen in the common under bar chart (table 3.3), homogenous subsets of similar overlapping means, showing 10 common groups, with treatment ‘EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4’ (table 3.3) having the lowest mean weight of ‘1.6521’ and treatment ‘Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8’ having the highest mean weight of ‘11.1690’. A common under bar chart of homogenous subsets was used instead of a pairwise comparison chart (table 3.4) as the former is a more succinct manner of displaying a larger amount of data, however a pairwise comparison of the weight of all treatments compared can be found in the appendix (Table 7.2).

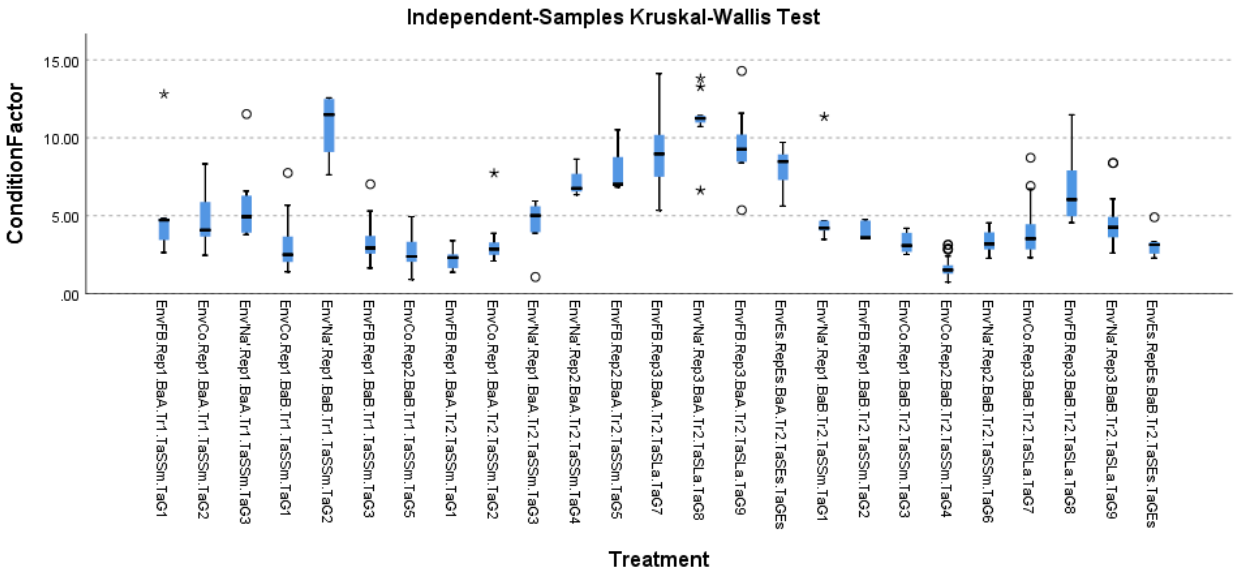


Figure 3.16: Box and whiskers graph, Condition Factor charted for each treatment.

Tank Descriptors	Category
Env = Environment	‘Na’= ‘Natural’, Co = Control, FB = Filter Brush
Rep = Replicate	1, 2, 3, Es = ‘Escapees’
Ba = Bank System	BaA = Bank A, BaB = Bank B
Tr = Trial	1,2
TaS = Tank Size	La = Large, Sm = Small, Es = ‘Escapees’
TaG = Tank Group	1,2,3,4,5,6,7,8,9, Es = ‘Escapees’

Table 3.3: Formerly table 3.1, Key to Abbreviations

			Subset									
Treatment	**No.**	**Means**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4	38	1.6521	■									
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1	21	2.2108	■	■								
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5	34	2.6766	■	■								
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1	25	3.0220	■	■	■							
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs	6	3.2249	■	■	■							
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3	11	3.2737	■	■	■							
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6	8	3.3411	■	■	■							
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3	17	3.3457	■	■	■							
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2	10	3.3525	■	■	■							
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	53	3.8327	■	■	■							
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	5	4.0382	■	■	■	■						
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	54	4.3664	■	■	■	■						
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	8	4.5188		■	■	■	■					
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	6	4.7531		■	■	■	■					
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	5	5.5537			■	■	■	■				
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	5	5.6872			■	■	■	■				
Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	8	5.6979			■	■	■	■				
EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	20	6.6176				■	■	■	■			
Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	3	7.2465					■	■	■	■		
EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	5	8.0035						■	■	■		
EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	3	8.1241						■	■	■	■	
EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	7	9.1147							■	■	■	■
EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	9	9.5075								■	■	■
Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	4	10.7906									■	■
Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	10	11.1690										■
Significance			0.64	.125	.075	.109	.061	.113	.148	.311	.078	.513

Table 3.4: Common under bar chart of homogenous subsets of treatments condition factors, with number of individuals, treatment means and significance. Pairwise comparison in 7. Appendix, Table 7.2.

3.1.2.2 Condition Factor Against Environment

A Kruskal-Wallis test of the comparison of condition factor data against environment ('Natural', Filter brush, Control and Escapees) produced a test summary of a significance level of 0.0001, which is below the level of 0.05, the null hypothesis: 'There will be no difference in the condition factor of individuals from different environments' can be rejected, showing there is a difference in the condition factor of individuals from differing environments. This test tested 375 individuals across 4 environments (figure 3.17).

Independent-Samples Kruskal-Wallis Test Summary

Total N	375
Test Statistic	76.459[a]
Degree Of Freedom	3
Asymptotic Sig.(2-sided test)	.000

a. The test statistic is adjusted for ties.

Figure 3.17: Test summary of total number of individuals and number of differing Environments (Deg. Of Freedom, N-1).

A box and whiskers of the condition factor across environments demonstrates the differences in condition factor that exist between them, condition factor ranging between 0.00 and 15.00, with the 'Escapees' having the highest mean condition factor and 'Control' having the lowest mean condition factor; indicating that in these trials, adding material to a tank does not produce disadvantageous effects in terms of producing a low condition factor (figure 3.18). In order to establish significance difference between differing environments a pairwise comparison of weights across environments was carried out. Figure 3.19, shows a pairwise comparison displaying adjusted significances, as the significances of 'Control-Filter Brush', 'Control-Escapees', 'Control-'Natural'' and 'Filter Brush-'Natural'' are below 0.05, a significant difference can be assumed between these environments, showing the greatest difference in condition factor between these environments.

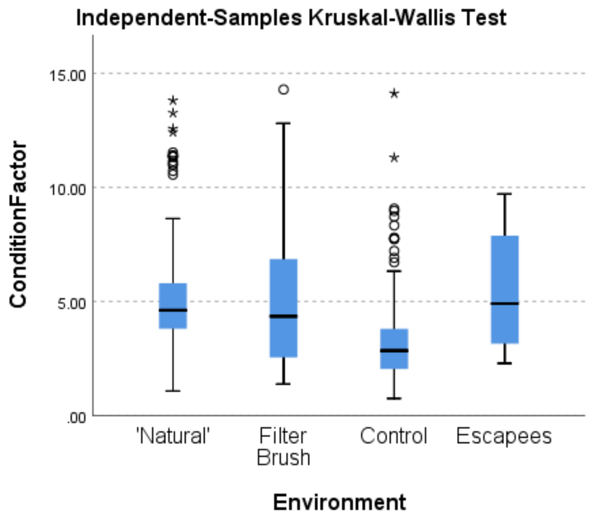


Figure 3.18: Box and whiskers graph, Condition Factor charted for each Environment.

Pairwise Comparisons of Environment

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
Control-Filter Brush	69.412	14.517	4.782	.000	.000
Control-Escapees	-94.998	33.646	-2.823	.005	.029
Control-'Natural'	112.308	13.467	8.340	.000	.000
Filter Brush-Escapees	-25.586	34.858	-.734	.463	1.000
Filter Brush-'Natural'	42.896	16.260	2.638	.008	.050
Escapees-'Natural'	17.310	34.434	.503	.615	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.
a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Figure 3.19: Kruskal-Wallis test pairwise comparison of Condition Factor in differing Environments.

3.1.2.3 Condition Factor Against Tank Size

A Kruskal-Wallis test of the comparison of condition factor data against tank size (large, small and Escapees) produced a test summary of a significance level of 0.0001, which is below the significance level of 0.05, the null hypothesis: 'There will be no difference in the condition factor of individuals from different tank sizes' can be rejected, showing there is a difference in the condition factor of individuals from differing environments. This test tested 375 individuals across 3 tank sizes (figure 3.20).

Independent-Samples Kruskal-Wallis Test Summary

Total N	375
Test Statistic	87.892[a]
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	.000

a. The test statistic is adjusted for ties.

Figure 3.20: Test summary of total number of individuals and number of differing Tank sizes (Deg. Of Freedom, N-1).

A box and whiskers of the condition factor across tank size demonstrates the differences in condition factor that exist between them, condition factor ranging between 0 and 15, with the 'Escapees' having the highest mean condition factor and 'Small' having the lowest mean condition factor (figure 3.21). In order to establish significance difference between different tank sizes a pairwise comparison of condition factor across tank size was carried out. Figure 3.22, shows a pairwise comparison displaying adjusted significances, as the significances of 'Small-Escapees' and 'Small-Large' are below 0.05, a significant difference can be assumed between these tank sizes, showing the greatest difference in condition factor between these tank sizes.

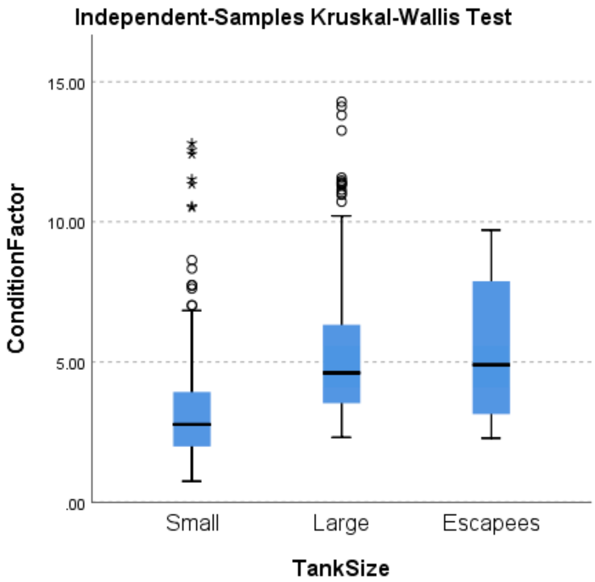


Figure 3.21: Box and whiskers graph, Condition Factor charted for each Tank size.

Pairwise Comparisons of TankSize

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
Small-Escapees	-93.682	33.524	-2.794	.005	.016
Small-Large	-106.567	11.510	-9.259	.000	.000
Escapees-Large	12.885	33.838	.381	.703	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Figure 3.22: Kruskal-Wallis test pairwise comparison of Condition Factor in differing Tank sizes.

3.2 Mortality in Tanks

3.2.1 Mortality within Treatments

Over the course of the experiments individuals died for variety of reasons. Both experiments started with 20 individuals per tank, Figure 3.23 shows the number of individuals left in each tank, each bank and each trial at the end of the experiment. Some treatments had no individuals left and others only had a few deaths, and some tanks had an increased number due to movement between tanks.

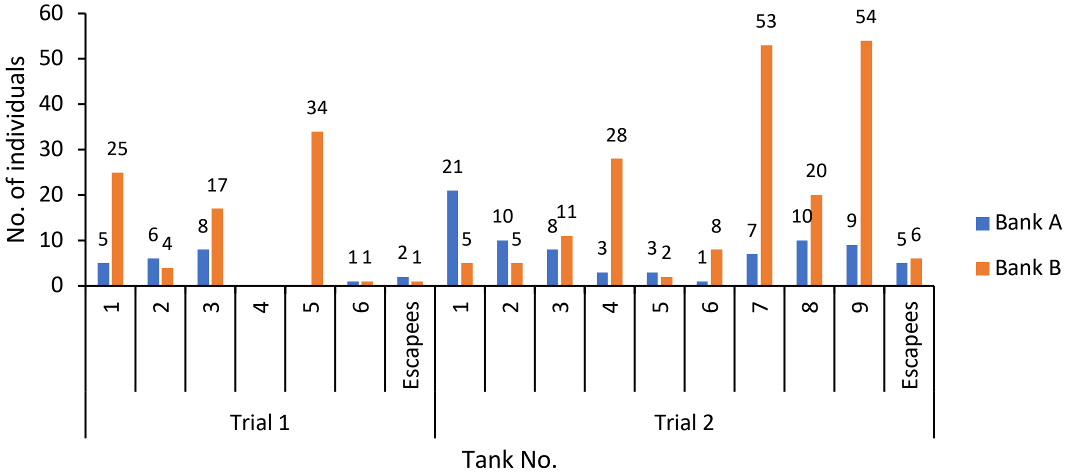


Figure 3.23: Number of individuals left in each Treatment at the end of both trials.

3.2.2 Mortality within Environments

Figure 3.24 shows the number of individuals left at the end of experiment divided into environment type, regardless of trial, tank or bank. The controlled environment had the highest amount of surviving individuals. Both the environment type Control and 'Natural', had tanks with large amount of movement into them.

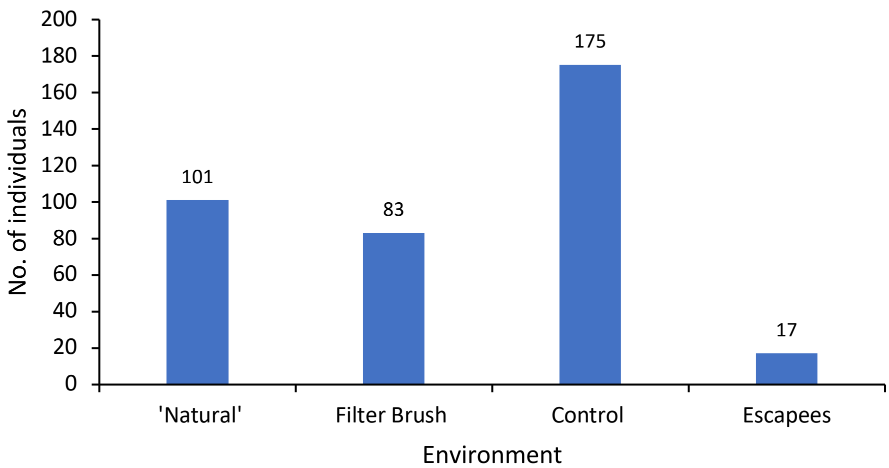


Figure 3.24: Number of individuals left at the end of both trials by environment.

3.2.3 Mortality with Tank Size

Figure 3.25 shows two values for the number of individuals left at the end of both the trials by tanks size. The value in blue is the actual number left based on the size of the tank they were placed in; the orange value is an adjusted one based on the number of individuals left and the amount of tanks of that type, as trial 1 only used small tanks, and both trials had a replicate small tank environments, whereas there was only one large tank for the environment type across each bank.

The adjusted figure for the large tank was worked out by the number individuals left (143) divided by the number of large tanks used in trial 2 across environment and bank (6), 143/6= 23.83. The adjusted figure for the small tanks was the number individuals left (206) divided by the number of small tanks used across the two trials, banks and environment (24), 206/24= 8.58. Adjusted value for the Escapees 14/4= 3.50.

Based on the adjusted figure more individuals survived in the larger tanks than the smaller tanks, showing a lower mortality of prawns in a tank with more space.

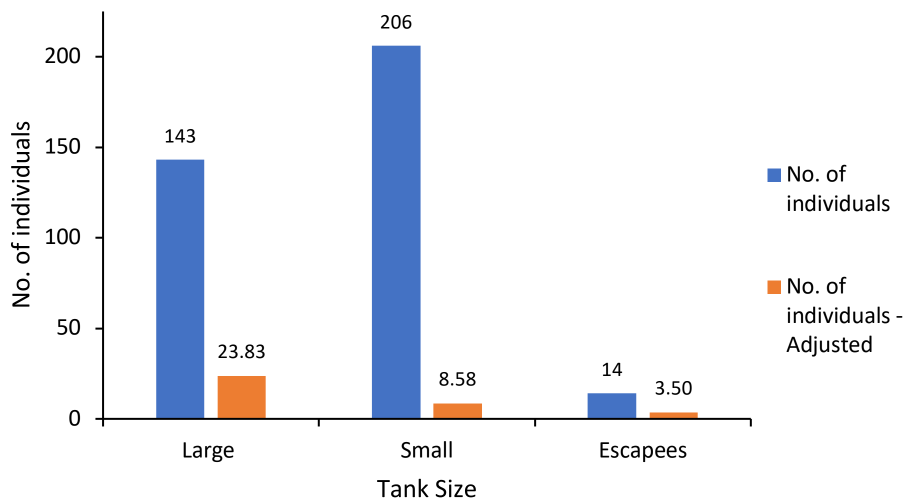


Figure 3.25: Number of individuals left at the end of both trials by tank size. Adjusted figure showing individuals left by no. of tanks.

3.3 Behavioural Analysis

3.3.1 Overall Tank Behaviour

Tanks were observed daily Before Feeding (BF) and After Feeding (AF) to observe what the principal behaviour was at those moments, displayed by the majority of individuals. Behaviour was divided into 4 categories:

1. Sitting on the bottom of the tank
2. Swimming
3. Interacting with added material
4. Eating

For definitions of these categories see section *Chapter 2: 2.1.2 Changes and additions for Trial 2*.

Figure 3.26 displays the behaviour on a tank by tank basis across the two bank (Bank A and Bank B) systems, the behaviour differences that are most obvious is the increase in individuals displaying the behaviour: Eating, after food has been added, this is expected. The tank data displayed in this figure (Figure 3.26) has be collated into figures based on Environment (Figure 3.27) and Tank Size (Figure 3.28 and 3.29) to more adequately display the effect of added material and tank size on behaviour. The summative main findings are increased activity after feeding, greater activity within tanks with added material, and greater activity of individuals within the Large tanks.

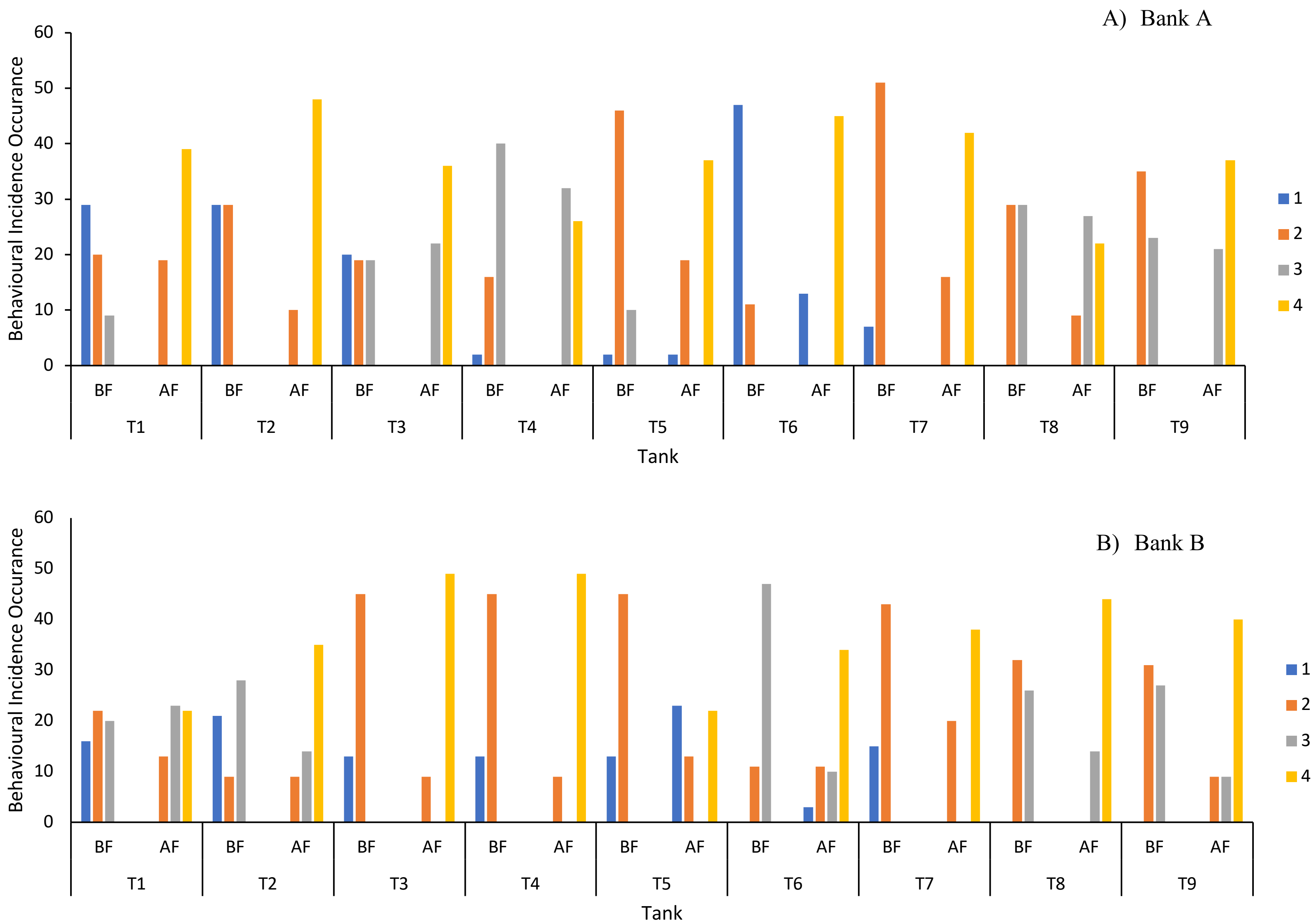


Figure 3.26 Occurrence of a particular behaviour within a tank before feeding (BF) and after feeding (AF) within A) Bank A (top) and B) Bank B (bottom). Behaviour key: 1.Sitting on the bottom of the tank, 2.Swimming , 3.Interacting with added material, 4.Eating.

3.3.2 Behaviour within Environment

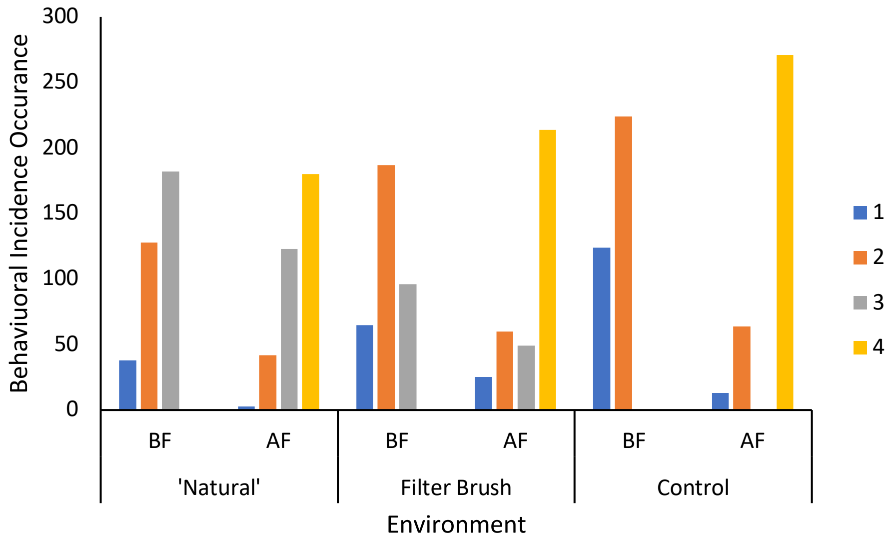


Figure 3.27: Occurrence of a particular behaviour within an environment before feeding (BF) and after feeding (AF). Behaviour key: 1.Sitting on the bottom of the tank, 2.Swimming , 3.Interacting with added material, 4.Eating.

Furthermore, Figure 3.27 shows an increase in the number of individuals eating after food has been added, it can also be seen that after feeding within the Control environment tanks has a higher number of individuals eating when food is added compared to the 'Natural' and Filter Brush tanks. This could be because the food is more visible or they are less distracted than in the added material environments, as these materials provide them with greater stimulation. The environments with added material, showed individuals interacting with the Filter Brushes or the artificial plants.

3.3.3 Behaviour within Tank Size

The trend of more individuals displaying the behaviour Eating after they have been fed continues into Tank Size, Figure 3.28 shows the tank data accumulated into Tank Size, however there are more Small tanks than Large tanks so an adjusted figure was produced. The data for the Small tanks were divided by 12 and the data for the Large tanks were divided by 6, to make a side by side comparison clearer, producing an average by witch clearer side by side comparisons could be made (Figure 3.29).

The major difference between the two tank sizes (Large and Small) in the behaviour displayed is the greater number of individuals that are displaying behaviour 2. Swimming, within the Large tank size compared to the Small, this is probably due to the increased amount of space, therefore individuals are more inclined to swim as they do not bump into others or tank walls. A continuation of this is the following contrast of the higher number of individuals displaying behaviour 1. Sitting on the bottom on the tank, in the Small tank contrasting to the Large tank.

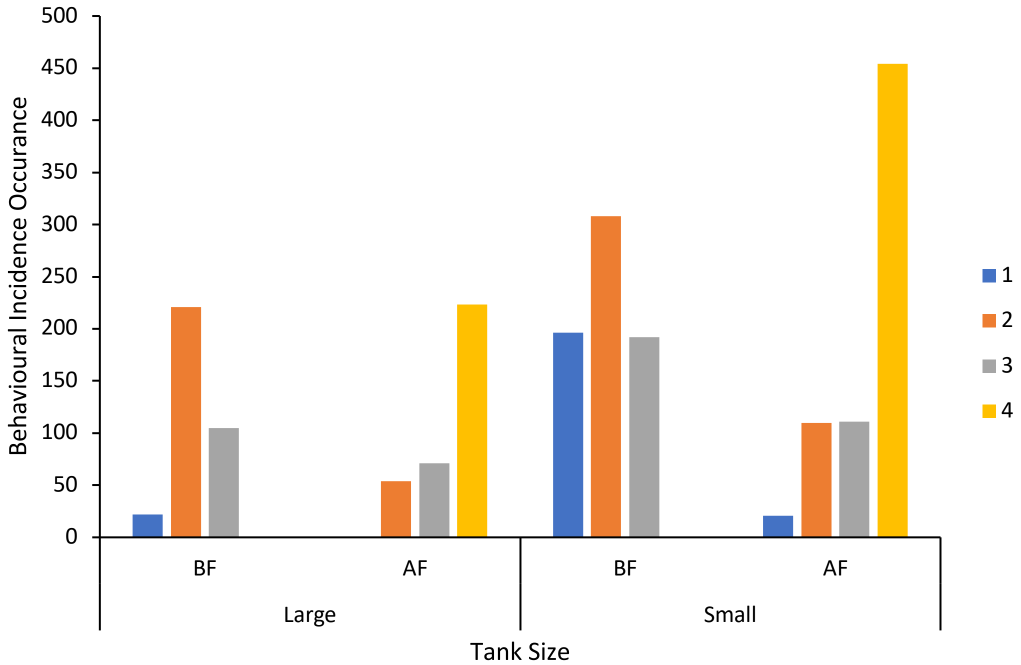


Figure 3.28: Occurrence of a particular behaviour within a tank size before feeding (BF) and after feeding (AF). Behaviour key: 1.Sitting on the bottom of the tank, 2.Swimming , 3.Interacting with added material, 4.Eating.

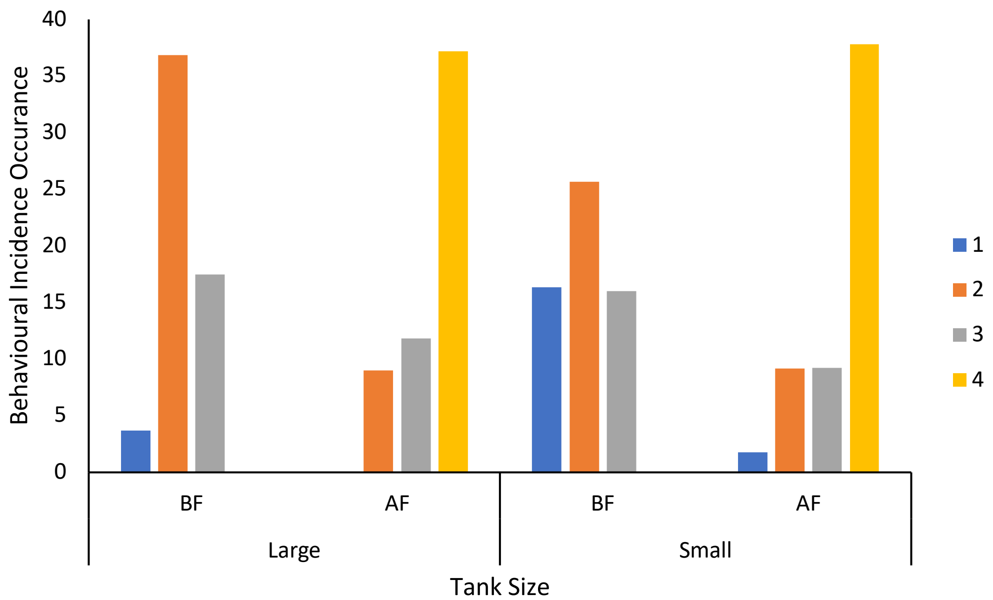


Figure 3.29: Occurrence of a particular behaviour within an environment before feeding (BF) and after feeding (AF). Values adjusted for number of tanks that size. Behaviour key: 1.Sitting on the bottom of the tank, 2.Swimming , 3.Interacting with added material, 4.Eating.

3.4 Visual Observations

Along with quantitative data on growth and qualitative observations on behaviour throughout the two trials, other behaviours were observed that are best displayed through photos.

3.4.1 Cannibalism

Over the two trials some individuals died for a variety of reasons, more individuals that the amount of carcases found. One explanation for this disparity can be found in what is demonstrated in Figure 3.30. The figure depicts an individual seen in the photo as a bright white object being eaten by several other individuals, the reason for the death of the individual is unknown, it is also unknown what stage of the moult cycle the individual is at.

Figure 3.30: A deceased prawn (white object) being eaten by several other prawns.

3.4.2 Shedding Consumption

As prawns grow they moult, their hard exoskeleton sheds revealing a larger individual underneath, this happens throughout life. As with the number of bodies found not matching up to the number of individuals dying, the number of shed exoskeletons found throughout the trials did not match up either. Figure 3.31 could provide an explanation for this. The figure shows an exoskeleton being held and eaten by another prawn, this was observed several times over the trials. The figure also shows other individuals in the shot, individuals would often ‘fight’ over these shed exoskeletons, with often as can be seen in the photo, larger individuals ‘winning’ the exoskeleton and then eating the majority of it before smaller prawns have an opportunity to eat it.

Figure 3.31: A prawn carcass (middle of the image) being held up and eaten by another prawn.

3.4.3 Movement

Figure 3.5 and 3.6 show the tank set ups of the trials and the individuals remaining at the end of the experimental periods. Movement occurred due to human influence and without it. During the first trial weight measurements were taken, this involved removing individuals for weighing and putting them back, this sometimes resulted in individuals in jumping, figure 3.32 shows two individuals on top of the glass of one of the tanks, after the disturbance of weighing making individuals want to ‘jump’ out. As the original tank location was known for these two individuals they could be placed back into that tank.

Individuals would also move and ‘jump’ without human influence, sometimes this lead to individuals ‘jumping’ up onto the tank glass and getting marooned and drying out and dying on the tank tops (Figure 3.33). Movement without human influence is also demonstrated by individuals being found parts of the system they were not put in, the sumps of the banks, and round the back of some of the tanks; these individuals became known as ‘Escapees’ and were left in their new locations as their original location was unknown they could not be placed into back into the trial.

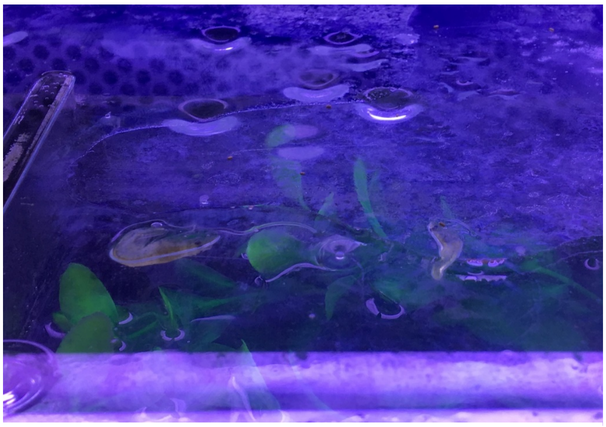


Figure 3.32: Two prawns on top of the tank glass- alive.

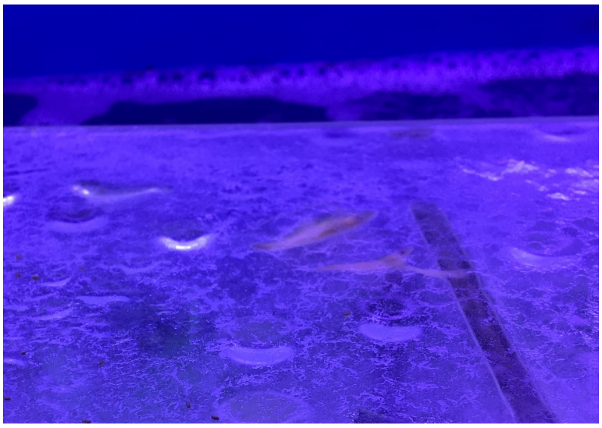


Figure 3.33: Two prawns on top of the tank glass- dead.

4. Discussion

The major findings of this study were the individuals in the added Environment tanks ('Natural' and Filter Brush) showed greater growth over the course of the experiments. In addition to this, individuals in the 'Large' Tank Size also displayed greater growth over two experimental periods. This discussion section will expand upon these findings and question their reliability in relation to other behaviour displayed, namely the movement of individuals between tanks.

4.1 Main Study

4.1.1 Weight Analysis

As shown in *Chapter 3: Results and Analysis* weight increased over the trials, this section will discuss changes in weight in different Environments and Tank Sizes, the reasons for this and why different groups experience greater weight changes than others.

The statistical analysis of Weight against Treatment in *Chapter 3: Results and Analysis* showed there is a difference in Weight of individuals from different Treatments, and the tanks placement played no difference in the outcome of individuals weights themselves, figures under section '*3.1.1.1 Weight Against Treatmen*t', display the differences in weight within and between each treatment. Due to the rejection of the Null Hypothesis further analysis of Weight v. Environment and Weight v. Tank Size could be undertaken, and the purpose of the trials i.e. the difference in weight of individuals in varying Environments and Tank Sizes could be analysed. Below is the analysis and discussion of these results displayed in the previous section *Chapter 3: Results and Analysis*.

4.1.1.1 Weight within Environment

The statistical analysis of Weight against Treatment in *Chapter 3: Results and Analysis* showed there is a difference in Weight of individuals from different Environments, therefore the Null Hypothesis: *'There will be no difference in the weight and condition factor of the individuals from differing environments'* is rejected. Figure 3.10 shows the distribution of weight across environment, with the mean of the 'escapees' being the highest and the 'control' environment having the lowest. The environments with added material placing in between, indicating that within these trials added materials did not have adverse effects in terms of the end weight of individuals. The greatest significant difference between environments was exhibited within 'Control-Filter

Brush' and 'Control-'Natural'', showing the growth disparity in terms of weight between the Control and added material environments.

Several studies have looked at the effect of artificial substrates, such as AquaMats within the aquaculture of a variety of prawn species on the weight of individuals. AquaMats and artificial substrates increase the surface area of a tank (Arnold *et al.*, 2006). Moss and Moss (2004) found the final weight of *L.vannamei* to be higher in treatments with substrate, 34.5% greater at one stocking density (figure 4.1). Moss and Moss (2004) hypothesed the greater growth rates to be due to larger availability of organic particular matter attached to the substrate, serving as an additional food source. Similarly, Zhang *et al.* (2010) found higher production rates: weight (g)- +5.45g, survival (%)- 86.5%, yield (g/m^{-2})- 2370.4g/m^{-2} in the treatment with the most pieces of artificial substrate (figure 4.2)(Zhang *et al.*, 2010).

Treatment	Final Weight (g)	Survival (%)	Production (kg/m^2)	FCR
778	1.69 ± 0.25	91.9 ± 2.6	1.21 ± 0.17	0.78 ± 0.04
7789S	2.13 ± 0.04	93.2 ± 1.9	1.54 ± 0.03	0.75 ± 0.09
1,167	1.44 ± 0..07	89.1 ± 3.1	1.50 ± 0.07	0.78 ± 0.00
1,167S	1.69 ± 0.03	93.0 ± 2.8	1.84 ± 0.09	0.73 ± 0.02
1,556	1.16 ± 0.11	91.8 ± 2.8	1.66 ± 0.21	0.89 ± 0.09
1,556S	1.56 ± 0.04	90.5 ± 2.3	2.20 ± 0.09	0.78 ± 0.07

Figure 4.1: Mean final weight (g), survival, production and feed conversion ration (FCR), S after treatment indicates the presence of substrate (Aquamats) in the treatment (Moss and Moss, 2004).

Treatments	Weight gain (g shrimp $^{-1}$)	Survival (%)	Yield (g m^{-2})
G0	3.35±0.18^{a}	63.50±0.02^{a}	1044.37±19.76^{a}
G1	3.73±0.16^{b}	70.50±0.02^{b}	1313.93±96.84^{b}
G2	4.41±0.11^{c}	76.00±0.01^{c}	1674.28±71.45^{c}
G3	5.45±0.06^{d}	86.50±0.02^{d}	2370.40±82.59^{d}

G0, G1, G2 and G3 denote the treatment with 0, 1, 2 and 3 pieces of artificial substrates, respectively. Different superscripts denote significant difference ($P<0.05$) between treatments.

Figure 4.2: Effects of artificial substrate on growth, survival and yield in *Litopeneaus vannamei* (Zhang *et al.*, 2010).

4.1.1.2 Weight within Tank Size

The statistical analysis of Weight against Treatment in *Chapter 3: Results and Analysis* showed there is a difference in Weight of individuals from different Tank Sizes, therefore the Null Hypothesis: *'There will be no difference in the weight and condition factor of individuals in different sized tanks'* is rejected. Figure 3.13 shows the distribution of weight across Tank Size, 'Escapees' having the highest mean weight and the 'Small' Tank Size having the lowest mean weight. The greatest significant difference between Tank Size was exhibited within the pairwise comparison of the 'Small- Large' Tank Sizes, demonstrating that in these trials Tank Size does effect the end weight of individuals.

This study demonstrates similarities to that of Araneda, *et al*, (2008), into the effect of different stocking ratios on weight of *Litopeneaus vannamei*, (figure 4.3) as the tanks with individuals at a lower stocking density of 90 m^{-2}, compared to 180 or 130 m^{-2}, had a higher average growth and end weight.

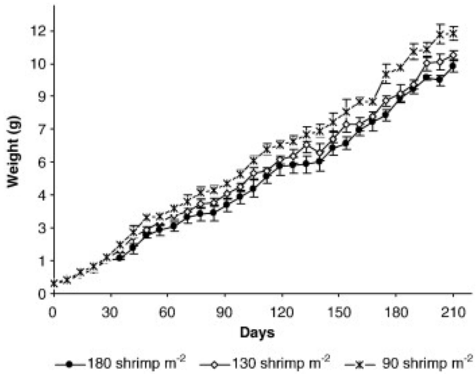


Figure 4.3: Average growth over a period of days of prawns stocked at different densities (Araneda, *et al*, 2008).

Many studies have found lower stocking densities produce larger individuals, the figure (4.4) below is an example of this (highlighted in red) however there are commercial interests in aquaculture so the production yield needs to be profitable, so although smaller densities may produce larger individuals with higher survival rates this may not translate into production yield, in this study the highest stocking density produced the greatest production yield (highlighted in blue), a desirable outcome for a commercial farm (Sookying *et al*., 2011).

Density	17 shrimp m^{-2*}	26 shrimp m^{-2*}	35 shrimp m^{-2}	45 shrimp m^{-2}
Yields(kg ha^{-1})	2660.8 + 510.25b	3052.8 + 4.25b	4612.5 + 810.51a	6149.6 + 715.79a
Final weight(g)	25.3 + 2.22	20.7 + 3.10	22.0 + 1.56	21.9 + 1.98
Weight gain (g)	25.3 + 2.22	20.7 + 3.10	21.9 + 1.57	21.8 + 1.98
FCR[a]	1.17 + 0.221	1.50 + 0.010	1.54 + 0.388	1.35 + 0.177
Survival (%)	61.5 + 6.40	58.0 + 8.73	59.5 + 10.14	65.1 + 11.19

[a] Feed conversion ratio = total feed offered/biomass increase.
* Two ponds were excluded from each of these treatments (n = 2) due to the blue green algae and alkalinity problem that resulted in poor survival.

Figure 4.4: Mean production parameters of *L. vannamei* over varying densities (Sookying *et al*., 2011). Red and blue boxes added by author.

This study found larger individuals dominating smaller ones whilst feeding and for space, this effect is amplified in high-density tanks, such as the Small Tanks in this study. While investigating the suitability of different species of prawns for intensive cultivation Forster and Beard (1974) found individuals in crowded environments had lower growth rates due to the behaviour of individuals towards causing additional stress. This effect brought on by social interaction in some cases brought on an inhibitory effect on the growth on individuals (Arnold *et al*., 2006). In order to reduce or negate the effect of high stocking density on the growth of *L.vannamei* artificial substrates could be used as mentioned above (Moss and Moss, 2004).

4.1.2 Condition Factor Analysis

As shown in *Chapter 3: Results and Analysis* there were differences in Condition Factor of individuals in different groups over the trials, this section will discuss these differences of Condition Factors within Environments and Tank Sizes, the reasons for this and why different groups display differences in Condition Factor.

The statistical analysis of Condition Factor against Treatment in *Chapter 3: Results and Analysis* showed there is a difference in Condition Factor of individuals from different Treatments, and the tanks themselves played no difference in the outcome of individuals Condition Factor, figures under section '*3.1.2.1 Weight Against Treatmen*t', display the differences in Condition Factor within and between each treatment. Due to the rejection of the Null Hypothesis further analysis of Condition Factor v. Environment and Condition Factor v. Tank Size could be undertaken, and the purpose of the trials i.e. the difference in Condition Factor of individuals in varying Environments and Tank Sizes could be analysed. Below is the analysis and discussion of these results displayed in the previous section *Chapter 3: Results and Analysis*.

4.1.2.1 Condition Factor with Environment

The statistical analysis of Condition Factor against Treatment in *Chapter 3: Results and Analysis* showed there is a difference in Condition Factor of individuals from different Environments, therefore the Null Hypothesis: *'There will be no difference in the weight and condition factor of the individuals from differing environments'* is rejected. Figure 3.18 shows the distribution of Condition Factor across environment, with the mean of the 'Escapees' being the highest and the 'Control' environment having the lowest. The environments with added material placing in between, indicating that within these trials added materials did not have adverse effects in terms of the end Condition Factor of individuals. The significant differences between environments was exhibited within 'Control-Filter Brush', 'Control-Escapees', 'Control-'Natural'' and 'Filter Brush-''Natural'', showing the disparity in terms of Condition Factor between the Control, 'Escapees' and added material environments.

4.1.2.2 Condition Factor with Tank Size

The statistical analysis of Condition Factor against Treatment in *Chapter 3: Results and Analysis* showed there is a difference in Condition Factor of individuals from different Tank Sizes, therefore the Null Hypothesis: *'There will be no difference in the weight and condition factor of individuals in different sized tanks'* is rejected. Figure 3.21 shows the distribution of Condition Factor across Tank Size, 'Escapees' having the highest mean Condition Factor and the 'Small' Tank Size having the lowest mean Condition Factor. The greatest significant difference between Tank Size was exhibited within the pairwise comparison of the 'Small-Escapees' and 'Small- Large' Tank Sizes, demonstrating that in these trials Tank Size does effect the end Condition Factor of individuals.

4.2 Mortality

Over the course of the two trials, individuals from across the two Bank systems died for a variety of reasons. This section will explore the deaths within the system by Treatment, Environment and Tank Size and the possible explanations for these deaths, within these groups and overall.

4.2.1 Mortality Analysis

Figures displayed under *3.3 Mortality in Tanks*, show the number of individuals left in the tanks at the end of the trial when 20 individuals were placed in each tank at the beginning of the two trials, split up by Treatment, Environment and Tank Size. By the end of the two experiments, some Treatments had no individuals left in them and some had an increased number of individuals, over the original 20 added at the beginning. The increase is due to movement of individuals between tanks (under their own volition).

4.2.1.1 Mortality within Environment

Under the categorisation of mortality within different environments, all tanks from both trials were split into 'Natural', Filter Brush, Control and Escapees. The controlled environment had the highest amount of surviving individuals at the end of the trials. However, both the environment type Control and 'Natural', had tanks with large amount of movement into them. It is therefore difficult to assess the effect differing Environments have on survival rate.

Other studies investigating the effect environment has on mortality rates, lend to survival rates being higher in treatments with added substrate or shelter. Studies by Moss and Moss (2004) (figure 4.5) and Zhang *et al.* (2010) (figure 4.6), it is thought added material increase the survival rate as they create more food through the organic matter that can grow on the added material and provide shelter from cannibalism (Moss and Moss 2004; Zhang *et al.* 2010).

Treatment	Final Weight (g)	Survival (%)	Production (kg/m^2)	FCR
778	1.69 ± 0.25	91.9 ± 2.6	1.21 ± 0.17	0.78 ± 0.04
7789S	2.13 ± 0.04	93.2 ± 1.9	1.54 ± 0.03	0.75 ± 0.09
1,167	1.44 ± 0..07	89.1 ± 3.1	1.50 ± 0.07	0.78 ± 0.00
1,167S	1.69 ± 0.03	93.0 ± 2.8	1.84 ± 0.09	0.73 ± 0.02
1,556	1.16 ± 0.11	91.8 ± 2.8	1.66 ± 0.21	0.89 ± 0.09
1,556S	1.56 ± 0.04	90.5 ± 2.3	2.20 ± 0.09	0.78 ± 0.07

Figure 4.5: Formerly 4.1. Mean final weight (g), survival, production and feed conversion ratio (FCR), S after treatment indicates the presence of substrate (Aquamats) in the treatment (Moss and Moss, 2004).

Treatments	Weight gain (g shrimp $^{-1}$)	Survival (%)	Yield (g m^{-2})
G0	3.35±0.18^{a}	63.50±0.02^{a}	1044.37±19.76^{a}
G1	3.73±0.16^{b}	70.50±0.02^{b}	1313.93±96.84^{b}
G2	4.41±0.11^{c}	76.00±0.01^{c}	1674.28±71.45^{c}
G3	5.45±0.06^{d}	86.50±0.02^{d}	2370.40±82.59^{d}

G0, G1, G2 and G3 denote the treatment with 0, 1, 2 and 3 pieces of artificial substrates, respectively. Different superscripts denote significant difference ($P<0.05$) between treatments.

Figure 4.6: Formerly 4.2. Effects of artificial substrate on growth, survival and yield in *Litopeneaus vannamei* (Zhang *et al.*, 2010).

4.2.1.2 Mortality within Tank Size

The Large tanks when adjusted for the number of tanks of that type had a higher survival rate of prawns compared to that of the Small tanks. Again as with the Mortality within Environment, it is difficult to get a true assessment of the effect Tank Size has on mortality due to the movement of individuals with the system. Aggression is density dependant, more densely populated environments increase the volume of interactions between individuals, increasing the likelihood of more of these interactions being aggressive, having negative effects (Abdussamad and Thampy 1994).

Several studies have investigated the effect of stocking densities on mortality, Araneda, *et. al*, (2008), found an approximate 10% increase in survival rate between their lowest stocked tanks and their highest; 76.1% ± 3.16 survival in 90m^{-2} stocked tanks, 65.9% ± 0.91 survival in 180m^{-2} stocked tanks (Figure 4.7). This difference in survival rates is once again thought to be due to the behaviour and social interactions amongst individuals, with conflicts over food and space, which are under greater pressure in reduced territory (Araneda, *et. al*, 2008).

Treatment (shrimp m^{-2})	Culture time (weeks)	Growth rate (g $week^{-1}$)	Survival (%)
90	30	0.38^{a} ±0.011	76.1^{a} ±3.16
130	30	$0.34^{a,b}$ ±0.007	$68.9^{a,b}$ ±0.78
180	30	0.33^{b} ±0.009	65.9^{b} ±0.91

Figure 4.7: Survival rates (%) of *L.vannamei* cultured at three different densities (Araneda, *et. al*, 2008).

4.3.1 Explanations for Prawn Mortality

4.3.1.1 Cannibalism

Cannibalistic behaviour has been observed in the commercial culture of many species of crustaceans and is undesirable in aquaculture because of its effect on the productivity of systems, producing varying levels of inefficiencies (Polis, 1981; Romano and Zeng, 2016). Cannibalism in prawn species is often because of high stocking densities (particulary in nursery stages); low food availability; insufficient water quality; and, diseased individuals (Romano and Zeng, 2016). Over the duration of both the studies, as figure 4.8 displays, some of the individuals were eating other individuals, whether they were already dead due to a variety of reasons (as will be discussed within this section), preyed upon whilst moulting (a vulnerable stage) or they were eating the carcases produced from moulting. Daily observation revealed that in a few cases larger individuals were predating on smaller prawns, especially when these have just moulted, at this point individuals are particularly vulnerable, as they have shed their hard exoskeleton, leaving a soft carapace underneath. Moulting increases the vulnerability of an individual because they become less to defend themselves from attack due to the newly formed soft exoskeleton, where they previously had a harder less penetrable carapace. Allowing for predation by individuals with a harder more impenetrable exoskelton (Soesanto *et al,* 1980). Low levels of dissolved oxygen within the culturing environment can lead to soft shell developing in populations, consequently making them more vulnerable to intraspecific predation (Schroeder *et al,* 2010). Additionally, individuals shed their exoskeletons as they become too big for them, this moulting in crustaceans, as with many of animals, produces a larger individual, which can be double their previous size. Once the exoskeleton has hardened, and they are less vulnerable to predation (Marshall *et al,* 2005).

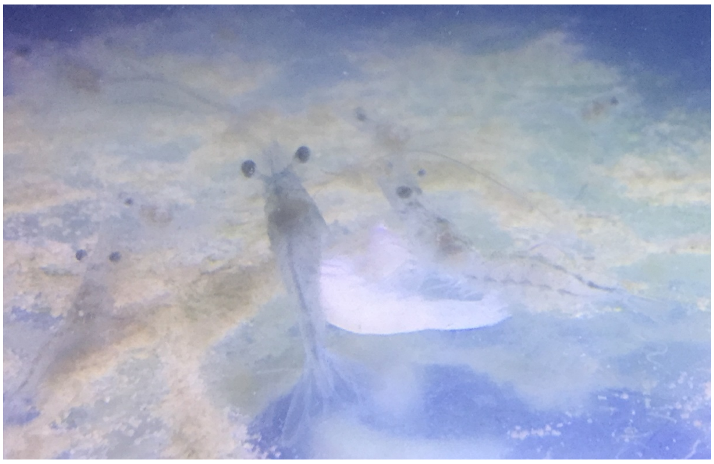


Figure 4.8: Formerly 3.30. A dead prawn (white-centre of the figure) being eaten by several other prawns.

As well as cannibalism reducing the economic output through predation, diseases such as white spot and yellow head virus can be transmitted through cannibalism, serving as a vector to the transmission of these diseases. These individuals are then unfit for consumption, undesirable for prawn farmers, as there is no economic worth to these individuals (Hamano *et al,* 2015; Soto *et al,* 2001).

There are several high technology methods being developed to reduce cannibalism in aquaculture such as using neurotransmitters to reduce aggressive tendencies and therefore lower predation within systems, reducing the effect cannibalism has on outputs. This is a high cost experimental method, lower cost methods would have been to provide shelter or reducing stocking densities to allow individuals to be protected while molting and decreasing the amount of interactions which could lead to aggressive behaviors reducing the likelihood of predation (Romano and Zeng, 2016; Soesanto *et al,* 1980).

A study by Abdussamad and Thampy (1994) explored the effect of different stocking densities and differing substrate types had on cannibalism within the nursery rearing stage of the Tiger shrimp (*Penaeus Monodon*). As displayed in Figure 4.9, higher rates of cannibalism were shown in higher population densities, the loss of individuals due to cannibalism ranged from 1.06% at the lowest stocking density of $25/m^2$ and 16.56% at the highest stocking density of $500/m^2$. This study concluded cannibalism is density dependent, as a more densely populated tank increases the amount of interactions between individuals therefore there is a higher chance of those interactions being aggressive, which could lead to cannibalism (Abdussamad and Thampy 1994).

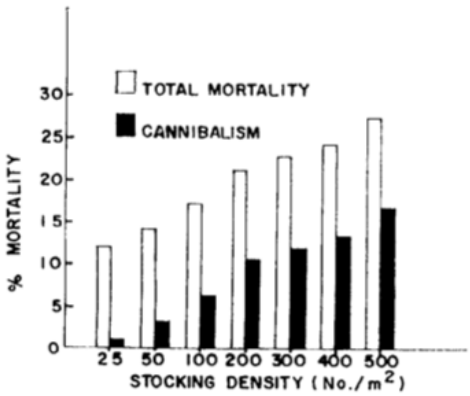


Figure 4.9: Effect of stocking density on cannibalism (Abdussamad and Thampy, 1994).

Abdussamad and Thampy (1994) also explored the effect tank substrate had on general mortality and cannibalism. Both the total mortality and the mortality from cannibalism was lower in the tanks with substrate provision compared to the control tank (G- highlighted in red) (figure 4.10). In the control tank, 18.65% of individuals died as a result of cannibalism, this number was lower in the tanks with substrate ranging from 5.3% to 10.17%. The rate of cannibalism decreased with age across all stocking densities and substrate type, this could be due to the greater availability of shelter, reducing the cases of interaction and providing a place for vulnerable individuals to hide, such as those who are small or molting (Abdussamad and Thampy 1994).

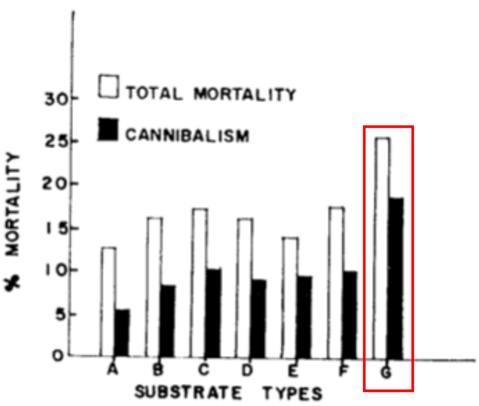


Figure 4.10: Effect of different substrate types on cannibalism: A- clam shell, B- round pebbles, C- PVC tube frame, D- polypropylene net frame, E- black polythene raffia, F- twigs, G- control (Abdussamad and Thampy, 1994). Red box added by author.

Abdussamad and Thampy (1994) also concluded cannibalism reduced with an increased feeding frequency, this amongst reduced stocking densities and introductions of substrates, would alleviate the effect of cannibalism. An increased feeding frequency is thought to reduce cannibalism as individuals spend less time moving around the tank searching for food, decreasing the amount of interactions and therefore the amount of negative interactions that could lead to cannibalistic behaviours.

4.3.1.2 Movement

Individuals moved between tanks, they were able to do this because the gaps within the system, the space between tank sides and the glass tops, as well as the gaps within the plastic discs between the glass sides that allow for the flow of water. The individuals were also able to move throughout the system as they can jump (figure 4.11), in some cases on to the glass tops, stranding themselves and subsequently drying out and dying. This movement is further demonstrated in *Chapter 3: Results and Analysis*, as *3.2.1 Mortality within Treatments*, displays move individuals in some treatments at the end of the trial than originally placed in those tanks (figure 3.23).

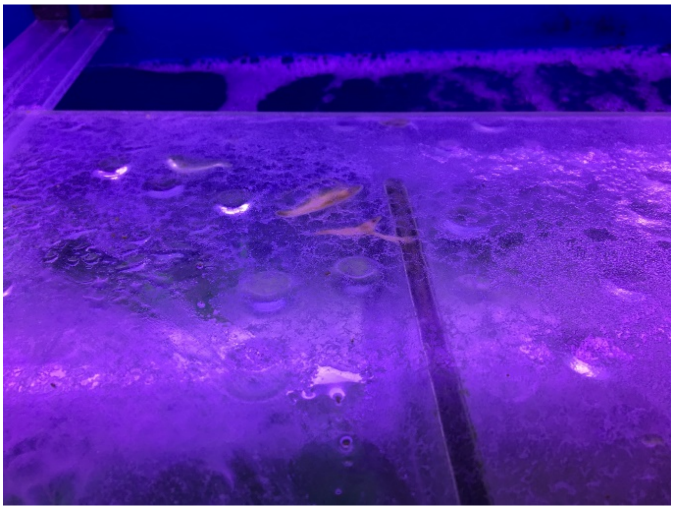


Figure 4.11: Showing individuals that have jumped up onto the glass top and become stranded, and subsequently died. Taken by Author.

4.3.1.3 Tank Conditions and Shock Events

4.3.1.3.1 Temperature Changes

There were small temperature fluctuations throughout both trials. Temperature is regarded as one of the most important factors to the survival of a marine organism, the further temperature strays from the organism natural parameters, the increase chance of mortality (Ponce-Palafox *et al,* 1997), high mortality is very undesirable in aquaculture as it leads to reduce profit margins. At consistent lower than ideal temperatures metabolism is slowed, over a prolonged period of time metabolic processes cease to function, leading to the death of an individual. At consistent higher than ideal temperatures proteins denature and membranes become fluid – metabolic dysfunction, leading to the death of an individual (Fast and Lester, 1992). The systems were in a temperature controlled room (about 10ºC) and water heaters were used to keep the water at 26-29ºC, optimum temperature for growth. However, within trial 2, there was a dip in the temperature of both the banks (figure 4.12 - highlighted in a green box), this was due to the cooling system not working properly and overly cooling the room, bringing the temperature down, straying from the ideal temperature range. As daily temperature measurements were taken this was quickly noticed and the cooling system was reprogrammed to address this and ensure the water temperature would not stray from 26-29ºC. The drop in temperature did not immediately lead to death of individuals however it could have effected their health and longevity.

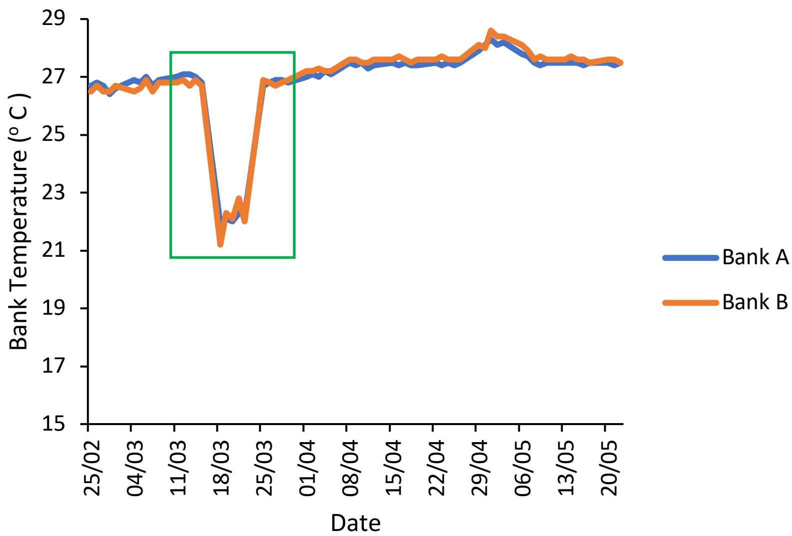


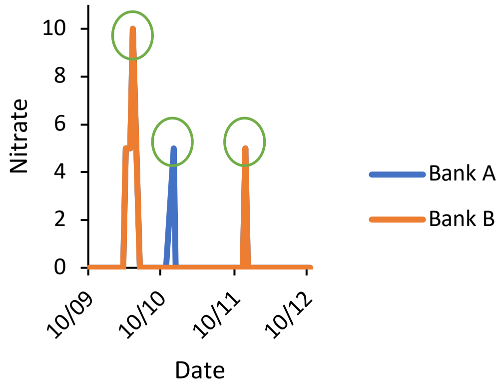
Figure 4.12: Formerly figure 2.10. Bank temperature across trial 2. Created by Author.

4.3.1.3.2 Changes in Salinity

Salinity is regarded as one of the most important factors to the survival of a marine organism, the further salinity strays from the organism natural parameters, the increase chance of mortality (Ponce-Palafox *et al,* 1997), high mortality is very undesirable in aquaculture as it leads to reduce profit margins. As shown in *Chapter 2: Methodology*, under *2.2.3 Regular Checks* (figure 2.11), the salinity fluctuated throughout both trials, however these changes in salinity were minimal and within the salinity tolerance of *L.vannamei*, this alongside the work of Jaffer *et al.* (2020), investigating the effect of low salinity on the growth and survival of *L.vannamei*, showing the species is able to maintain homeostasis at a variety of salinity without an impact on growth or mortality is why salinity fluctuations within the systems over the two trials can be excluded as a possible reason for prawn mortality (Jaffer *et al.*, 2020). The fluctuations shown in figure 2.11, were due to water evaporating increasing the salinity and then the salinity decreasing as the system was topped up with freshwater, to ensure optimum salinity but also so the system did not run dry.

4.3.1.3.3 Nitrogen Levels and Algal Blooms

As shown in *Chapter 2: Methodology*, under *2.2.3 Regular Checks* (figures 2.13 to 2.15), the nitrogen levels (ammonia, nitrite and nitrate) fluctuated throughout both trials. The growth of the prawn aquaculture and demands of the industry, has led to more farm pursuing intensive farming methods, with farms adding high protein feeds *ad libitum*, for the fastest growth of individuals as possible. The use of feed in this way can led to high levels of nitrates existing in systems because of food waste and excrement (Lovell, 1989). Nitrate at high concentrations is toxic to *L.vannamei*, high levels can cause slow growth, lethargy and in very high concentrations death; however, if high concentrations are noticed within systems prawns can partially recover if these levels are reduced through either the removal of waste (food and excrement) or water changes, highlighted by a green circle (figure 4.13), to remove waste water and introduce freshwater into the system. Although nitrate levels have been reduced to a safe level, nitrate shock events can led to reduce growth rates (Gross *et al,* 2004; Boyd and Tucker, 1998). Taking daily nitrate levels identified when nitrate levels spiked, however it was difficult to quantify the effect of nitrate levels on mortality, as there was no dead individuals found after these events, however due to there being fewer individuals at the end of the trial than deaths identified throughout the two trials it is possible that these spikes could be responsible for more individuals mortality than previously thought.



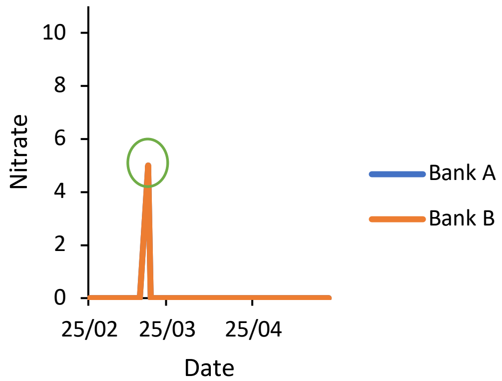


Figure 4.13: Formerly figure 2.15. Nitrate across the trials. Above Trial 1, Below Trial 2.

At the beginning of both the trials the sumps at the bottom of the bank systems were seeded with bacteria (supplied by FloGro Fresh), along with water changes, and aeration throughout the systems to allow dissolved O_2 into the systems, these actions were to reduce and prevent cyanobacteria build up and subsequent algal blooms (Blanchard, 2014). Despite these actions an algal bloom still developed, at the beginning of trial 2 during the period of tank equilibration (figure 4.14). This build-up of blue-green algae occurred before the second trial commenced, it is thought the bloom occurred due to not all the waste still the water being removed from trial 1, when the systems were drained, and the water flow running at a reduced level therefore reducing water circulation. To prevent the bloom moving across into the other banks, the system was drained and tanks cleaned, along with the materials used during the cleaning process to not contaminate the other tanks. Prawns for the second trial were only introduced when water quality levels were at safe parameters.

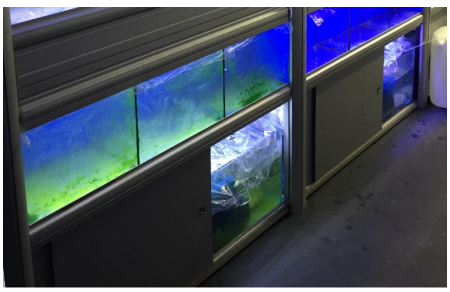


Figure 4.14 Algal bloom in the banks, bank 1 (left) has a larger build up.

4.3.1.4 Water Circulation

Between the experimental tanks (tanks on the middle row) there are flow vents to allow for water to move freely, exchanging waste water with freshwater (figure 4.15). However, the holes in the flow vents were large enough for early PLs to escape through, netting was placed between to reduce the possibility of movement. The netting had an unwanted side effect of providing a surface for algae to grow on, this subsequently created a barrier and reduced the ability of water to flow in and out, leading to an increase in ammonia (figure 4.16), high levels of which are toxic. When this was observed the old netting with large algal growth was replaced with new netting to allow water flow and reduce the possibility of harmful chemicals building up. No carcasses were found after this event, there was no direct link between this increase in ammonia and death however as mentioned previously, such increases could have contributed to the death of individuals and reduced their growth rates (Gross *et al,* 2004; Boyd and Tucker, 1998).

Figure 4.15: Mesh between tanks.

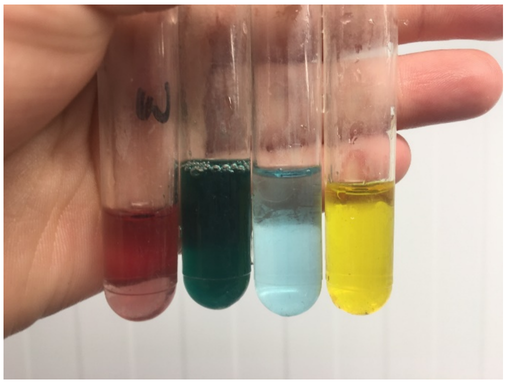


Figure 4.16 Water quality samples, green is showing high ammonia. Taken by Author.

4.3 Behavioural Study

As shown in *Chapter 3: Results and Analysis* there were differences in Behaviour of individuals in different groups over the trials, this section will discuss these differences of Behaviour within Environments and Tank Sizes, the reasons for this and why different groups display differences in Behaviour.

In order to explore the effect Environment and Tank Size on may have on behaviour; prawns were observed daily Before Feeding (BF) and After Feeding (AF) to observe what the principal behaviour was at those moments, displayed by the majority of individuals. Behaviour was divided into 4 categories.

4.3.1 Behavioural Analysis

Figures under subheading *3.3.1 Overall Tank Behaviour* (figure 3.26), show the overall behaviour of all tanks across Bank A and Bank B Before and After Feeding in Trial 2. The most obvious trend is the increase in individuals eating After Feeding; this is due to more food being added to the tanks, enabling individuals to eat more than the other sparse food sources e.g. algae, exoskeletons and other individuals. As the purpose of the trial is to investigate the effect Environment and Tank Size has on individuals, further graphs were produced to show the behaviour displayed by individuals due to Environment (figure 3.27) and Tank Size (figure 3.28 and 3.29).

4.3.1.1 Behaviour within Environment

The Figure 4.17 displays as discussed above the increase in individuals eating After Feeding. Additionally, as has been mentioned previously the Control environment tanks has a higher number of individuals eating when food is added compared to the 'Natural' and Filter Brush tanks. It could be suggested the food is more visible, due to there not being objects and additional materials blocking the view of food being added or perhaps they are less distracted than in the added material environments. As these materials provide them with greater stimulation, this stimulation could mean they are more occupied and less alert on to changes in their Environment. Tanks with added material showed individuals interacting with the Filter Brushes or the artificial plants, an option of stimulus unavailable to the 'Control' tanks.

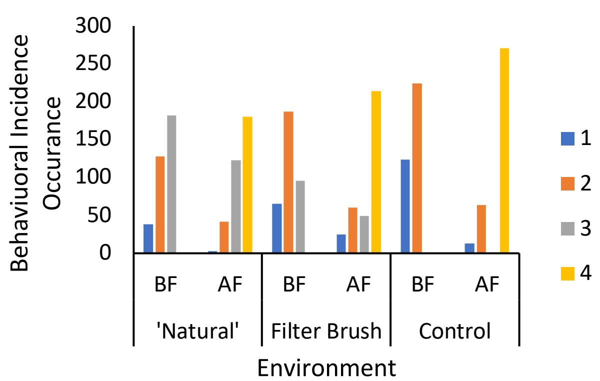


Figure 4.17: Cited previously as figure 3.27. Occurrence of a particular behaviour within an environment before feeding (BF) and after feeding (AF). Behaviour key: 1.Sitting on the bottom of the tank, 2.Swimming , 3.Interacting with added material, 4.Eating.

4.3.1.2 Behaviour within Tank Size

The figures under *3.3.3 Behaviour within Tank Size*, as with previous figures under *3.3.1 Overall Tank Behaviour* the trend of more individuals displaying the behaviour Eating after they have been fed continues into Tank Size (figure 4.18). The major difference between the two tank sizes (Large and Small) in the behaviour displayed is the greater number of individuals that are displaying behaviour *2. Swimming* within the Large tanks. This is in part due to the increased amount of space within the Large tanks, the lower stocking density within these tanks could mean individuals are more inclined to swim as they do not bump into others, an action that could be considered 'aggressive' or into tank walls. Within the Small tanks, a continuation of the above is the following contrast of the more individuals displaying behaviour *1. Sitting on the bottom on the tank*, in the Small tank contrasting to the Large tank.

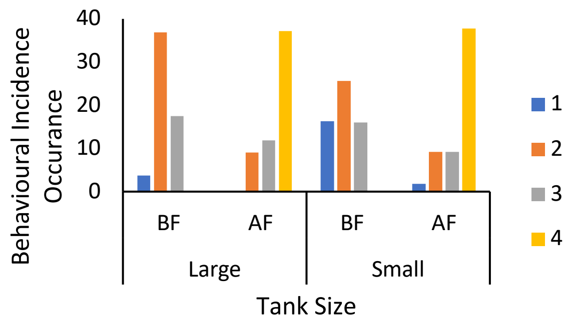


Figure 4.18: Cited previously as figure 3.29. Occurrence of a particular behaviour within an environment before feeding (BF) and after feeding (AF). Values adjusted for number of tanks that size. Behaviour key: 1.Sitting on the bottom of the tank, 2.Swimming , 3.Interacting with added material, 4.Eating.

4.4 Supplementary Feed

Throughout the two trials, additional food sources were unintentionally on offer supplementing the diets of individuals through prawn eating other dead individuals or their shed carcasses, but also through the growth of algae within the systems.

4.4.1 Cannibalism and Shedding

As mentioned throughout this thesis individuals in both the trials ate dead individuals and their shed carcasses. Both the consumption of deceased individuals and the carcasses of those as they grow contributed to the nutrition of the prawns. Their consumption may also allude to something missing in their diet that is not provided for in their formulated feed (figure 4.19 and 4.20).

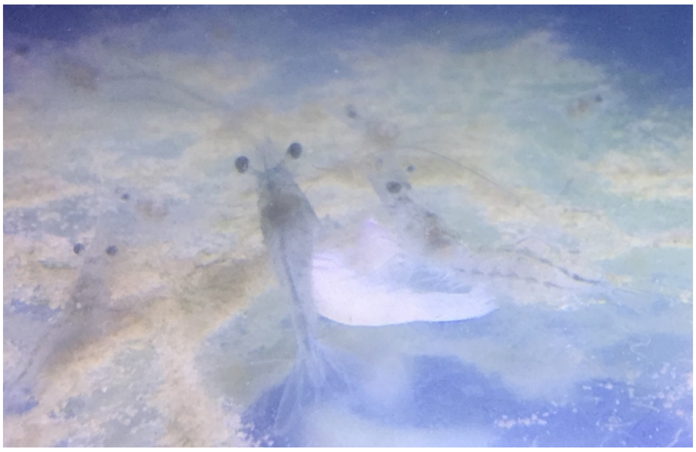


Figure 4.19: Formerly 3.30 and 4.8. A dead prawn (white-centre of the figure) being eaten by several other prawns.

Figure 4.20: Formerly 3.31. A prawn carcass (middle of the image) being held up and eaten by another prawn.

Such behaviour was noted as interesting by the suppliers (FloGro Fresh), it was able to be observed due the experimental set up including glass fronts, giving a clear view of a variety of behaviours. Behaviour such as cannibalism is difficult for industry to document due to the fabric and scale of the tanks used commercially. FloGrow Fresh noted that they thought cannibalism within their system was a possibility and occurred because fewer carcasses and shed carapaces are found than to be expected, leading to cannibalism being speculated as an answer to this query.

4.4.2 Algae

Adding material such as Filter Brushes and creating a ‘Natural’ environment with a substrate and artificial plants, increase the surface area algae has to grow on. Individuals were observed feeding on the algae, previously observed by Arnold *et al.* (2005), brown tiger shrimp (*Penaeus esculentus*) eating the epiphytic biota that began to colonise the AquaMat artificial substrate, demonstrating that prawns in cultured environment will graze on additional food sources, as well suggesting there may be something missing within their diet that the consumption of algae fulfils (Arnold *et al.*, 2005).

Demonstration of consumption of algae can be seen through the figure below (figure 4.21), the left image shows the tanks within the bank system and the right is a zoomed in image of tank 4, showing algal growth within a tank with no individuals in. It can then be suggested as the other tanks with individuals in display less algal growth, individuals are eating the algae suspended in the tanks and attached to the tank walls. The consumption of algae is also shown through the end of trial photos (*7.Appendix (7.1 End of trial photos)* displaying the escapees. These individuals were not directly fed, they fed on the suspended material and algae within the tank system. The photos (figures 7.5 to 7.9) also show individuals being 'greener' in colour suggesting a greater abundance of chlorophyll within their system, this could be due to algae being a larger part of their diet than the individuals that were also fed their formulated feed.

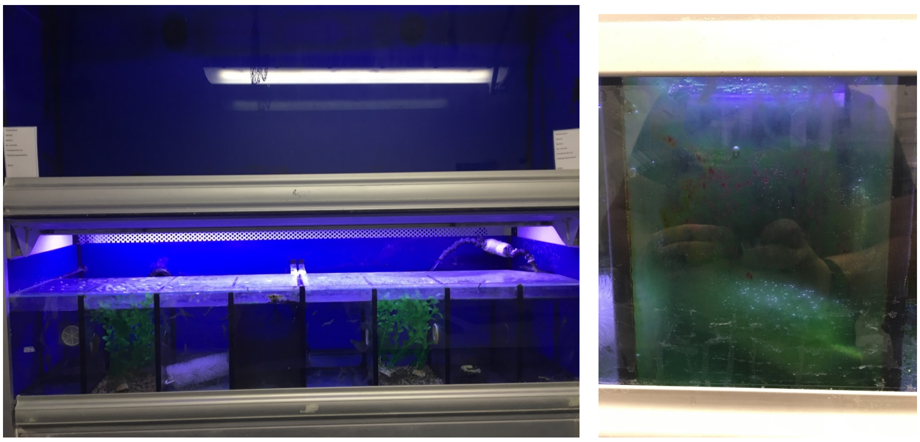


Figure 4.21: Left- tank set up. Right- zoomed in image of tank 4, showing algal growth. Taken by Author.

4.5 Critique of Experimental Design

Over the two trials, there were some issues that could be attributed to the design of the experiments, figure 4.22 to 4.26 depict some of these issues. Over the course of the two trials individuals escaped to different parts of the tank system, this is illustrated in figure 4.22. As well as, individuals being stranded on the glass tops, they moved between tanks, they were able to do this through gaps within the banks and due to their natural ability to jump, illustrated in figures 4.22 to 4.26. In the future these gaps and areas they could jump through could be limited by netting or made to measure covers, preventing individuals moving around the system and getting stranded.

Figure 4.23 shows a flow vent between the experimental tanks, mesh was added to inhibit the movement of individuals between the experimental tanks, however this mesh also provided a surface for algae to grow on, and their growth then reduced the flow of water between tanks. This led to a decrease in water quality in the some of the tanks and therefore could be an explanation for high mortality within those tanks. This problem could be alleviated in the future by having replacement flow vents discs, with reduced hole sizes to avoid movement between tanks, or to regularly replace the mesh reducing the ability for algae to build up and reduce water flow between tanks.

The addition of these improvements in further trials would help to reduce mortality and produce wider understanding of the effect tank Environment and Size play on the growth and mortality of *L. vannamei*, through reducing the number of mortalities due to design issues.

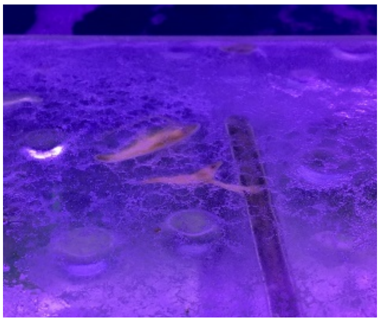


Figure 4.22: Showing individuals that have jumped up onto the glass top and become stranded, and subsequently died.

Figure 4.23: Mesh between tanks.

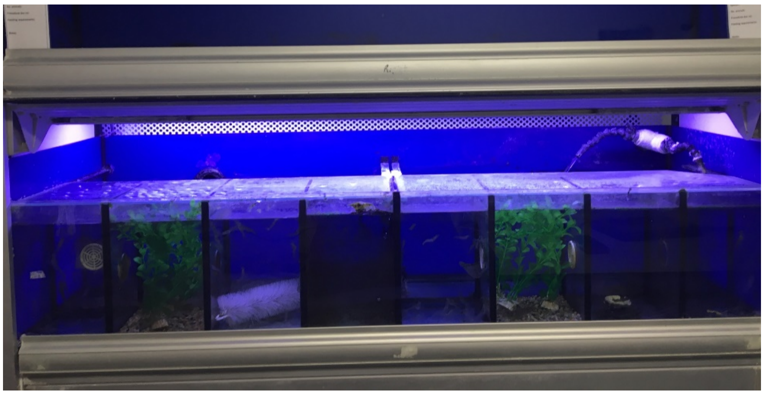


Figure 4.24: Image of the front of the experimental tanks in a bank.

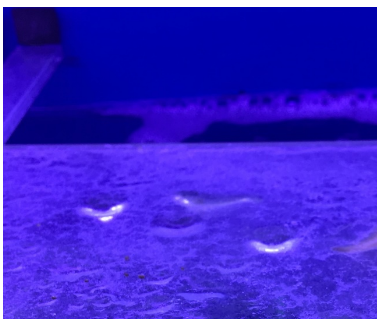


Figure 4.25: Showing the space behind the front experimental tanks.

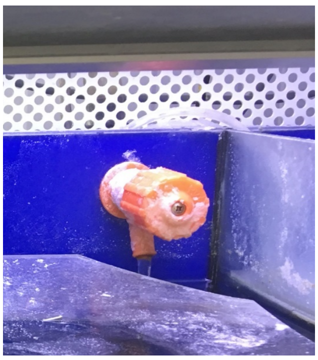


Figure 4.26: Glass divider on the right between the Large tanks.

4.6 Implications for the Industry

This work is of value to the industry as it demonstrates culturing *Litopenaeus vannamei* in added material environments can produce increased growth. This is desirable to the industry as it enables individuals to be of marketable size quicker, this efficiency in growth, increases the sustainability of prawn aquaculture within run for profit farms.

5. Conclusions and Further work

5.1 Summary of Findings

These two trials investigated whether a 'natural environment' increased growth and reduced the mortality of *Litopenaeus vannamei* within a closed aquaculture system. This aim was tested through the successful creation of 3 different environments: 'Natural', 'Filter Brush' and 'Control'. Throughout both trials the water quality parameters (temperature, salinity, pH, ammonia, nitrite and nitrate) were measured daily and maintained within acceptable limits for the growth of *Litopenaeus vannamei*. At the end of each trial weight and length measurements of all individuals were recorded. At the end of each trial mortality was noted within each environment. Throughout both trials the behaviour of individuals was noted.

Additionally, for trial 2 the effect of Tank Size was investigated. 'Large' Tanks of 3 different environments: 'Natural', 'Filter Brush' and 'Control', were successfully created. The weight and length measurements of all individuals in both 'Large' and 'Small' tank sizes were recorded. At the end of each trial mortality was noted within each Tank Size. Throughout trial 2 the behaviour of individuals was noted within Tank Size.

The results showed individuals grew in each of the environments: 'Natural', 'Filter Brush' and 'Control'. The statistical analysis showed there was a significant difference in the growth of these 3 environments: 'Natural', 'Filter Brush' and 'Control'. The analysis showed there was increased growth in the added material environments ('Natural' and 'Filter Brush') compared to the 'Control' environment. An analysis showed there was a significant difference in the growth of individuals in the differing tank sizes, with the 'Large' tank environments showing the greatest growth. More individuals were recorded in the 'Control' environments compared to that of the 'Natural' and 'Filter Brush' environments at the end of both trials. More individuals were recorded in the 'Large' tanks compared to that of the 'Small' tanks at the end of trial 2. The behaviour of individuals within the added material environments ('Natural' and 'Filter Brush') exhibited more active behaviour than that of the control. The behaviour of individuals within the 'Large' tank size exhibited more active behaviour than that of the 'Small' tank size.

5.2 Further Work

In the future, there are several different directions in which this work could be taken and developed further. Firstly, the trial could be replicated with just a change in the length of the trial, the trial could be extended until individuals are 4-5 months old, in which time individuals will reach market size (25g).

Investigating which Environment grows the majority of individuals to that size first; or which Environment enables the most amount of individuals to survive to that size. As well as to see the effect of tanks size has on individuals as they grow and get older, observing the differences in behaviour amongst individuals over the longer trial period, as well observing the behaviour as they get larger and see if there any changes in behaviour with size.

Secondly, more variables could be introduced, that could be replicated easily within aquaculture systems such as large boulders or rocks, with cut out areas to serve as hiding places providing more shelter from larger individuals or to individuals when they are vulnerable during moulting, which this trial has highlighted as important to reducing cannibalism. The behaviour study also showed the difference in movement between treatments with textured and non-textured tank bottoms, different textured tank bottoms could also been investigated to make recommendations of what would be best for industry, and most viable within a run for profit farm. Additionally, trials like this could have larger growth rates and decreases in the mortality rates by the addition of Ultraviolet (UV) light to light the tanks as it reduces stress responses (Fei *et al*., 2020).

The majority of prawns are cultured in monoculture systems of varying intensities, where there is a reliance on high protein feed of low digestibility which leds to reduced nutrient uptake efficiency from individuals. Some see integrated multitrophic aquaculture (IMTA) as a way of improving nutrient efficiencies as well combating the increasing cost of feed, improving the profitability of prawn aquaculture. Co-culture is an emerging interest in aquaculture, growing several species alongside each other. Omout *et al.* (2020) investigated the of culturing *L.vannamei* in an integrated system with the Pacific Oyster (*Crassostrea gigas*), co-cultured individuals had a higher mean growth weight at the end of the trial, due to the ability of *C.gigas* to maintain optimum water quality parameters through the biofiltering ability of oysters, removing phytoplankton, bacteria, suspended solids and waste products which could led to eutrophication (Omout *et al.*, 2020). As well as using one species to improve the health of the desired species, culturing two commercially viable species such as macroalgae (*Gracilaria tikvahiae*) and *L.vannamei* alongside each other increases the profitability of production (Samocha *et al.*, 2015).

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7. Appendix

Figure 7.1: Aquarium Checklist (Side A)

Aquarium checklist (please complete both sides)

Ue

Week commencing Monday 11 of (month) March (year) 2019

Health, safety and room maintenance checks											
Day (add in date)	Sign in with staff (or security if weekend)	Time	Sign out with staff (or security if weekend)	Time	Refrigeration unit works?	Fans work?	Record temp on display	Ice build-up on refrigeration unit?	Dehumidifier works?	Trip/slip hazards on floor?	Initial
M	✓	15:17	✓	15:31	✓	✓	16.1	/	✓	/	AmB
Tu	✓	10:41	✓	11:37	✓	✓	15.8	/	✓	/	AmB
W	✓	/	✓	/	✓	✓	16.1	/	✓	/	AmB
Th	✓	/	✓	/	✓	✓	16	/	✓	/	AmB
F	✓	/	✓	/	✓	✓	15	/	✓	/	AmB
Sa											
Su											

System maintenance checks											
Day (add in date)	Tank heaters work?	Tank bulbs work?	Tank pumps work?	Top up water	Feed animals	Do animals look/behave healthy?	Inlets/outlets free of algae with good flow?	Pipes at back free of algae with good flow?	Any leaks?	Other notes	Initial
M	✓	✓	✓	✓	✓	✓	✓	✓	/	/	AmB
Tu	✓	✓	✓	✓	✓	✓	✓	✓	/	/	AmB
W	✓	✓	✓	✓	✓	✓	✓	✓	/	/	AmB
Th	✓	✓	✓	✓	✓	✓	✓	✓	/	/	AmB
F	✓	✓	✓	✓	✓	✓	✓	✓	/	/	AmB
Sa											
Su											

Figure 7.1: Aquarium Checklist (Side A)

Figure 7.2: Aquarium Checklist (Side B)

Daily checks (please fill in day done, date and values – interval of testing will depend on individual experiments)

Day	Time	Notes	*Temperature*			*Salinity*			*pH*						Initial
			Left 1	Mid 2	Right 3	Left 1	Mid 2	Right 3	Left 1	Mid 2	Right 3	Left 1	Mid 2	Right 3	
M	15		21.9	21.2		22	21		8.4	8.4					
Tu	10		22.1	22.3		21	20		8.4	8.4					
W			22	22.1		21	21		8.4	8.4					
Th			22.3	22.8		21	21		8.4	8.4					
F			22.2	22		22	22		8.4	8.4					
Sa															
Su															

Day	Time	Notes	*Ammonia*			*Nitrate*			*Nitrite*						Initial
			Left 1	Mid 2	Right 3	Left 1	Mid 2	Right 3	Left 1	Mid 2	Right 3	Left 1	Mid 2	Right 3	
M	15		0	0		0	0		0	0					
Tu	10		~~[illegible]~~ 0	~~[illegible]~~ 0		0	0		0	0					
W			0	0		0	0		0	0					
Th			0	0		0	0		0	0					
F			0.25	0.25		0	0		0	0					
Sa															
Su															

2x weekly maintenance activities (please fill in day done, date and tick if done)

Day	Date	Time	Scrape inner algae build-up on fronts	Clean outer glass fronts	Wipe down and de-salt banks	Clean tank outflows	Check/feed phytoplankton culture	General tidy and mop of room			Initial
Tu	20th	10:[illegible]	✓	✓	✓	✓		✓			AmB
Thu	22nd	✓	✓	✓	✓	✓		✓			AmB

Any notes? used Bottle Brush.

Please complete a new form every week. **Please submit completed forms to your supervisor every Monday.**

Page **2** of **2**

Figure 7.2: Aquarium Checklist (Side B)

Figure 7.3: Prawn Observation (Side A)

Prawn Activity Observation

Week commencing Monday 1 of (month) April (year) 2019

Daily Observations: Bank A									
Day	Time	Notes	Tank 1		Tank 2		Tank 3		Initial
			BF	AF	BF	AF	BF	AF	
M	9:00	/	1	4	1	4	8	3	AmB
Tu	1400	/	1	4	1	4	1	4	AmB
W	12:00	/	1	4	1	4	3	3	AmB
Th	13		1	4	1	4	3	4	AmB
F	13		2	2	2	2	2	3	

Daily Observations: Bank A									
Day	Time	Notes	Tank 4		Tank 5		Tank 6		Initial
			BF	AF	BF	AF	BF	AF	
M			1	4	1	1	2	4	AmB
Tu			3	3	2	4	1	1	AmB
W			3	4	2	4	1	4	AmB
Th			2	4	2	4	1	4	AmB
F			3	3	5	4	1	4	AmB

Daily Observations: Bank A									
Day	Time	Notes	Tank 7		Tank 8		Tank 9		Initial
			BF	AF	BF	AF	BF	AF	
M			2	4	2	4	2	3	AmB
Tu			2	4	3	4	3	4	AmB
W			2	4	2	3	2	4	AmB
Th			1	2	3	4	2	4	AmB
F			2	4	2	3	2	3	AmB

Key:	
BF	Before Feeding
AF	After Feeding
1	Sitting on the bottom of the tank
2	Swimming
3	Interacting with added material
4	Eating

Figure 7.3: Prawn Observation (Side A)

Figure 7.4: Prawn Observation (Side B)

Prawn Activity Observation

Week commencing Monday 1 of April 2019

Daily Observations: Bank B									
Day	Time	Notes	Tank 1		Tank 2		Tank 3		Initial
			BF	AF	BF	AF	BF	AF	
M	9:00		3	~~3~~ 3	3	4	2	4	AmB
Tu	14:00		1	~~3~~ 4	3	3	2	4	AmB
W	12:00		3	~~0~~ 4	1	4	2	4	AmB
Th	12		1	4	1	4	2	4	AmB
F	15		3	3	3	4	2	4	AmB

Daily Observations: Bank B									
Day	Time	Notes	Tank 4		Tank 5		Tank 6		Initial
			BF	AF	BF	AF	BF	AF	
M			3	3	2	1	3	1	AmB
Tu			2	4	2	4	3	3	AmB
W			3	4	2	4	3	4	AmB
Th			3	4	2	4	3	4	AmB
F			3	3	2	1	2	2	AmB

Daily Observations: Bank B									
Day	Time	Notes	Tank 7		Tank 8		Tank 9		Initial
			BF	AF	BF	AF	BF	AF	
M			1	4	2	4	3	3	AmB
Tu			1	4	2	3	2	4	AmB
W			2	4	3	4	2	3	AmB
Th			2	4	2	4	2	4	AmB
F			1	2	3	4	3	4	AmB

Key:	
BF	Before Feeding
AF	After Feeding
1	Sitting on the bottom of the tank
2	Swimming
3	Interacting with added material
4	Eating

Figure 7.4: Prawn Observation (Side B)

Table 7.1: Pairwise comparison of the weight of all treatments

Pairwise Comparisons of Treatment

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs	-64.474	47.612	-1.354	.176	1.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1	74.200	29.470	2.518	.012	1.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5	108.371	25.585	4.236	.000	.007
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6	-118.974	42.160	-2.822	.005	1.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3	124.769	37.108	3.362	.001	.232
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1	134.124	27.910	4.806	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2	147.124	38.520	3.819	.000	.040
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	-150.743	23.038	-6.543	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3	162.077	31.624	5.125	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	182.824	51.560	3.546	.000	.117
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	186.924	51.560	3.625	.000	.087
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-192.437	22.949	-8.385	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	229.224	42.160	5.437	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	237.974	47.612	4.998	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	242.124	51.560	4.696	.000	.001
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	269.724	42.160	6.398	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-277.824	29.941	-9.279	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	308.509	44.578	6.921	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	308.557	64.998	4.747	.000	.001

EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	313.279	40.179	7.797	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	313.390	64.998	4.822	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	315.324	51.560	6.116	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	330.224	38.520	8.573	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	336.974	56.972	5.915	.000	.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1	9.726	50.171	.194	.846	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5	43.897	47.992	.915	.360	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6	54.500	58.533	.931	.352	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3	60.295	55.006	1.096	.273	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1	69.650	49.271	1.414	.157	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2	82.650	55.968	1.477	.140	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	86.269	46.684	1.848	.065	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3	97.603	51.466	1.896	.058	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	118.350	65.629	1.803	.071	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	122.450	65.629	1.866	.062	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	127.963	46.640	2.744	.006	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	164.750	58.533	2.815	.005	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	173.500	62.575	2.773	.006	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	177.650	65.629	2.707	.007	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	205.250	58.533	3.507	.000	.136
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	213.350	50.449	4.229	.000	.007

EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	244.036	60.298	4.047	.000	.016
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	244.083	76.638	3.185	.001	.434
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	248.806	57.122	4.356	.000	.004
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	248.917	76.638	3.248	.001	.349
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	250.850	65.629	3.822	.000	.040
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	265.750	55.968	4.748	.000	.001
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	272.500	69.960	3.895	.000	.029
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5	34.171	30.081	1.136	.256	1.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6	-44.774	45.030	-.994	.320	1.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3	-50.569	40.339	-1.254	.210	1.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1	59.924	32.082	1.868	.062	1.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2	-72.924	41.642	-1.751	.080	1.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	-76.543	27.946	-2.739	.006	1.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3	87.877	35.360	2.485	.013	1.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	-108.624	53.932	-2.014	.044	1.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	-112.724	53.932	-2.090	.037	1.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-118.237	27.873	-4.242	.000	.007
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	-155.024	45.030	-3.443	.001	.173
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	163.774	50.171	3.264	.001	.329
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	167.924	53.932	3.114	.002	.554
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	195.524	45.030	4.342	.000	.004

EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-203.624	33.863	-6.013	.000	.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-234.310	47.302	-4.953	.000	.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	-234.357	66.895	-3.503	.000	.138
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-239.079	43.181	-5.537	.000	.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-239.190	66.895	-3.576	.000	.105
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-241.124	53.932	-4.471	.000	.002
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-256.024	41.642	-6.148	.000	.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	262.774	59.127	4.444	.000	.003
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6	-10.603	42.589	-.249	.803	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3	-16.398	37.595	-.436	.663	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1	25.753	28.555	.902	.367	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2	-38.753	38.989	-.994	.320	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	-42.372	23.814	-1.779	.075	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3	53.706	32.194	1.668	.095	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	-74.453	51.912	-1.434	.152	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	-78.553	51.912	-1.513	.130	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-84.066	23.728	-3.543	.000	.119
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	-120.853	42.589	-2.838	.005	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	129.603	47.992	2.700	.007	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	133.753	51.912	2.577	.010	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	161.353	42.589	3.789	.000	.045

EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-169.453	30.542	-5.548	.000	.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-200.139	44.984	-4.449	.000	.003
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	-200.186	65.277	-3.067	.002	.649
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-204.908	40.629	-5.043	.000	.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-205.020	65.277	-3.141	.002	.506
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-206.953	51.912	-3.987	.000	.020
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-221.853	38.989	-5.690	.000	.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	228.603	57.290	3.990	.000	.020
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3	5.795	50.361	.115	.908	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1	15.150	44.025	.344	.731	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2	28.150	51.410	.548	.584	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	-31.769	41.109	-.773	.440	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3	43.103	46.468	.928	.354	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	63.850	61.787	1.033	.301	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	67.950	61.787	1.100	.271	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-73.463	41.059	-1.789	.074	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	110.250	54.191	2.034	.042	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	119.000	58.533	2.033	.042	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	123.150	61.787	1.993	.046	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	150.750	54.191	2.782	.005	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-158.850	45.340	-3.504	.000	.138

Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	189.536	56.093	3.379	.001	.218
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	189.583	73.375	2.584	.010	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	194.306	52.664	3.690	.000	.067
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	194.417	73.375	2.650	.008	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	196.350	61.787	3.178	.001	.445
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	211.250	51.410	4.109	.000	.012
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	218.000	66.370	3.285	.001	.306
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1	9.355	39.214	.239	.811	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2	22.355	47.356	.472	.637	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	-25.973	35.910	-.723	.469	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3	37.307	41.939	.890	.374	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	58.055	58.457	.993	.321	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	62.155	58.457	1.063	.288	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-67.668	35.853	-1.887	.059	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	104.455	50.361	2.074	.038	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	113.205	55.006	2.058	.040	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	117.355	58.457	2.008	.045	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	144.955	50.361	2.878	.004	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-153.055	40.684	-3.762	.000	.051
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	183.740	52.402	3.506	.000	.136
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	183.788	70.594	2.603	.009	1.000

EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	188.510	48.714	3.870	.000	.033
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	188.621	70.594	2.672	.008	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	190.555	58.457	3.260	.001	.335
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	205.455	47.356	4.339	.000	.004
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	212.205	63.282	3.353	.001	.240
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2	-13.000	40.553	-.321	.749	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	-16.619	26.296	-.632	.527	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3	-27.953	34.071	-.820	.412	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	-48.700	53.096	-.917	.359	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	-52.800	53.096	-.994	.320	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-58.313	26.218	-2.224	.026	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	-95.100	44.025	-2.160	.031	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	103.850	49.271	2.108	.035	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	108.000	53.096	2.034	.042	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	135.600	44.025	3.080	.002	.621
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-143.700	32.515	-4.420	.000	.003
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-174.386	46.346	-3.763	.000	.050
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	-174.433	66.223	-2.634	.008	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-179.156	42.131	-4.252	.000	.006
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-179.267	66.223	-2.707	.007	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-181.200	53.096	-3.413	.001	.193

EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvNa'.Rep3.BaA.Tr2.TaSLa.TaG8	-196.100	40.553	-4.836	.000	.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	-202.850	58.366	-3.476	.001	.153
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	-3.619	37.367	-.097	.923	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3	14.953	43.193	.346	.729	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	-35.700	59.363	-.601	.548	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	-39.800	59.363	-.670	.503	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-45.313	37.312	-1.214	.225	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	-82.100	51.410	-1.597	.110	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	90.850	55.968	1.623	.105	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	95.000	59.363	1.600	.110	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	122.600	51.410	2.385	.017	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-130.700	41.976	-3.114	.002	.554
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-161.386	53.411	-3.022	.003	.754
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	-161.433	71.346	-2.263	.024	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-166.156	49.798	-3.337	.001	.254
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-166.267	71.346	-2.330	.020	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-168.200	59.363	-2.833	.005	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-183.100	48.470	-3.778	.000	.048
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	189.850	64.120	2.961	.003	.920
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3	11.334	30.210	.375	.708	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	32.081	50.705	.633	.527	1.000

EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-EnvNa'.Rep1.BaB.Tr2.TaSSm.TaG1	36.181	50.705	.714	.475	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-41.694	20.956	-1.990	.047	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	78.481	41.109	1.909	.056	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	87.231	46.684	1.869	.062	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	91.381	50.705	1.802	.072	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	118.981	41.109	2.894	.004	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-127.081	28.442	-4.468	.000	.002
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	157.767	43.586	3.620	.000	.088
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	157.814	64.321	2.454	.014	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	162.537	39.075	4.160	.000	.010
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	162.648	64.321	2.529	.011	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	164.581	50.705	3.246	.001	.351
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	179.481	37.367	4.803	.000	.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	186.231	56.199	3.314	.001	.276
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	-20.747	55.139	-.376	.707	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	-24.847	55.139	-.451	.652	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-30.360	30.142	-1.007	.314	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	-67.147	46.468	-1.445	.148	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	75.897	51.466	1.475	.140	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	80.047	55.139	1.452	.147	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	107.647	46.468	2.317	.021	1.000

EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-115.747	35.754	-3.237	.001	.362
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-146.433	48.673	-3.008	.003	.788
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	-146.480	67.872	-2.158	.031	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-151.203	44.679	-3.384	.001	.214
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-151.314	67.872	-2.229	.026	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-153.247	55.139	-2.779	.005	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-168.147	43.193	-3.893	.000	.030
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	174.897	60.230	2.904	.004	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	4.100	68.547	.060	.952	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-9.613	50.664	-.190	.850	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	46.400	61.787	.751	.453	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	55.150	65.629	.840	.401	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	59.300	68.547	.865	.387	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	86.900	61.787	1.406	.160	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-95.000	54.191	-1.753	.080	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	125.686	63.462	1.980	.048	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	125.733	79.151	1.589	.112	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	130.456	60.453	2.158	.031	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	130.567	79.151	1.650	.099	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	132.500	68.547	1.933	.053	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	147.400	59.363	2.483	.013	1.000

EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-EnvNa'.Rep1.BaB.Tr1.TaSSm.TaG2	154.150	72.705	2.120	.034	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-5.513	50.664	-.109	.913	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	42.300	61.787	.685	.494	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	51.050	65.629	.778	.437	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	55.200	68.547	.805	.421	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	82.800	61.787	1.340	.180	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-90.900	54.191	-1.677	.093	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	121.586	63.462	1.916	.055	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	121.633	79.151	1.537	.124	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	126.356	60.453	2.090	.037	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	126.467	79.151	1.598	.110	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	128.400	68.547	1.873	.061	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	143.300	59.363	2.414	.016	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	150.050	72.705	2.064	.039	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	36.787	41.059	.896	.370	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	45.537	46.640	.976	.329	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	49.687	50.664	.981	.327	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	77.287	41.059	1.882	.060	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	85.387	28.370	3.010	.003	.784
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	116.073	43.539	2.666	.008	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	116.120	64.289	1.806	.071	1.000

Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	120.843	39.022	3.097	.002	.587
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	120.954	64.289	1.881	.060	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	122.887	50.664	2.426	.015	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	137.787	37.312	3.693	.000	.067
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	144.537	56.162	2.574	.010	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	8.750	58.533	.149	.881	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	12.900	61.787	.209	.835	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	40.500	54.191	.747	.455	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-48.600	45.340	-1.072	.284	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-79.286	56.093	-1.413	.158	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	-79.333	73.375	-1.081	.280	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-84.056	52.664	-1.596	.110	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-84.167	73.375	-1.147	.251	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-86.100	61.787	-1.393	.163	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-101.000	51.410	-1.965	.049	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	107.750	66.370	1.623	.104	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	4.150	65.629	.063	.950	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	-31.750	58.533	-.542	.588	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-39.850	50.449	-.790	.430	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-70.536	60.298	-1.170	.242	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	-70.583	76.638	-.921	.357	1.000

EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-75.306	57.122	-1.318	.187	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-75.417	76.638	-.984	.325	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-77.350	65.629	-1.179	.239	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-92.250	55.968	-1.648	.099	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	-99.000	69.960	-1.415	.157	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	-27.600	61.787	-.447	.655	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-35.700	54.191	-.659	.510	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-66.386	63.462	-1.046	.296	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	-66.433	79.151	-.839	.401	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-71.156	60.453	-1.177	.239	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-71.267	79.151	-.900	.368	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-73.200	68.547	-1.068	.286	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-88.100	59.363	-1.484	.138	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	-94.850	72.705	-1.305	.192	1.000
Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-8.100	45.340	-.179	.858	1.000
Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-38.786	56.093	-.691	.489	1.000
Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	-38.833	73.375	-.529	.597	1.000
Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-43.556	52.664	-.827	.408	1.000
Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-43.667	73.375	-.595	.552	1.000
Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-45.600	61.787	-.738	.461	1.000
Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-60.500	51.410	-1.177	.239	1.000

Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	-67.250	66.370	-1.013	.311	1.000
EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	30.686	47.597	.645	.519	1.000
EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	30.733	67.104	.458	.647	1.000
EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	35.456	43.503	.815	.415	1.000
EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	35.567	67.104	.530	.596	1.000
EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	37.500	54.191	.692	.489	1.000
EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	52.400	41.976	1.248	.212	1.000
EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	59.150	59.363	.996	.319	1.000
EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	.048	74.791	.001	.999	1.000
EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-4.770	54.620	-.087	.930	1.000
EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	4.881	74.791	.065	.948	1.000
EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-6.814	63.462	-.107	.914	1.000
EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-21.714	53.411	-.407	.684	1.000
EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	28.464	67.932	.419	.675	1.000
Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-4.722	72.255	-.065	.948	1.000
Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-4.833	88.494	-.055	.956	1.000
Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-6.767	79.151	-.085	.932	1.000
Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-21.667	71.346	-.304	.761	1.000
Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	28.417	82.778	.343	.731	1.000
EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	.111	72.255	.002	.999	1.000
EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-2.044	60.453	-.034	.973	1.000

EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	16.944	49.798	.340	.734	1.000
EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	23.694	65.130	.364	.716	1.000
EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-1.933	79.151	-.024	.981	1.000
EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-16.833	71.346	-.236	.813	1.000
EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	23.583	82.778	.285	.776	1.000
EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	14.900	59.363	.251	.802	1.000
EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	21.650	72.705	.298	.766	1.000
Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	6.750	64.120	.105	.916	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Table 7.1: Pairwise comparison of the weight of all treatments

Table 7.2: Pairwise comparison of the weight of all treatments

Pairwise Comparisons of Treatment

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1	34.143	29.474	1.158	.247	1.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5	71.603	25.589	2.798	.005	1.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1	90.760	27.914	3.251	.001	.345
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2	105.400	38.525	2.736	.006	1.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs	-111.667	47.619	-2.345	.019	1.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3	114.441	31.629	3.618	.000	.089
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3	120.091	37.113	3.236	.001	.364
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6	-123.875	42.166	-2.938	.003	.992
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	-150.519	23.041	-6.533	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	176.400	51.568	3.421	.001	.187
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	190.333	47.619	3.997	.000	.019
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-191.898	22.952	-8.361	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	196.600	51.568	3.812	.000	.041
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	198.875	42.166	4.716	.000	.001
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	213.200	51.568	4.134	.000	.011
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	233.500	42.166	5.538	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-267.650	29.945	-8.938	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	286.000	65.007	4.400	.000	.003
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	293.000	65.007	4.507	.000	.002

EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	293.800	51.568	5.697	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	300.286	44.585	6.735	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	305.556	40.184	7.604	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	315.500	56.980	5.537	.000	.000
EnvCo.Rep2.BaB.Tr2.TaSSm.TaG4-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	317.800	38.525	8.249	.000	.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5	37.460	30.085	1.245	.213	1.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1	56.617	32.086	1.765	.078	1.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2	-71.257	41.648	-1.711	.087	1.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs	-77.524	50.178	-1.545	.122	1.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3	80.298	35.365	2.271	.023	1.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3	-85.948	40.345	-2.130	.033	1.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6	-89.732	45.036	-1.992	.046	1.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	-116.376	27.950	-4.164	.000	.009
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	-142.257	53.940	-2.637	.008	1.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	156.190	50.178	3.113	.002	.556
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-157.755	27.877	-5.659	.000	.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	162.457	53.940	3.012	.003	.779
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	-164.732	45.036	-3.658	.000	.076
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	-179.057	53.940	-3.320	.001	.270
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	199.357	45.036	4.427	.000	.003
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-233.507	33.868	-6.895	.000	.000

EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1- Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	-251.857	66.904	-3.764	.000	.050
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1- EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-258.857	66.904	-3.869	.000	.033
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1- EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-259.657	53.940	-4.814	.000	.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1- EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-266.143	47.308	-5.626	.000	.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1- EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-271.413	43.187	-6.285	.000	.000
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1- Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	281.357	59.136	4.758	.000	.001
EnvFB.Rep1.BaA.Tr2.TaSSm.TaG1- Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-283.657	41.648	-6.811	.000	.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5- EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1	19.157	28.558	.671	.502	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5- EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2	-33.797	38.995	-.867	.386	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5- EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs	-40.064	47.999	-.835	.404	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5- EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3	42.838	32.199	1.330	.183	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5- EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3	-48.488	37.600	-1.290	.197	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5- Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6	-52.272	42.595	-1.227	.220	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5- EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	-78.916	23.818	-3.313	.001	.277
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5- EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	-104.797	51.919	-2.018	.044	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5- EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	118.730	47.999	2.474	.013	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5- Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-120.295	23.731	-5.069	.000	.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5- EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	124.997	51.919	2.408	.016	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5- Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	-127.272	42.595	-2.988	.003	.843
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5- Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	-141.597	51.919	-2.727	.006	1.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5- Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	161.897	42.595	3.801	.000	.043

EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-196.047	30.546	-6.418	.000	.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	-214.397	65.286	-3.284	.001	.307
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-221.397	65.286	-3.391	.001	.209
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-222.197	51.919	-4.280	.000	.006
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-228.683	44.991	-5.083	.000	.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-233.953	40.634	-5.758	.000	.000
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	243.897	57.298	4.257	.000	.006
EnvCo.Rep2.BaB.Tr1.TaSSm.TaG5-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-246.197	38.995	-6.314	.000	.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2	-14.640	40.559	-.361	.718	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs	-20.907	49.278	-.424	.671	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3	-23.681	34.076	-.695	.487	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3	-29.331	39.220	-.748	.455	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6	-33.115	44.031	-.752	.452	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	-59.759	26.300	-2.272	.023	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	-85.640	53.104	-1.613	.107	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	99.573	49.278	2.021	.043	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-101.138	26.222	-3.857	.000	.034
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	105.840	53.104	1.993	.046	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	-108.115	44.031	-2.455	.014	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	-122.440	53.104	-2.306	.021	1.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	142.740	44.031	3.242	.001	.356

EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-176.890	32.519	-5.440	.000	.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	-195.240	66.232	-2.948	.003	.960
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-202.240	66.232	-3.054	.002	.679
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-203.040	53.104	-3.823	.000	.039
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-209.526	46.353	-4.520	.000	.002
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-214.796	42.137	-5.098	.000	.000
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	-224.740	58.374	-3.850	.000	.035
EnvCo.Rep1.BaB.Tr1.TaSSm.TaG1-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-227.040	40.559	-5.598	.000	.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs	-6.267	55.976	-.112	.911	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3	9.041	43.199	.209	.834	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3	-14.691	47.362	-.310	.756	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6	-18.475	51.417	-.359	.719	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	-45.119	37.372	-1.207	.227	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	-71.000	59.372	-1.196	.232	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	84.933	55.976	1.517	.129	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-86.498	37.317	-2.318	.020	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	91.200	59.372	1.536	.125	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	-93.475	51.417	-1.818	.069	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	-107.800	59.372	-1.816	.069	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	128.100	51.417	2.491	.013	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-162.250	41.982	-3.865	.000	.033

EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvNa'.Rep2.BaA.Tr2.TaSSm.TaG4	-180.600	71.356	-2.531	.011	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-187.600	71.356	-2.629	.009	1.000
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-188.400	59.372	-3.173	.002	.452
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-194.886	53.419	-3.648	.000	.079
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-200.156	49.805	-4.019	.000	.018
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvNa'.Rep1.BaB.Tr1.TaSSm.TaG2	210.100	64.129	3.276	.001	.316
EnvCo.Rep1.BaA.Tr2.TaSSm.TaG2-EnvNa'.Rep3.BaA.Tr2.TaSLa.TaG8	-212.400	48.477	-4.381	.000	.004
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3	2.775	51.473	.054	.957	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3	8.424	55.014	.153	.878	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvNa'.Rep2.BaB.Tr2.TaSSm.TaG6	12.208	58.541	.209	.835	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	38.852	46.691	.832	.405	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	64.733	65.638	.986	.324	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	78.667	62.583	1.257	.209	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvNa'.Rep3.BaB.Tr2.TaSLa.TaG9	80.231	46.647	1.720	.085	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	84.933	65.638	1.294	.196	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvNa'.Rep1.BaA.Tr2.TaSSm.TaG3	87.208	58.541	1.490	.136	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvNa'.Rep1.BaB.Tr2.TaSSm.TaG1	101.533	65.638	1.547	.122	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvNa'.Rep1.BaA.Tr1.TaSSm.TaG3	121.833	58.541	2.081	.037	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	155.983	50.456	3.091	.002	.598
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvNa'.Rep2.BaA.Tr2.TaSSm.TaG4	174.333	76.649	2.274	.023	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	181.333	76.649	2.366	.018	1.000

EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	182.133	65.638	2.775	.006	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	188.619	60.307	3.128	.002	.529
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	193.889	57.130	3.394	.001	.207
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	203.833	69.970	2.913	.004	1.000
EnvEs.RepEs.BaB.Tr2.TaSEs.TaGEs-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	206.133	55.976	3.683	.000	.069
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3	-5.650	41.945	-.135	.893	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6	-9.434	46.475	-.203	.839	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	-36.078	30.214	-1.194	.232	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	-61.959	55.147	-1.124	.261	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	75.892	51.473	1.474	.140	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-77.457	30.146	-2.569	.010	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	82.159	55.147	1.490	.136	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	-84.434	46.475	-1.817	.069	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	-98.759	55.147	-1.791	.073	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	119.059	46.475	2.562	.010	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-153.209	35.759	-4.285	.000	.005
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	-171.559	67.881	-2.527	.011	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-178.559	67.881	-2.630	.009	1.000
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-179.359	55.147	-3.252	.001	.343
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-185.845	48.680	-3.818	.000	.040
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-191.114	44.685	-4.277	.000	.006

EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	201.059	60.238	3.338	.001	.253
EnvFB.Rep1.BaB.Tr1.TaSSm.TaG3-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-203.359	43.199	-4.707	.000	.001
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6	-3.784	50.368	-.075	.940	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	-30.428	35.915	-.847	.397	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	56.309	58.465	.963	.335	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	70.242	55.014	1.277	.202	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-71.807	35.858	-2.003	.045	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	76.509	58.465	1.309	.191	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	78.784	50.368	1.564	.118	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	93.109	58.465	1.593	.111	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	113.409	50.368	2.252	.024	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-147.559	40.690	-3.626	.000	.086
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	165.909	70.603	2.350	.019	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	172.909	70.603	2.449	.014	1.000
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	173.709	58.465	2.971	.003	.890
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	180.195	52.409	3.438	.001	.176
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	185.465	48.721	3.807	.000	.042
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	195.409	63.290	3.087	.002	.606
EnvCo.Rep1.BaB.Tr2.TaSSm.TaG3-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	197.709	47.362	4.174	.000	.009
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7	-26.644	41.115	-.648	.517	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	52.525	61.796	.850	.395	1.000

Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	66.458	58.541	1.135	.256	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-68.023	41.065	-1.656	.098	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	72.725	61.796	1.177	.239	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	75.000	54.199	1.384	.166	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	89.325	61.796	1.445	.148	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	109.625	54.199	2.023	.043	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-143.775	45.346	-3.171	.002	.456
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	162.125	73.385	2.209	.027	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	169.125	73.385	2.305	.021	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	169.925	61.796	2.750	.006	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	176.411	56.101	3.145	.002	.499
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	181.681	52.672	3.449	.001	.169
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	191.625	66.380	2.887	.004	1.000
Env'Na'.Rep2.BaB.Tr2.TaSSm.TaG6-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	193.925	51.417	3.772	.000	.049
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2	25.881	50.712	.510	.610	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	39.814	46.691	.853	.394	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-41.379	20.959	-1.974	.048	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	46.081	50.712	.909	.364	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	48.356	41.115	1.176	.240	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	62.681	50.712	1.236	.216	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	82.981	41.115	2.018	.044	1.000

EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-117.131	28.446	-4.118	.000	.011
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	135.481	64.330	2.106	.035	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	142.481	64.330	2.215	.027	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	143.281	50.712	2.825	.005	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	149.767	43.592	3.436	.001	.177
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	155.037	39.080	3.967	.000	.022
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	164.981	56.207	2.935	.003	1.000
EnvCo.Rep3.BaB.Tr2.TaSLa.TaG7-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	167.281	37.372	4.476	.000	.002
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2	13.933	65.638	.212	.832	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-15.498	50.671	-.306	.760	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	20.200	68.557	.295	.768	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	22.475	61.796	.364	.716	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	36.800	68.557	.537	.591	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	57.100	61.796	.924	.355	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-91.250	54.199	-1.684	.092	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	109.600	79.162	1.384	.166	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	116.600	79.162	1.473	.141	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	117.400	68.557	1.712	.087	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	123.886	63.471	1.952	.051	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	129.156	60.461	2.136	.033	1.000
EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	139.100	72.715	1.913	.056	1.000

EnvFB.Rep1.BaB.Tr2.TaSSm.TaG2-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	141.400	59.372	2.382	.017	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9	-1.565	46.647	-.034	.973	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	6.267	65.638	.095	.924	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	-8.542	58.541	-.146	.884	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	-22.867	65.638	-.348	.728	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	-43.167	58.541	-.737	.461	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-77.317	50.456	-1.532	.125	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	-95.667	76.649	-1.248	.212	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-102.667	76.649	-1.339	.180	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-103.467	65.638	-1.576	.115	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-109.952	60.307	-1.823	.068	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-115.222	57.130	-2.017	.044	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	-125.167	69.970	-1.789	.074	1.000
EnvCo.Rep1.BaA.Tr1.TaSSm.TaG2-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-127.467	55.976	-2.277	.023	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1	4.702	50.671	.093	.926	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	6.977	41.065	.170	.865	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	21.302	50.671	.420	.674	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	41.602	41.065	1.013	.311	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	75.752	28.374	2.670	.008	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	94.102	64.298	1.464	.143	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	101.102	64.298	1.572	.116	1.000

Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	101.902	50.671	2.011	.044	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	108.388	43.545	2.489	.013	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	113.657	39.028	2.912	.004	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	123.602	56.170	2.200	.028	1.000
Env'Na'.Rep3.BaB.Tr2.TaSLa.TaG9-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	125.902	37.317	3.374	.001	.222
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3	-2.275	61.796	-.037	.971	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	-16.600	68.557	-.242	.809	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	-36.900	61.796	-.597	.550	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-71.050	54.199	-1.311	.190	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	-89.400	79.162	-1.129	.259	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-96.400	79.162	-1.218	.223	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-97.200	68.557	-1.418	.156	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-103.686	63.471	-1.634	.102	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-108.956	60.461	-1.802	.072	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	-118.900	72.715	-1.635	.102	1.000
EnvFB.Rep1.BaA.Tr1.TaSSm.TaG1-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-121.200	59.372	-2.041	.041	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1	-14.325	61.796	-.232	.817	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	34.625	54.199	.639	.523	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-68.775	45.346	-1.517	.129	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	-87.125	73.385	-1.187	.235	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-94.125	73.385	-1.283	.200	1.000

Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-94.925	61.796	-1.536	.125	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-101.411	56.101	-1.808	.071	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-106.681	52.672	-2.025	.043	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	116.625	66.380	1.757	.079	1.000
Env'Na'.Rep1.BaA.Tr2.TaSSm.TaG3-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-118.925	51.417	-2.313	.021	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3	20.300	61.796	.329	.743	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-54.450	54.199	-1.005	.315	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	72.800	79.162	.920	.358	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	79.800	79.162	1.008	.313	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	80.600	68.557	1.176	.240	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	87.086	63.471	1.372	.170	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	92.356	60.461	1.528	.127	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	102.300	72.715	1.407	.159	1.000
Env'Na'.Rep1.BaB.Tr2.TaSSm.TaG1-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	104.600	59.372	1.762	.078	1.000
Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3-EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8	-34.150	45.346	-.753	.451	1.000
Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	-52.500	73.385	-.715	.474	1.000
Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-59.500	73.385	-.811	.417	1.000
Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-60.300	61.796	-.976	.329	1.000
Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-66.786	56.101	-1.190	.234	1.000
Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-72.056	52.672	-1.368	.171	1.000
Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	-82.000	66.380	-1.235	.217	1.000

Env'Na'.Rep1.BaA.Tr1.TaSSm.TaG3-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-84.300	51.417	-1.640	.101	1.000
EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8-Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4	18.350	67.113	.273	.785	1.000
EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	25.350	67.113	.378	.706	1.000
EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	26.150	54.199	.482	.629	1.000
EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	32.636	47.603	.686	.493	1.000
EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	37.906	43.509	.871	.384	1.000
EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	47.850	59.372	.806	.420	1.000
EnvFB.Rep3.BaB.Tr2.TaSLa.TaG8-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	50.150	41.982	1.195	.232	1.000
Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4-EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5	-7.000	88.506	-.079	.937	1.000
Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-7.800	79.162	-.099	.922	1.000
Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-14.286	74.801	-.191	.849	1.000
Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-19.556	72.265	-.271	.787	1.000
Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	29.500	82.790	.356	.722	1.000
Env'Na'.Rep2.BaA.Tr2.TaSSm.TaG4-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-31.800	71.356	-.446	.656	1.000
EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5-EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs	-.800	79.162	-.010	.992	1.000
EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	-7.286	74.801	-.097	.922	1.000
EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-12.556	72.265	-.174	.862	1.000
EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	22.500	82.790	.272	.786	1.000
EnvFB.Rep2.BaA.Tr2.TaSSm.TaG5-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-24.800	71.356	-.348	.728	1.000
EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7	6.486	63.471	.102	.919	1.000
EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	11.756	60.461	.194	.846	1.000

EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	21.700	72.715	.298	.765	1.000
EnvEs.RepEs.BaA.Tr2.TaSEs.TaGEs-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	24.000	59.372	.404	.686	1.000
EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7-EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9	-5.270	54.627	-.096	.923	1.000
EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	15.214	67.942	.224	.823	1.000
EnvFB.Rep3.BaA.Tr2.TaSLa.TaG7-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-17.514	53.419	-.328	.743	1.000
EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9-Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2	9.944	65.139	.153	.879	1.000
EnvFB.Rep3.BaA.Tr2.TaSLa.TaG9-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	12.244	49.805	.246	.806	1.000
Env'Na'.Rep1.BaB.Tr1.TaSSm.TaG2-Env'Na'.Rep3.BaA.Tr2.TaSLa.TaG8	-2.300	64.129	-.036	.971	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Table 7.2: Pairwise comparison of the weight of all treatments

7.1 End of Trial Photos

7.1.1 Trial 1

7.1.1.1 Bank A

The following images catalogue the euthanised prawns at the end of each trial, and offer a global illustration of the specimen’s size and condition.

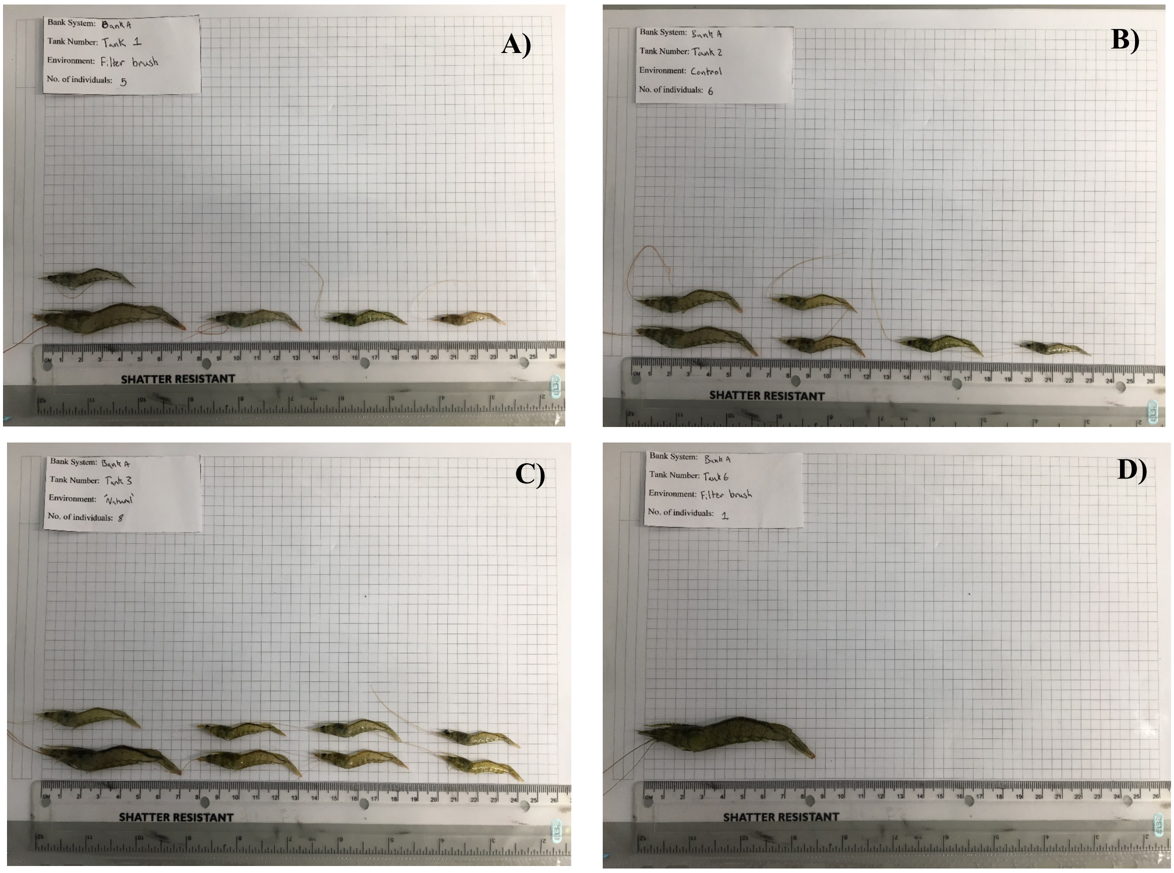

Figure 7.5: Prawns from each of the tanks classed in their environments from Bank A. Clockwise starting with Photo A- Filter Brush, B- control, C- ‘natural’, D- Filter Brush.

7.1.1.2 Bank B

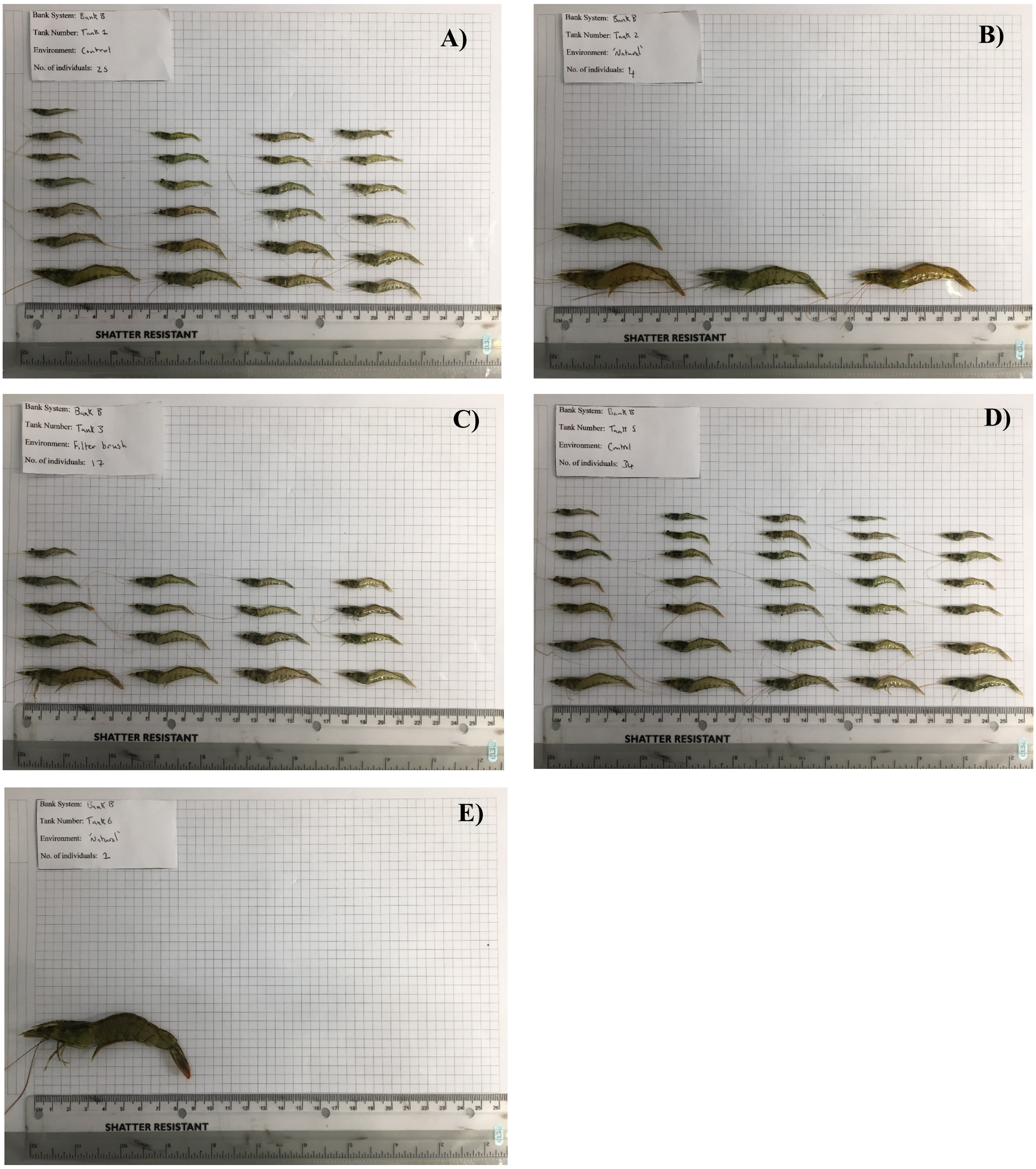

Figure 7.6: Prawns from each of the tanks classed in their environments from Bank A. Clockwise starting with Photo A- Control, B- 'natural', C- Filter Brush, D- Control, E- 'Natural'.

7.1.1.3 Escapees from Both Banks

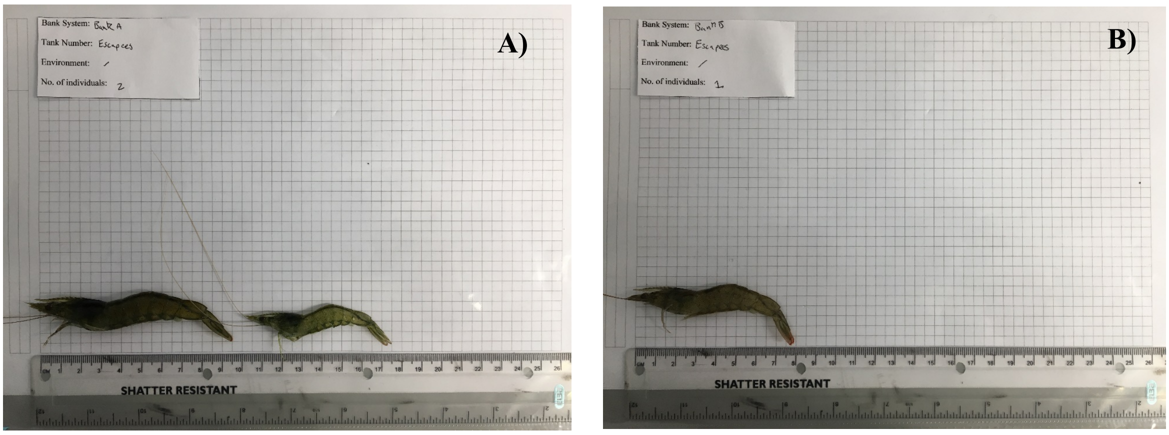

Figure 7.7: Escaped Prawns found in the back of the banks. A from Bank A, B- Bank B.

7.2.2 Trial 2

7.2.2.1 Bank A

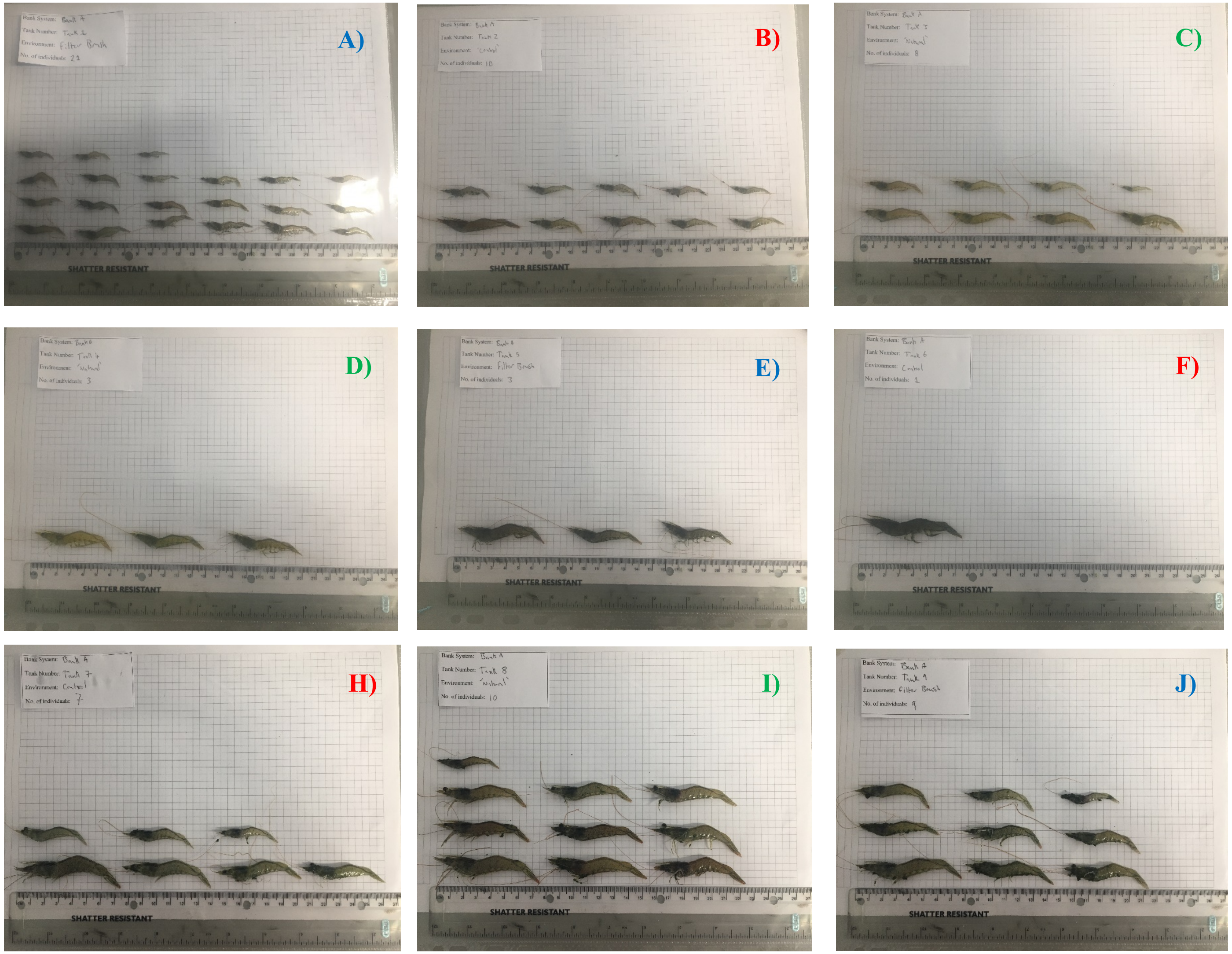

Figure 7.8: Prawns from each of the tanks classed in their environments from Bank A. Clockwise, from A to J:

A- Filter Brush,

B- Control,

C- 'Natural',

D- 'Natural',

E- Filter Brush,

F- Control,

H- Control,

I- 'Natural',

J- Filter Brush.

Red Letter- Control

Blue Letter- Filter Brush

Green Letter- 'Natural'

7.2.2.2 Bank B

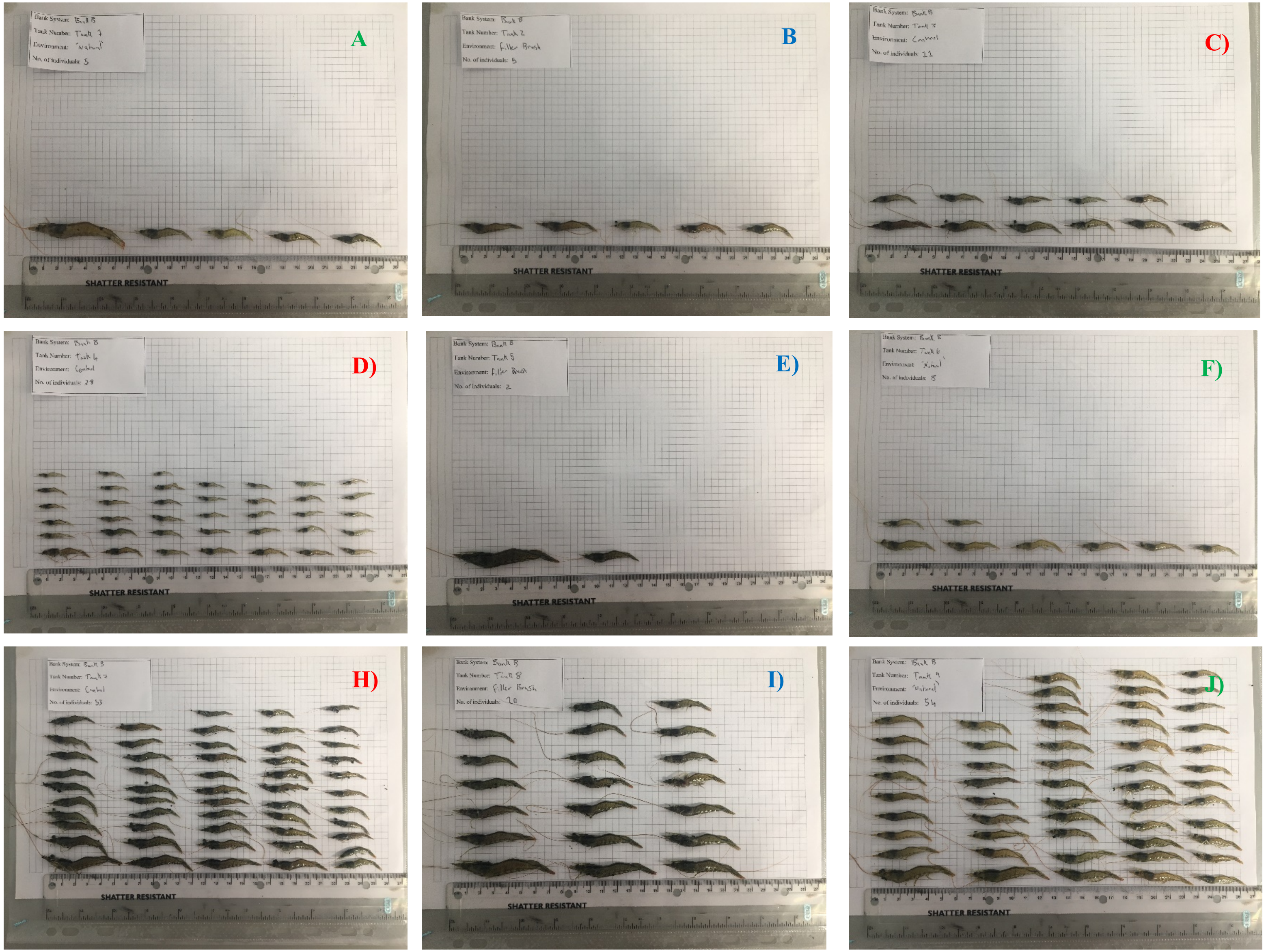

Figure 7.8: Prawns from each of the tanks classed in their environments from Bank B. Clockwise in rows, from A to J:
A- ‘Natural’,
B- Filter Brush,
C- Control,
D- Control,
E- Filter Brush,
F- ‘Natural’,
H- Control,
I- Filter Brush,
J- ‘Natural’.
Red Letter- Control
Blue Letter- Filter Brush
Green Letter- ‘Natural’

7.2.2.3 Escapees from Both Banks

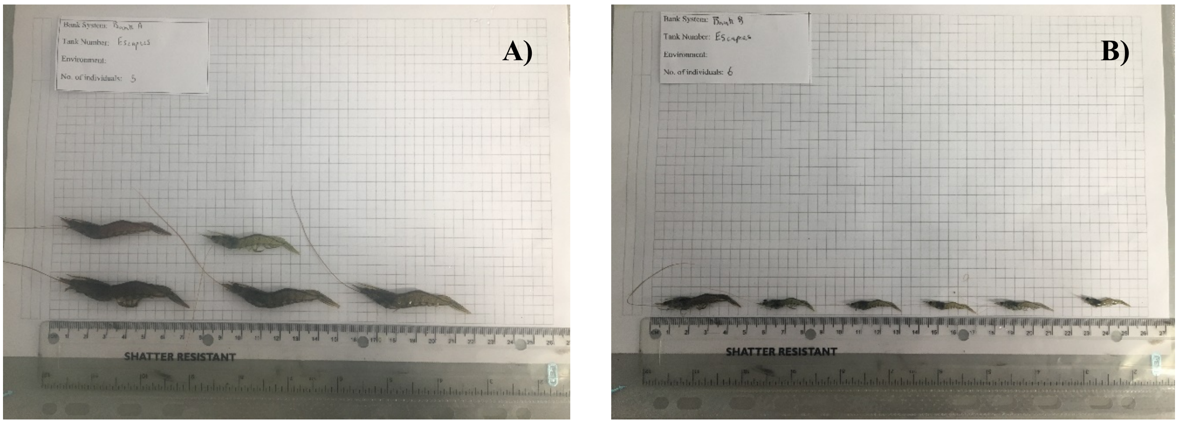

Figure 7.9: Escaped Prawns found in the back of the banks. A from Bank A, B- Bank B.