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Investigations into the potential for mixed cultures of banana prawns *Penaeus merguiensis*, sea mullet *Mugil cephalus* and rabbitfish *Siganus nebulosus* for bioremediation of aquaculture waste

P J Palmer[1], C Morrison[1], B W Rutherford[12] and B K D Pledger[13]
[1]Department of Primary Industries and Fisheries, Bribie Island Aquaculture Research Centre, Bribie Island, Queensland, Australia
[2]Present address: Queensland Sea Scallop Ltd., Burnett Heads, Queensland, Australia
[3]Present address: Gold Coast Marine Aquaculture, Woongoolba, Queensland, Australia

Correspondence: Dr P. J. Palmer, Bribie Island Aquaculture Research Centre, PO Box 2066 Bribie Island, Queensland, 4507 Australia. Email: paul.palmer@dpi.qld.gov.au

Abstract

To experimentally investigate the potential of mixed species polycultures for bioremediation of nutrient rich prawn farm effluent, a series of experiments was performed with banana prawns *Penaeus* (*Fenneropenaeus*) *merguiensis*, sea mullet *Mugil cephalus* and rabbitfish *Siganus nebulosus* to determine their compatibilities during particular life stages. Rabbitfish demonstrated a high tendency to prey upon banana prawn juveniles when no other food was available. Mullet of various sizes did not appear to prey upon banana prawn postlarvae (PL16) or juveniles in a fed or unfed environment. The study confirms the good potential for mullet and banana prawn polycultures.

Keywords: banana prawns, mullet, rabbitfish, predation

Introduction

Banana prawns, *Penaeus* (*Fenneropenaeus*) *merguiensis*, have been shown to successfully grow into a marketable food grade product in Australian prawn farm effluent treatment systems (Palmer *et al.*, 2002). They are therefore a potentially profitable inclusion in mixed species cultures for nutrient cycling within a wider bioremediation strategy. Whilst at least one fish species, the milkfish *Chanos chanos,* is widely known to be compatible with prawns in culture, most marine fish are likely to prey on prawns during particular life stages. For example, prawn nauplii are generally small (approx. 300 µm) and vulnerable to predation. For a short period they are part of planktonic food sources that are available to fish in natural environments, and as they grow their abilities to avoid some predators improves.

Both the sea mullet (*Mugil cephalus*) and the rabbitfish (*Siganus nebulosus*) have also been proposed as secondary crops that can be generated in effluent treatment systems for prawn farms (Erler *et al.* 2000). Whilst sea mullet and rabbitfish would mainly be expected to consume detritus and benthic algal growth respectively in sedimentation ponds, it was uncertain to what degree these fish species would also prey on banana prawns if they were free-ranging in the same ponds.

Each of these fish species is known to feed on small planktonic crustaceans in artificial systems (eg: copepods, brine shrimp). Even though sea mullet and rabbitfish have both been successfully cultured with banana prawns (stocked as PL15) in ponds fed artificial diets at the Bribie Island Aquaculture Research Centre, it is unknown how large the prawns would have to be to evade or be overlooked by foraging mullet and/or rabbitfish in systems where food sources are more limited (eg: in an unfed settlement pond). Given that predictably high survival would be necessary for maintenance of prescribed mixtures of species for nutrient cycling, and to maximise harvestable biomasses (fish and prawns) for nutrient removal and profitability, further knowledge on the compatibilities of these species in mixed cultures was required.

The objective of these trials was to investigate the potential for banana prawns, rabbitfish and sea mullet to co-inhabit a prawn farm wastewater remediation environment. We focussed this work on life stages that are likely to be used to create mixed cultures under commercial operating conditions.

Materials and methods

Two replicated experiments (A and B) were designed to expose banana prawns to potential predation by fish.

Experimental systems

Experiment A was conducted in 12 x 1000 litre fibreglass tanks (1300 mm diameter and 830 mm deep). These tanks (Figure 1) were supplied with flow-through (12 litres per min) filtered (20 µm) seawater (35 ppt., 24 - 26˚C), and equipped with screened outlets to prevent animals escaping. Each tank was also equipped with four airstones delivering a very slow level of aeration, and covered with one layer of 70% green nylon shadecloth.

Figure 1. Fibreglass tanks used in experiment A

Experiment B was undertaken in 12 nylon mesh (500 µm) floating cages arranged in a 10,000 litre conical-bottom tank with central overflow drawing from the tank bottom (see Figure 2). These cages had a 300 x 300 mm square base, a height of 800 mm and were completely covered at the top with foam rubber floatation. Each cage was supplied with a periodic (1.5 hr per day) flowthrough (approx. 7 litres per min) of prawn-culture-pond water containing microalgal bloom and suspended particulate matter. The water qualities of pond water used for exchanges in the study are provided in Appendix A. Each cage also had a single airstone positioned on the central bottom delivering very slow aeration to provide continuous water circulation and gaseous exchange. A 3 kw titanium bar heater maintained the water temperature in the tank at 26-28˚C.

Figure 2. Cages and tank set-up used in experiment B

Experimental design

Experiment A was designed to assess whether juvenile banana prawns would be preyed on by a variety of mullet and rabbitfish size classes when no other food sources were available. Two replicates for each of 6 treatments (including control) were used as follows with a completely randomised design:

1) Prawns only (control)
2) Small sized mullet + prawns
3) Medium sized mullet + prawns
4) Large sized mullet + prawns
5) Small to medium sized rabbitfish + prawns
6) Medium to large sized rabbitfish + prawns

Juvenile banana prawns (500) with an average weight of 0.072 g (range from 0.047 to 0.115 g) were removed from a nursery system (4,000 litre fibreglass tank) for Experiment A (3rd January 2002). Thirty prawns were randomly selected from this pool and immediately and gently introduced into each of the 12 experimental tanks. On the same day mullet and rabbitfish were also gently introduced into tanks in the experiment as described in Table 1.

Table 1. Distribution of fish types, their relative numbers and mean weights used in Experiment A.

Tank	**Treatment No.** (as described above)	**Fish types and sizes**	**Numbers of fish added**	**Total wet weight of fish per tank (g)**	**Mean wet weight per fish (g)**
E1	2 – replicate 1	Mullet – small	10	20	2
E2	4 – replicate 1	Mullet – large	3	501	167
E3	5 – replicate 1	Rabbitfish – small to medium	5	90	18
E4	1 – replicate 1	Prawns only (control)	0	-	-
E5	3 – replicate 1	Mullet – medium	5	225	45
E6	6 – replicate 1	Rabbitfish – medium to large	3	156	52
E7	3 – replicate 2	Mullet – medium	5	230	46
E8	1 – replicate 2	Prawns only (control)	0	-	-
E9	2 – replicate 2	Mullet – small	10	25	2.5
E10	6 – replicate 2	Rabbitfish – medium to large	3	186	62
E11	4 – replicate 2	Mullet – large	3	534	178
E12	5 – replicate 2	Rabbitfish – small to medium	5	95	19

Following the addition of juvenile prawns and fish, each tank was covered with shade cloth and left undisturbed for one week. On the 10th January, each tank was drained to recover and count all live prawns to calculate their percentage survival. Further to this trial, general observations were made for 50 juvenile prawns as they were offered to large mullet and rabbitfish being held at BIARC as broodstock. These two sets of broodstock were in separate 2-metre-deep tanks with high clarity seawater flow-through, making it possible to observe any potential feeding responses exhibited by the fish following the addition of the juvenile prawns (25 prawns offered per tank).

Experiment B was designed to test whether mullet would prey on young prawns at the size that they would most likely be stocked into bioremediation ponds. Postlarval 16 (16 days after metamorphosis) banana prawns were harvested from an intensive culture at BIARC on 11th December 2002, and within 2 hours 50 were randomly selected from the harvested pool and gently stocked into each cage. The next day, 60 mullet juveniles (approximately 50 mm long) were randomly netted from a nursery-rearing tank at BIARC, and following mild sedation with clove oil (10 drops in 10 litres) they were randomly distributed between cages according to the prescribed design. The design included 3 replicates for each of 4 treatments as follows using a completely randomised design:

1) Unfed prawns only
2) Unfed prawns + mullet
3) Fed prawn only
4) Fed prawns + mullet

Fed cages received approximately 0.07 g of Frippak Ultra PL+300 (200-400µm) 3 times per day following the stocking of prawns. The prawns were accustomed to this feed and feeding schedule, and the amounts offered were several fold more than that recommended for 50 PL16 prawns to ensure ample food availability for the prawns in the presence of mullet.

This experiment ran for 1 week when each cage was harvested and the surviving prawns were counted and weighed on bulk for survival and weight gain comparisons. The mullet in each cage were also harvested and euthanased with benzocaine before weighing *en mass*, and measuring each fish's total length to provide an accurate measure of fish sizes utilised in the study.

<u>Statistical analyses</u>
Binary survival data were analysed using a generalized linear model (McCullagh and Nelder, 1989) with the binomial distribution and logit link (GenStat, 2000), followed by protected t-tests to determine significant differences between the treatments.

Results

Experiment A

The survival rates of juvenile banana prawns in the first experiment are provided in Figure 3. Raw data are given in Appendix 2. No prawns survived in the tanks with small or large rabbitfish. No significant differences (P>0.05) in survival were found between the "prawns only" tanks, and prawns in tanks with various sized mullet.

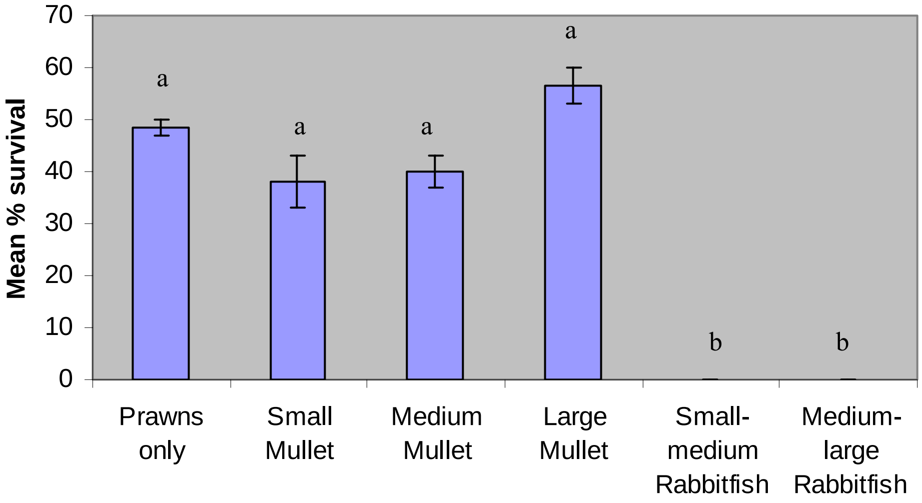

Figure 3. Mean (± se) percentage survival of banana prawns exposed to various sized mullet and rabbitfish for 7 days. Columns with different letters are significantly different (P<0.05).

The addition of juvenile banana prawns to mullet broodstock tanks at BIARC resulted in no observable predation. The mullet displayed no differences in behaviour before, during or after the addition of prawns. Similarly, no predation of prawns by large rabbitfish was observed following introduction into their broodstock tank. Although the rabbitfish displayed typical feeding behaviour before the addition of banana prawns (mouthing at the surface), after the addition of banana prawns (and not the pellet feed that they were accustomed to), they became uninterested and moved to the bottom of the tank whilst the prawns remained clearly visible in the water column and on tank surfaces.

Experiment B

Total lengths of mullet used in the second experiment ranged from 45 to 73 mm and their weights at the end of the experiment ranged from 1.95 to 2.89 g (raw data and summary given in Appendix 3). The survival of banana prawns in cages is shown in Table 2 below. No significant differences (P>0.05) were found between the survival rates of fed or unfed prawns with or without the presence of mullet.

Table 2. Survival of banana prawn postlarvae with and without mullet juveniles in fed and unfed cages.

Treatment	**Number of prawns surviving**			**Percentage of prawns surviving**			**Mean ± se % survival***
	Rep 1	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**	
Unfed prawns only	48	48	48	96	96	96	96 ± 0.0a
Unfed prawns + mullet	44	50	46	88	100	92	93 ± 3.5a
Fed prawn only	47	46	42	94	92	84	90 ± 3.1a
Fed prawns + mullet	43	45	45	86	90	90	89 ± 1.3a

*Means followed by different letters are significantly different (P<0.05)

As an adjunct to these survival results, the weights of prawns in each treatment (raw data shown in Appendix 4) suggests that the growth of prawns was slowed by the presence of mullet in fed cages, and possibly also in unfed cages (see summary in Figure 4 below). Although adding feed to the cages increased the growth of prawns when they were on their own, the presence of mullet appears to have reduced its availability to prawns.

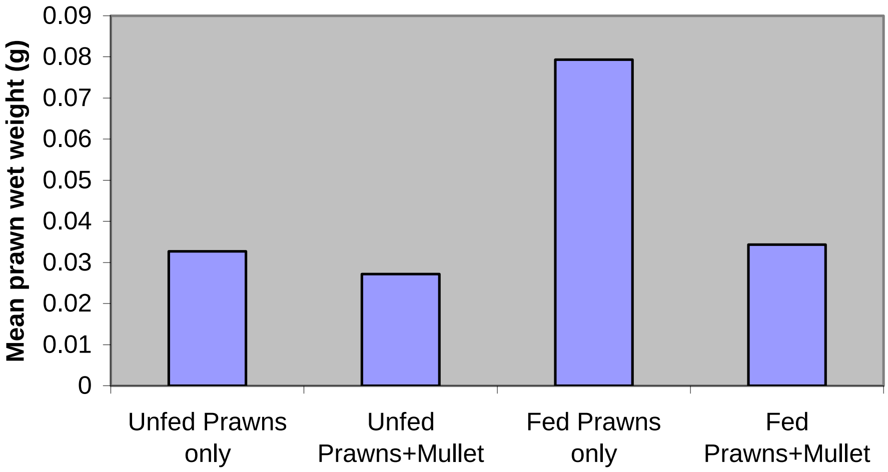

Figure 4. Mean prawn wet weights from different treatments in experiment B.

Discussion

Banana prawns and sea mullet

The feeding habits of juvenile Mugilidae has been reported to change with size from carnivorous to herbivorous (De Silva, 1980). During larval stages they are known to feed mainly on mircocrustaceans in the zooplankton, but postlarval stages of *M. cephalus* up to 40 mm in length have been observed to mainly consume phytoplankton including diatoms and blue-green algae (De Silva and Wijeyaratne, 1977). These studies have shown that the ingestion of sand and detritus begins at a length of 25 mm, and that from 50 mm they are almost exclusively vegetarian. Whilst various studies have found metazoans like polychaetes, crustacean larvae and molluscs in the gut contents of *M. cephalus* collected from the wild (eg: Rao and Sivani, 1996 – 31% polychaete; Wells, 1984 – 7% snails in freshwater), these food items are thought to be ingested with localised sediments rather than through targeted foraging or food-oriented habitat preferences.

There is no reference in literature so far sighted that postlarval or juvenile prawns are consumed by *M. cephalus*, and results from the present study support this assumption. Maguire and Bell (1981) investigated the effects of sea mullet on school prawns (*Metapenaeus macleayi*) in 3.3 m^2 net pens in brackish water ponds in New South Wales. They found prawn survival to be unaffected by mullet stocking with up to 25 x 15-42 g (55-155 mm caudal fork length) fish per net pen. The present results suggest that banana prawns as young as PL16 could be cultured with sea mullet from a size of approximately 50mm (or 2 g) without the potential for predation causing prawn losses. These prawn and mullet sizes are typical of seed stocks for these species that are presently commercially available in Australia, suggesting that the creation of such polycultures is immediately practically achievable.

Larger sea mullet (mean length of 439 mm) in aquaculture ponds have been shown to obtain 75% of their food from the water column (Cardona and Castello, 1994). This planktophagous behaviour was thought to enhance organic matter transfer from the water column to the sediment, which could therefore be expected to improve the growth of bottom feeding animals like prawns. The results from experiment B however, do not support this assertion, where the presence of mullet may have marginally slowed the increase of prawn biomass in unfed cages. Maguire and Bell (1981) however, showed that *M. cephalus* had little effect on the growth of *M. macleayi*, and suggested that these two species may utilise different components of the detritus.

Penaeids and mullet are both known to derive considerable nutrition from ingested bacteria. Moriarty (1976) found that bacteria and diatoms were equally important to mullet feeding over seagrass beds, but in muddy habitats, bacteria and possibly protozoa were relied on more for this fishes energy needs. In the same work, Moriarty also reported that the detritus feeding greasyback prawn *Metapenaeus bennettae* was more selective against algae, whilst digesting and assimilating at least 5 species of bacteria. If prawns and fish in a polyculture environment were each competing for the same pool of available nutrients (eg: micro-organisms), slower growth could be expected in either or both if these reserves were limited. This effect could be pronounced if one were more successful at collection and processing, or if one were more selective in their preferred natural diets.

Additionally, sea mullet are well known to grow quickly in semi-intensive prawn culture ponds in Australia, because they feed voraciously on the artificial prawn feeds that are normally applied. As mullet in such polycultures do not restrict their feeding activities to settled organics and other potentially wasted nutrients, farmers question the usefulness of their inclusion because it is thought to create unnecessary competition for expensive prawn feeds. Further replicated experimental work and farm based trials testing banana prawn growth with different mullet sizes are therefore necessary to clarify the potential benefits of growing these species together.

Banana prawns and rabbitfish

Up to 30 species of rabbitfishes are known in the world (Shokita *et al*., 1991). Of the 26 species reported from the tropical Indo-West Pacific by Woodland (1983), one is estuarine, and the rest live in the vicinity of coral reefs. Larval and immediate post-larval stages are planktivorous, but later stages are particularly omnivorous as demonstrated by their ability to be reared successfully in captivity using artificial diets from a very early age (Parazo, 1991).

Adults in natural habitats are reported to generally feed on seaweeds, benthic algae, and sea grasses (Shokita *et al*., 1991; Woodland, 1983), but Bwathondi (1982) reports one species' (*Siganus canaliculatus*) feeding capabilities to also include amphipods, copepods, sponges, Foraminifera, crustaceans, and brittle stars. Other prey items that this species is also reported to sometimes consume includes mysids and crabs, but red, brown, and green algae have mainly been found in the stomach contents of wild adults (Shokita *et al*., 1991). These later authors found *S. guttatus* preferred *Schizomeris* and *Ulva* to other plants, but such diets resulted in weight losses compared with slight growth from artificial diets. Similarly these authors reported that *S. canaliculatus* increased in weight when fed *Schizomeris* or artificial feed, but decreased when fed *Ulva* or *Sargassum*.

Only scant information could be found regarding the feed preferences of the rabbitfish species used in the present study. Shokita *et al*. (1991) reported that *S. spinus* larvae increased in weight when fed formulated feeds but that growth was poor when they were fed the algae *Ulva pertusa*. Specimens utilised in the present study were 1st generation progeny of animals sourced from coastal areas in, and immediately north of Moreton Bay in Southern Queensland. Although this species, which is commonly known along the Queensland coast as happy moments or black trevally is classified as *S. spinus* (Linnaeus) by Grant (1982), it is more recently recognised as *S. nebulosus* (Yearsley *et al*. 1999). Despite the group's reported herbivorous nature, rabbitfish in Queensland are commonly taken on hooks baited with prawn flesh. In fact, this is how broodstock at BIARC have been sourced in the past, from around rocky headlands or near rock walls in artificial canals. In contrast, sea mullet are seldom if ever taken with prawn flesh baits. Bread or dough is more commonly use by mullet anglers.

Although the predatory nature of rabbitfish on juvenile banana prawns demonstrated in this study suggests they are unsuitable for mixed cultures, this combination has been moderately successful at producing significant prawn biomass in pond trials at BIARC. This previous work involved stocking PL15 banana prawns into a culture pond that contained large mullet and rabbitfish. However, the fish in this case, like the broodstock that were offered prawns in this study, were likely to have been more accustomed to feeding on pelleted fish food. They probably did not immediately recognise the prawns offered as potential feed. Additionally, the fish in the culture pond had a range of other potential food sources such as benthic algae and detritus, and so were not so heavily challenged with low availability of suitable feed in the presence of available prawn biomass. Greater niche space and more refuges would also aid prawn survival in the culture pond's ecosystem. However, increasing pressures of low natural feeds from higher rabbitfish stocking densities could, as suggested in experiment A, cause high levels of predation of rabbitfish on juvenile banana prawns.

Bioremediation pond ecosystems

Species stocked into bioremediation ponds are typically not fed with artificial diets. Rather, they are expected to find sufficient food from different components of the pond ecosystem they inhabit, thereby stripping nutrients and incorporating a portion in their biomass for later harvest/removal. Ideally, each species in such a prescribed mix sources different resources with little overlap, so that the inclusion of additional species makes use of nutrients not being utilised to give an overall increase in total harvestable biomass. Such complimentary polycultures are practiced to varying degrees around the world (eg: Wahab *et al*., 2002), but less is known about the attributes of Australian species to make this possible.

In experiment A, tanks were set up the day before starting the experiment so that natural biofilms had little time to develop to provide fish with an alternative food source to the juvenile prawns. This was undertaken to fully evaluate the fishes potential to prey on these prawns. The pond water used for exchanges in experiment B was typical of discharge from prawn culture ponds. This procedure supplied microscopic food sources to the banana prawns and mullet that were similar in nature to food available in the water column of a prawn farm settlement pond. The effects of well developed substrate biofilms and the soil-water interface was not factored in to these experiments, and such alternative food sources for these species could change their predatory potentials displayed herein.

Finally, these experiments were not designed to assess mullet growth under the trial's conditions. Accurate measurements of fish weights and sizes were undertaken after the weeklong trials rather than before, so that stress levels in fish were minimised to better facilitate normal feeding behaviours during the trial. Stress minimisation was considered at all operational stages of this work because it was well recognised that stressed animals would not perform according to their natural tendencies. Furthermore, the feeds that the postlarval prawns were previously accustomed to was used in experiment B to ensure that they did not die for reasons related to nutrition or an inability to change feed types. Amounts offered to prawns were also several-fold more than the recommended feeding level for 50 PL16 prawns, because the cage design prevented an accumulation of uneaten feed to cause environmental fouling problems, and to ensure that prawns were getting a reasonable opportunity to acquire feed when mullet were also foraging and eating the feed added to the cage.

The results show that rabbitfish are probably not suitable for inclusion in polycultures with banana prawns. On the other hand, sea mullet do not appear to present a risk to banana prawn seedstock, but appear to feed on similar natural and artificial diets which may reduce the advantages of their mixed culture.

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Appendix 1. Water quality and management details for the 1600 m^2 prawn culture pond at BIARC (G3) used as a water source in experiment B.

Date	Time	pH	Temp (ºC)	Secchi depth (cm)	Feed added (kg/d)	Salinity (ppt)	Comments (eg: bloom colour; weather; pond-water exchange rate)
10/12/03	1000 1500	8.57 8.58	25.2 27.2	>120 >120	6.0	35.9	Green; overcast; 5% exchange
11/12/03	0900 1630	8.49 8.65	24.5 26.2	>120 >120	6.0	34.4	Green; partially overcast and windy; 5% exchange
12/12/03	1000 1530	8.60 8.71	25.2 26.2	>120 >120	6.0	35.0	Green; fine; 5% exchange
13/12/03	0800 1700	8.57 8.75	25.3 27.2	>120 >120	6.0	35.3	Green; fine; 5% exchange
14/12/03	0700 1730	8.58 8.76	25.6 27.7	>120 -	6.0	35.4	Green; fine; 5% exchange
15/12/03	-	-	-	-	6.0	-	-
16/12/03	1030 1630	8.57 8.75	27.4 28.9	100 80	6.0	35.5	Green; fine; 5% exchange
17/12/03	0800 1830	8.58 8.70	27.2 27.9	120 80	6.0	35.7	Green; fine; 5% exchange
18/12/03	0630 1600	8.52 8.69	27.1 28.7	>120 75	6.0	35.6	Green; fine; 5% exchange

Appendix 2. Raw data for experiment A. Number of living prawns removed from tanks containing mullet and rabbit fish of various sizes and their relative survival percentage after 7 days.

Tank No.	Fish Type	Number of live prawns removed	Prawn survival (%)
E1	Mullet – small	10	33
E2	Mullet – large	18	60
E3	Rabbit Fish – small	0	0
E4	Prawns Only	14	47
E5	Mullet – medium	13	43
E6	Rabbit fish – large	0	0
E7	Mullet – medium	11	37
E8	Prawns Only	15	50
E9	Mullet – small	13	43
E10	Rabbit fish – large	0	0
E11	Mullet – large	16	53
E12	Rabbit fish –small	0	0

Appendix 3. Sizes of mullet used in experiment B. Measurements were taken at the end of the experiment.

Treatment/ replicate	Total lengths (mm)	Mean length (min-max) (mm)	Bulk weight (g)	Mean weight (g)
Unfed prawns + mullet / rep 1	69 67 62 52 62 61 55 50 48 48	57.4 (48-69)	23.4	2.34
Unfed prawns + mullet / rep 2	50 55 64 55 65 45 48 50 48 50	53.0 (45-65)	19.5	1.95
Unfed prawns + mullet / rep 3	68 58 65 55 60 65 50 60 61 51	59.3 (50-68)	24.3	2.43
Fed prawns + mullet / rep 1	67 54 73 70 53 71 62 60 60 53 48 *	61.0 (48-73)	31.8	2.89
Fed prawns + mullet / rep 2	66 50 52 65 52 59 68 70 49 60	59.1 (49-70)	25.1	2.51
Fed prawns + mullet / rep 3	55 57 54 52 68 55 55 65 59 56	57.6 (52-68)	22.3	2.23

* Note 11 mullet were in this replicate

Appendix 4. Bulk weights of prawns at the end of experiment B. All replicates were pooled prior to weighing total prawn biomass in each treatment.

Treatment	Bulk wet weight (g)	Number of prawns	Mean prawn wet weight (g)
Unfed prawns only	4.71	144	0.033
Unfed prawns + mullet	3.80	140	0.027
Fed prawn only	10.71	135	0.079
Fed prawns + mullet	4.57	133	0.034