

## Survival and Immunity of Marron *Cherax cainii* (Austin, 2002) Fed *Bacillus mycooides* Supplemented Diet under Simulated Transport

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### Abstract

The present study examined the effect of simulated transport on marron, *Cherax cainii*, (Austin, 2002) after a 10 week feeding trial using basal diet or customised probiotic, *Bacillus mycooides* supplemented diet by measuring intestinal bacterial population, total haemocyte count (THC), bacteraemia, morbidity, dehydration and mortality.

Packing steps followed the established standard packing method for live transportation of marron. Each treatment group consisted of six polystyrene boxes (65 × 30 × 40 cm<sup>3</sup>), and each box contained 30 marron from each feeding group. The sealed boxes were placed on a trolley at room temperature to give the effect of transportation. Boxes were opened at 24<sup>th</sup> and 48<sup>th</sup> hour post simulated transport and marron from each treatment group were returned to the culture tank. After temperature acclimation, the marron were observed for mortality and samples were collected to assess marron health and immunity.

The results demonstrated that no mortality was observed at 24 h of transport both in basal diet and probiotic diet fed marron, however at 48h of transport the survival of probiotic fed marron was significantly higher (100 ± 0.0%) compared to survival (93.3 ± 2.8%) of basal diet fed marron. The higher survival rate of probiotic fed marron was also sustained by superior health and immune status indicated by higher intestinal bacterial population, higher total haemocyte count and lower haemolymph bacteria (bacteraemia) level. In brief, supplementation with host origin customized probiotic *B. mycooides* significantly improved marron tolerance to a live transport stress test, which resulted in no mortality up to 48h of transport.

**Keywords:** Simulate transport; Intestinal bacteria population; THC; Bacteraemia; Mortality

### Introduction

Practices and methods used for crustacean handling and live trade may lead to serious physiological stress responses in aquatic animals [1-3] including marron [4-6]. Marron, *Cherax cainii* (Austin, 2002), is endemic to Western Australia and recently was introduced into South Africa, Zimbabwe, Japan, USA, China and the Caribbean as a commercial aquaculture species [6-8].

The reasons for live trade of crustaceans include for consumption, grow-out, restocking, and the aquarium trade; hence survival and quality of the animals is extremely important for welfare and economic reasons. The duration of the stressors encountered in the live trade process leads to short and long term changes in immune parameters as stress response shifts from adaptive to maladaptive [2,9]. Beyond this point the physiological stress response may reduce disease resistance and growth reduced quality and eventually death [2,10]. Therefore, improving immunity, stress tolerance and optimising health conditions of crustaceans during storage and live transport is of fundamental importance [3].

The successful culture and stocking of marron relies on better understanding the factors affecting their well-being during transport and their recovery afterwards [5]. Marron are an economically important aquaculture species in Western Australia and they show significant environmental stress tolerance post handling and live transport. Jussila observed no mortality of marron post 24 h handling and simulated transport, whereas detected no mortality of marron up to 36 h under simulated transport [5,6]. Moreover, marron may undergo live transport up to 72 h without mortality, however longer periods of transportation resulted in an average dehydration of 4.5% of body weight [4,5].

Feed additives such as probiotics and prebiotics can improve stress tolerance and immunity of aquatic animals including marron [6,11-16]. Our previous studies indicated that *Bacillus mycooides*, a marron origin customised probiotic, possessed a number of favourable probiotic properties [17], significantly improved gastrointestinal (GIT) health of marron [18] and improved immunity against the pathogenic bacterium *Vibrio mimicus* [19], a dominant bacterial pathogen of freshwater crayfish in aquaculture [20-22].

Cruz et al. suggested that aquatic animals should be treated with probiotics before exposure to transport and environmental stressors [23]. To date, improved stress tolerance and immunity by feeding probiotics have been documented in fish [11,24,25], shrimps [26-29] and molluscs [30,31] however information on probiotic-fed marron under practical transport conditions is not available. The present study evaluated the effect of simulated transport conditions on marron fed the probiotic *B. mycooides* by measuring intestinal bacterial population, total haemocyte count (THC), bacteraemia, morbidity, dehydration and mortality.

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## Material and Methods

### Acclimation and feeding trial

Marron juveniles were supplied by Blue Ridge Western Australia Pty Ltd and then acclimated to the culture tanks, fed using basal diet for 2 weeks, and distributed into six experimental culture tanks at a density of 12 marron per tank.

The experimental system consisted of three standing units of steel racks with three shelves in each unit. The experimental units were cylindrical plastic tanks (80 cm diameter and 50 cm high and 250 L in capacity) filled with freshwater running continuously at a rate of approximately 3 L/min. using a recirculating biological filtration system (Fluval 205, Askoll, Italy). Each tank was supplied with constant aeration and equipped with a submersible thermostat set to 24°C. PVC pipes of appropriate diameter were added to the tanks as shelters for the marron.

Prior to the simulated transport test, a feeding trial using basal and probiotic supplemented diet was conducted for 10 weeks. Each tank was stocked with 12 marron where each treatment consisted of five replicate tanks. The test diets were given to marron every day in the late afternoon at a rate of 1% of the total biomass and adjusted weekly after determination of biomass at the end of each week.

### Experimental diet and set up

The experimental diets used in this feeding trial were (1) basal diet of a marron commercial feed supplied by Specialty Feed Pty Ltd, WA and (2) the basal diet supplemented with customised probiotic *B. mycoides*. Before use, the pelleted diet was homogenised with a blender to obtain a desirable pellet size before supplementation with the *B. mycoides* at 10<sup>8</sup> cfu/g of feed. The density of *B. mycoides* was based on the density used in other *Bacillus* species studies [32-36] and from results of our previous studies [18,19].

Supplementation of the probiotic followed established methods [37]. In brief, the basal diet was placed on tray covered with sterilised aluminium foil and sprayed with 20 mL of fish oil per kg of feed to improve attachment of probiotic bacteria. The feed was wrapped in individual sterilised aluminium foil packs containing the amount adjusted to marron biomass for each tank, and stored at 4°C until used. The diet was prepared each week and the feeding rate adjusted according to the marron weight.

### Simulated live transport

After feeding with the test diet for 10 weeks, the animals were subjected to a simulated live transport following the "Code of Practice for the Harvest and the Post-Harvest Handling of Live Marron for Food" established by Department of Fishery Western Australia [38] and the standard packing of marron commonly used by marron growers. Feeding ceased one day before the commencement of simulated transport trial.

In brief, healthy marron of equal size (12.3 ± 0.5 g) from probiotic fed and a control basal diet were selected and placed in a polystyrene box (60 × 40 × 30 cm) for 24 h and 48 h simulated transport. Each box contained sufficient ice-gel bags covered by a moist foam layer and a temperature data logger (Onset HOBO). Marron from each treatment group were placed in a ventilated container prior to placing in the polystyrene boxes. Placing the marron in the ventilated container not only avoided the marron from mixing with different treatment groups, but also protected the marron from the moist foam layer and

ice-gel bags, and was based on the method used in a previous study [6]. Subsequently, another moist foam layer and ice bag were placed over the marron ventilated containers, before the outer polystyrene box was sealed with a lid. The sealed boxes were placed on a trolley at room temperature and being moved intermittently to give simulated transport effects.

Twenty four and forty eight hours post simulated live transport, the animals were returned to the culture tanks once the parameters for intestinal bacteria population, total haemocyte count (THC), bacteraemia, morbidity, dehydration and mortality were measured and recorded.

### Measurements of the parameters

**Intestinal bacteria population:** Bacterial population of the marron was measured before and during simulated transport at 24h and 48h. In brief, marron from each treatment group were sacrificed by placing them at -20°C for 5 minutes before aseptic removal of the GIT. The marron dorsal shell was cut-off horizontally from tail to head until the intestines were exposed. The intestine from individual animals was collected aseptically and placed in a sterilised pestle, weighed and then homogenised. The homogenates of intestines were serially (10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup>) diluted using sterile normal saline. Fifty microliters of each serial dilution was inoculated onto a blood agar (BA) plate and incubated overnight in a CO<sub>2</sub> incubator at 25°C. A colony count was performed for each dilution to determine the total number of aerobic bacteria [39].

**Total haemocyte count (THC):** The total haemocyte count was measured following the established methods used in western rock lobsters *Panulirus cygnus* George [40] and marron [6]. In brief, 0.5 mL of haemolymph withdrawn from the second last ventral segment of marron was inserted into a haemocytometer (The Neubauer Enhanced Line, Munich, Germany) counting chamber and immediately viewed under 100-fold magnification on a camera-equipped microscope and images were taken for THC counts. Cells were counted in both grids, and the mean was used as the haemocyte count. For each treatment group, the procedure was repeated using ten different animals. The total haemocyte count was calculated as THC = (cells counted x dilution factor × 1000)/volume of grid (0.1 mm<sup>3</sup>).

**Haemolymph bacteria (Bacteraemia):** Bacteraemia of marron was determined following the established method described by sang et al. [6] with a minor modification. Briefly, the haemolymph was withdrawn into a sterile syringe and placed onto a sterile glass slide to avoid bubbles before a 0.05 mL aliquot was lawn inoculated onto a BA plate. The plates were placed in a sterilised container and incubated overnight at 25°C. The total colony forming units (CFU) for each plate and CFU/mL were calculated on the basis of a total volume of 0.05 mL/plate.

**Dehydration:** Dehydration of marron was measured using the established method [5]. Ten marron from each treatment group of equal size were weighed prior to the commencement of the simulated transport, then weighed at 24 h and 48 h post transport and the percentage of weight loss was recorded.

### Morbidity and survival rate (%)

Morbidity (vigour index) of marron was measured following the established method proposed by Jussila et al. [5]. In this study, morbidity of marron was identified based on the response to stimuli at a time after simulated transport, and the time of recovery was recorded after being returned to the culture tanks.

Mortality of marron from each treatment group was measured at 24 h and 48 h post-simulated transport up to one day they were returned to the culture tanks. Determination of survival rate following the established equation;

$$SR = (N_t/N_0) \times 100$$

where SR is the survival rate (%);  $N_t$  is the number of marron at time  $t$  and  $N_0$  is the number of marron at the commencement (0), respectively.

### Data analysis

The data were analysed using SPSS statistical package version 22.0 for Windows and Microsoft Excel. The difference between means was determined using one way analysis of variance (ANOVA) and a t-test. All significant tests were performed at  $P < 0.05$  level. All data were presented as mean  $\pm$  SE, unless otherwise indicated.

## Results

### Intestinal bacteria population

Intestinal bacterial population of marron declined at 24 and 48 h simulated transport both for basal diet and probiotic fed marron. Reduction of the intestinal bacterial population occurred at 24 h and 48 h of transport; however a significant reduction of more than half the initial population levels were observed at 48 h, both in basal diet and probiotic fed marron (Table 1). This result suggests that the longer the stress disturbance, the greater the reduction of intestinal bacterial population. Nevertheless, at 48 h post-transport, the bacterial population ( $646 \pm 16.4$ ) of probiotic fed marron was comparable to the initial bacterial population ( $626.7 \pm 19.7$ ) of basal diet fed marron.

### Total haemocyte count (THC)

Prior to the simulated transport test, the THC of marron fed probiotic and basal diets for 10 weeks was measured. The THC of marron fed probiotic supplemented diet was significantly higher compared to THC of basal diet fed marron at the commencement of simulated transport test (Figure 1). This result indicates that the health status of marron fed probiotic was higher at the initiation of the simulated transport test.

After 24 h and 48 h post transport, the THC in both treatment groups declined indicating that transport affects the THC in marron.

### Haemolymph bacteria (Bacteraemia)

Haemolymph bacteria were observed in basal diet and probiotic fed marron after feeding with the test diets for ten weeks prior to the simulated transport test. Total bacterial count in the haemolymph of probiotic fed marron was significantly ( $P < 0.05$ ) lower compared to basal fed marron, indicating the customised probiotic of host origin may induce protection from bacteria and other foreign particles in the haemolymph. This result was strongly related to THC of marron in each treatment group, as haemocytes play an important role in removal of bacteria and foreign particles from the haemolymph of crayfish [41,42].

### Dehydration

Dehydration occurred in marron fed either the basal diet or the probiotic supplemented diet. During the first 24 h, dehydration in the basal diet fed marron was 3.8 percent, whereas in probiotic fed marron it ranged between 3.0 and 3.7%. After 48 h of transport, the dehydration still occurred in both treatment groups but in the basal diet it was 0.45% while in probiotic fed marron the extra dehydration was 0.55%. There

was no significant different ( $P > 0.05$ ) in the mean dehydration between basal diet and probiotic fed marron after 24h and 48h of transport.

### Morbidity and survival rate (%)

Marron showed a very weak response to stimuli after 24 h of transport and this condition was more noticeable after 48 h of transport. Marron started to show response to stimuli after 30 minutes during the temperature acclimation in the boxes. As most marron in the boxes were actively crawling and swimming, they were returned to the tanks for mortality observation. No mortality was observed in marron fed probiotic supplemented diet; while in basal diet fed marron mortality (6.7%) occurred at 48 h of transport. No clinical signs were observed on dead marron shells (Figure 2).

## Discussion

A number of immune and physiological parameters are involved in the stress response following handling and live transport of crustaceans and fish such as behaviour, morbidity and vigour, THC, blood glucose, dehydration, oxygen uptake, blood composition, pH, hormones and ion [1,3]. In marron, the common selected parameters for testing following handling and simulated transport include dehydration [4] THC, haemolymph/plasma glucose, serum protein, dehydration [5], proportion of granule cells, clotting time and bacteraemia [6].

The circulating haemocytes of crustaceans are an essential part of the immune system, and perform functions such as phagocytosis, encapsulation, and lysis of foreign cells and much research in the defensive role of haemocyte in crustacean is being conducted [26,40,43]. The results suggest total haemocyte count (THC) is a reliable indicator of stress in crustaceans [2,44] including in marron [5,6].

In the present study, THC was investigated as an indicator for stress tolerance. THC of marron fed probiotic was significantly higher compared to basal diet fed marron both at 24 h and 48 h post simulated transport, indicating that the customised probiotic *B. mycoides* was able to improve marron immunity. Enhancement of THC in probiotic fed marron leads to increased stress tolerance and diseases resistance, which results in a significantly higher survival rate (100%) over 24 h and 48 h post live transport. Higher THC of probiotic fed marron also provides better protection to the gill from parasites and bacteria pathogens which may cover and reduce respiration efficiency. Tinh et al. [45] suggested that probiotic bacteria can also be active on the gills and skin of the host. Inefficient respiration during handling and live transport may contribute to marron mortality in basal diet fed marron. Thus prior to transport, purging is generally essential in freshwater crayfish [3] to evacuate the GIT content and clean the gills and skin.

Handling and live transport creates physiological stresses which reduce THC in many crustacean species such as American lobster *Homarus americanus* [1], crab *Cancer pagurus* [2], western rock lobster *Panulirus cygnus* [2,46] marron [5,6], and a mollusc abalone *Haliotis tuberculata* [47]. Therefore, increasing the THC by feeding probiotic supplemented diets may improve stress tolerance and protect the animals from pathogens. This has been demonstrated in crayfish, *Pacifastacus leniusculus*, where a higher THC corresponded

Treatment	Intestinal bacteria population (million CFU/g of gut)		
	0h	24h	48h
Basal diet	$626.7 \pm 19.7^{a,1}$	$531.1 \pm 15.7^{a,1}$	$260.0 \pm 67.1^{b,1}$
<i>B. mycoides</i> diet	$1656 \pm 167.7^{a,2}$	$1318 \pm 131.5^{a,2}$	$646 \pm 16.4^{b,2}$

**Table 1:** Intestinal bacteria population (million CFU  $\pm$  SE) of marron fed different diets at 24 h and 48 h post transport.

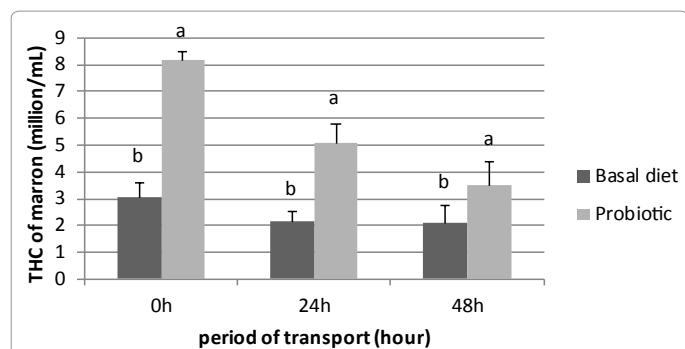


Figure 1: THC of marron fed basal and probiotic diets at 24h and 48h of transport.

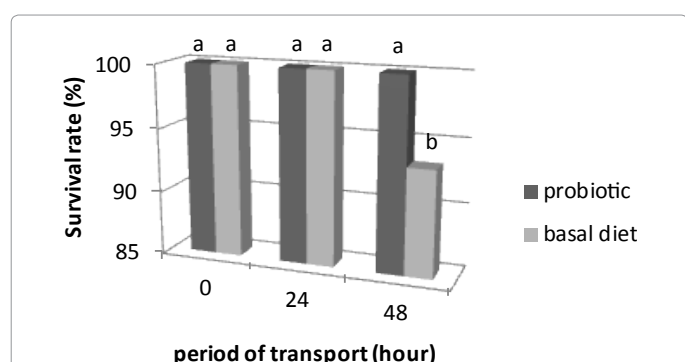


Figure 2: Survival rate (%) of marron fed basal and probiotics diets at 24 h and 48 h of transport.

to improved defence reactions of the animal when infected with the Oomycete fungus *Aphanomyces astaci* [48] and in marron where a higher THC corresponded to reduced bacteraemia and improved immunity against the pathogen *Vibrio mimicus* [6,19].

Bacterial population and diversity in the GIT is an important health component for aquatic animals [49,50] significantly affected by acute stress [51]. Stress due to high stocking density could also affect the performance of probiotics [24]. In the present study, the bacterial population level decreased both in probiotic and basal diet fed marron at 24h and a significant reduction was observed at 48h of transport indicating that prolonged stress significantly reduces intestinal bacteria of the animal. However, the higher bacterial population level of probiotic fed marron prior to transport resulted in the bacterial population remaining higher at 48h of transport compared to the population level of basal diet fed marron at the initiation of the transport test. The reduction of intestinal bacterial population due to handling and transport stress was also determined in other species. In Atlantic salmon, *Salmo salar* and rainbow trout, *Oncorhynchus mykiss* (Walbaum), adherent bacterial population in the mid-gut and hindgut were significantly reduced following acute handling stress, whereas the level increased in faeces, which suggests that considerable amounts of mucus was lost following stress [51,52]. This significant reduction of the intestinal bacterial population implies decline in the health status of the animal. The microbiota within the GIT can be considered a metabolically active organ, is an essential health component, provides protection against infection and instructs mucosal immunity [50]. Beneficial bacteria such as lactobacilli and bifidobacteria decrease following a stress response [50] and this may provide opportunistic pathogens to become established [2,44,53].

Haemolymph bacteria (bacteraemia) of marron fed probiotic diet was significantly lower compared to basal diet fed marron suggesting that greater THC plays an important role in reducing bacteria and foreign particles in marron haemolymph. Once pathogens or foreign particles enter the haemocoel, the haemocytes initiate phagocytosis [54]. In crayfish, hyaline cells are chiefly involved in phagocytosis, whereas semi-granular cells are active in encapsulation [41]. Crustacean haemocytes contain antibacterial activity [55,56] which can reduce the viable count of bacteria within 4 hours, however the antibacterial potency (per unit protein) varies from species to species [42].

Morbidity and mortality post live transport often occurs as a result of stress [3]. In the present study, morbidity and weakness indicated by no response to stimuli were observed both in basal diet and probiotic fed marron at 24 h and 48 h post simulated transport. However, after returning to the culture tanks the probiotic fed marron fully recovered and swam normally in less than 30 minutes, whereas basal diet fed marron took more time to recover and had several mortalities.

Other than mortality, marron also could be losing weight through dehydration from tissue and gill chambers while out of water during handling and live transport [3-5]. The present study indicated that dehydration of marron was observed in both test diets at 24 h and 48 h post transportation, however the dehydration was not significantly different between the two treatments. The results revealed that the dehydration of marron was comparable to the previous marron handling and live transport studies indicating that dehydration of 4 to 5% of the body weight over 24 to 72 h transportation is a common phenomenon. Jussila et al. observed a minor dehydration of marron during the first 4 hours that remained at 4.0 to 4.5% up to 24 h post handling and transportation, whereas Morrissy et al. observed wet dehydration of 3.9% during the first 24 h, with a further additional loss of 0.4% over the next 24 h [4,5]. Acute stress requires high energy which could reduce the hepatosomatic indices and contribute to the dehydration of the animal [23]. In marron, hepatopancreas significantly reduced after 24 h of transport [5]. Therefore, dehydration should be considered when crayfish are going to be transported for a long distance [5].

Probiotics have improved stress tolerance and immunity of various aquatic animal groups such as fish species including sea bream, *Sparus aurata* [11], grouper *Epinephelus coioides* [57], Nile tilapia *Oreochromis niloticus* [58]; shrimp such as western king prawns, *Penaeus latissulcatus* [59] shrimp, *Litopenaeus vannamei* [60-62], and tiger shrimp, *Penaeus monodon* [63]. Among the bacterial genera evaluated for probiotic use, *Bacillus* species have been successful in improving the stress tolerance in these aquatic animal hosts and more recently in black swordtail, *Xiphophorus helleri* [16]. Our previous work using *B. mycoides* also showed improved marron immunity when experimentally challenged with the pathogen *Vibrio mimicus* [19].

Overall, the present study demonstrated that supplementation with host origin customized probiotic *B. mycoides* significantly improved the health status of marron by increasing their tolerance to a live transport stress test, which resulted in no mortality up to 48 h of simulated live transport.

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## References

- Lorenzon S, Giulianini PG, Martinis M, Ferrero EA (2007) Stress effect of different temperatures and air exposure during transport on physiological profiles in the American lobster *Homarus americanus*. *Comp Biochem Physiol* 147: 94-102.
- Lorenzon S, Giulianini PG, Libralato S, Martinis M, Ferrero EA (2008) Stress effect of two different transport systems on the physiological profiles of the crab *Cancer pagurus*. *Aquaculture* 278: 156-163.
- Fotedar S, Evans L (2011) Health management during handling and live transport of crustaceans: A review. *Journal of Invertebrate Pathology* 106: 143-152.
- Morrissy NM, Walker PCF, Moore W (1992) An investigation of dehydration of marron (*Cherax tenuimanus*) in air during live transportation to market. Bernard Bowen Fisheries Research Institute, Western Australia Marine Research Laboratories. Fisheries Department of Western Australia. Fisheries Research Report 99: 1-21.
- Jussila J, Paganini M, Mansfield S, Evans LH (1999) On physiological responses, plasma glucose, total hemocyte count and dehydration, of marron *Cherax tenuimanus* (Smith) to handling and transportation under simulated conditions. *Freshwater Crayfish* 12: 154-167.
- Sang HM, Le KYT, Fotedar R (2009) Dietary supplementation of mannan oligosaccharide improves the immune responses and survival of marron, *Cherax tenuimanus* (Smith, 1912) when challenged with different stressors. *Fish shellfish immunology* 27: 341-8.
- Morrissy NM, Evans L, Huner JV (1990) Australian freshwater crayfish: Aquaculture species. *Journal of the World Aquaculture Society* 21: 113-122.
- Rouse DB, Kartamulia I (1992) Influence of salinity and temperature on molting and survival of the Australian freshwater crayfish (*Cherax tenuimanus*). *Aquaculture* 105: 47-52.
- Barton BA, Iwama GK (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annu Rev Fish Dis* 1: 3-26.
- Ige BA (2013) Probiotics use in intensive fish farming. *African Journal of Microbiology Research* 7: 2701-2711.
- Rollo A, Sulpizio R, Nardy M, Silvi S, Orpianesi C, et al. (2006) Live microbial feed supplement in aquaculture for improvement of stress tolerance. *Fish Physiol Biochem* 32: 167-177.
- Zhang J, Liu Y, Tian L, Yang H, Liang G, et al. (2012) Effects of dietary mannan oligosaccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific white shrimp, *Litopenaeus vannamei*. *Fish & Shellfish Immunology* 33: 1027-1032.
- Soleimani N, Hoseinifar SH, Merrifield DL, Barati M, Abadi ZH (2012) Dietary supplementation of fructooligosaccharide (FOS) improves the innate immune response, stress resistance, digestive enzyme activities and growth performance of Caspian roach (*Rutilus rutilus*) fry. *Fish & Shellfish Immunology* 32: 316-321.
- Lund I, Skov PV, Hansen BW (2012) Dietary supplementation of essential fatty acids in larval pikeperch (*Sander lucioperca*); short and long term effects on stress tolerance and metabolic physiology. *Comp Biochem Physiol A Mol Integr Physiol* 162: 340-348.
- Wongsasak U, Chaijamrus S, Kumkhong S, Boonanuntanasarn S (2015) Effects of dietary supplementation with  $\beta$ -glucan and synbiotics on immune gene expression and immune parameters under ammonia stress in the Pacific white shrimp. *Aquaculture* 436: 179-187.
- Hoseinifar SH, Roosta Z, Hajimoradloo A, Vakili F (2015) The effects of *Lactobacillus acidophilus* as feed supplement on skin mucosal immune parameters, intestinal microbiota, stress resistance and growth performance of black swordtail (*Xiphophorus helleri*). *Fish & Shellfish Immunology* 42: 533-538.
- Ambas I, Buller N, Fotedar R (2015a) Isolation and screening of probiotic candidates from marron, *Cherax cainii* (Austin, 2002) gastrointestinal tract (GIT) and commercial probiotic products for the use in marron culture. *Journal of Fish Diseases* 38: 467-476.
- Ambas I, Fotedar R, Buller N (2015b) *Bacillus mycoides* Improves Health of Gastrointestinal Tract in Marron (*Cherax cainii*, Austin 2002). *J Aquac Mar Biol* 2: 1-7.
- Ambas I, Suriawan A, Fotedar R (2013) Immunological responses of customised probiotics-fed marron, *Cherax tenuimanus*, (Smith 1912) when challenged with *Vibrio mimicus*. *Fish & shellfish immunology* 35: 262-270.
- Eaves LE, Ketterer PJ (1994) Mortalities in red claw crayfish *Cherax quadricarinatus* associated with systemic *Vibrio mimicus* infection. *Diseases of Aquatic Organism* 19: 233-237.
- Wong FYK, Fowler K, Desmarchelier PM (1995) Vibriosis due to *Vibrio mimicus* in Australian freshwater crayfish. *Journal of Aquatic Animal Health* 7: 284-291.
- Evans L, Edgerton BF (2002) Pathogens, Parasites and Commensals, in *Biology of Freshwater Crayfish*, Blackwell Science Ltd, Osney OX2 0EL, UK.
- Cruz PM, Ibanez AL, Hermosillo OAM, Saad HCR (2012) Use of Probiotics in Aquaculture. *ISRN Microbiology*.
- Nayak SK (2010) Probiotics and immunity: A fish perspective. *Fish and shellfish immunology* 29: 2-14.
- Tapia-Paniagua ST, Vidal S, Lobo C, Prieto-Alamo MJ, Jurado J, et al (2014) The treatment with the probiotic *Shewanella putrefaciens* Pdp11 of specimens of *Solea senegalensis* exposed to high stocking densities to enhance their resistance to disease. *Fish shellfish immunology* 41: 209-221.
- Bachere E (2000) Shrimp immunity and disease control - Introduction. *Aquaculture* 191: 3-11.
- Farzanfar A (2006) The use of probiotics in shrimp aquaculture. *FEMS Immunology and Medical Microbiology* 48: 149-158.
- Ninawe AS, Selvin J (2009) Probiotics in shrimp aquaculture: Avenues and challenges. *Critical Reviews in Microbiology* 35: 43-66.
- Lakshmi B, Viswanath B, Gopal DVRS (2013) Probiotics as Antiviral Agents in Shrimp Aquaculture. *Journal of Pathogens*.
- Kesarcodi-Watson A (2009) Screening for probiotics of Green shell mussel larvae, *Perna canaliculus*, using a larval challenge bioassay. *Aquaculture* 296: 159-164.
- Kesarcodi-Watson A, Kaspar H, Lategan MJ, Gibson L (2012) Performance of single and multi-strain probiotics during hatchery production of Greenshell™ mussel larvae, *Perna canaliculus*. *Aquaculture* 354-355: 56-63.
- Keysami MA, Saad CR, Daud HM, Sijam K, Ar A (2007) Effect of *Bacillus subtilis* on growth development and survival of larvae *Macrobrachium rosenbergii* (de Man). *Aquaculture nutrition* 13: 131-136.
- Keysami M, Mohammadpour M, Saad CR (2012) Probiotic activity of *Bacillus subtilis* in juvenile freshwater prawn, *Macrobrachium rosenbergii* (de Man) at different methods of administration to the feed. *Aquaculture international* 20: 499-511.
- Li J, Tan B, Mai K (2009) Dietary probiotic *Bacillus OJ* and isomaltooligosaccharides influence the intestine microbial populations, immune responses and resistance to white spot syndrome virus in shrimp (*Litopenaeus vannamei*). *Aquaculture* 291: 35-40.
- Zhang Q, Tan B, Mai K, Zhang W, Ma H, et al. (2011) Dietary administration of *Bacillus* (*B. licheniformis* and *B. subtilis*) and isomaltooligosaccharide influences the intestinal microflora, immunological parameters and resistance against *Vibrio alginolyticus* in shrimp, *Penaeus japonicus* (Decapoda: Penaeidae). *Aquaculture research* 42: 943-952.
- Liu CH, Chiu CH, Wang SW, Cheng W (2012) Dietary administration of the probiotic, *Bacillus subtilis* E20, enhances the growth, innate immune responses, and disease resistance of the grouper, *Epinephelus coioides*. *Fish shellfish immunology* 33: 699-706.
- Hai NV, Buller N, Fotedar R (2010) Effect of Customized Probiotics on the Physiological and Immunological Responses of Juvenile Western King Prawns (*Penaeus latisulcatus* Kishinouye, 1896) Challenged with *Vibrio harveyi*. *Journal of Applied Aquaculture* 22: 321-36.
- Fisheries WA (2002) Code of Practice for the Harvest and the Post Harvest Handling of Live Marron for Food.
- Buller NB (2004) *Bacteria from Fish and Other Aquatic Animals: A Practical Identification Manual*. CABI Publishing, Oxfordshire, UK.
- Fotedar S, Tsvetnenko E, Evans L (2001) Effect of air exposure on the immune system of the rock lobster *Panulirus cygnus*. *Marine and Freshwater Research* 52: 1351-1355.

41. Johansson MW, Keyser P, Sritunyalucksana K, Soderhall K (2000) Crustacean haemocytes and haematopoiesis. *Aquaculture* 191: 45-52.
42. Chisholm JRS, Smith VJ (1995) Comparison of antibacterial activity in the hemocytes of different crustacean species. *Camp Biochem Physiol A Physiol* 110: 39-45.
43. Soderhall K, Cerenius L (1992) Crustacean immunity. *Annu Rev Fish Dis* 2: 3-23.
44. Lorenz S, Francese M, Smith VJ, Ferrero EA (2001) Heavy metals affect the circulating haemocyte number in the shrimp *Palaemon elegans*. *Fish shellfish immunology* 11: 459-472.
45. Tinh NT, Dierckens K, Sorgeloos P, Bossier P (2008) A review of the functionality of probiotics in the larviculture food chain. *Mar. Biotechnol* 10: 1-12.
46. Jussila J, McBride S, Jago J, Evans LH (2001) Hemolymph clotting time as an indicator of stress in western rock lobster (*Panulirus cygnus* George). *Aquaculture* 199: 185-193.
47. Cardinaud M, Offret C, Huchette S, Moraga D, Paillard C (2014) The impacts of handling and air exposure on immune parameters, gene expression, and susceptibility to vibriosis of European abalone *Haliotis tuberculata*. *Fish shellfish immunology* 36: 1-8.
48. Persson M, Cerenius L, Soderhall K (1987) The Influence of Haemocyte Number on The Resistance of The Freshwater Crayfish, *Pasifastacus leniusculus* Dana, to The Parasitic Fungus, *Aphanomyces astacii*. *Journal of fish diseases* 10: 471-477.
49. Gomez GD, Balcazar JL (2008) A review on the interactions between gut microbiota and innate immunity of fish. *FEMS Immunol Med Microbiol* 52:145-154.
50. Gaggia F, Mattarelli P, Biavati B (2010) Review Probiotics and prebiotics in animal feeding for safe food production. *International Journal of Food Microbiology* 141: S15-28.
51. Olsen RE, Sundell K, Mayhew TM, Myklebust R, Ringø E (2005) Acute stress alters intestinal function of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquacult* 250: 480-495.
52. Olsen RE, Sundell K, Hansen T, Hemre GI, Myklebust R, et al. (2002) Acute stress alters the intestinal lining of Atlantic salmon, *Salmo salar* L.: An electron microscopical study *Fish Physiology and Biochemistry* 26: 211-221.
53. Ringo E, Lovmo L, Kristiansen M, Bakken Y, Salinas I, et al. (2010) Lactic acid bacteria vs. pathogens in the gastrointestinal tract of fish: a review. *Aquaculture Research* 41: 451-467.
54. Li CC, Yeh ST, Chen JC (2010) Innate immunity of the white shrimp *Litopenaeus vannamei* weakened by the combination of a *Vibrio alginolyticus* injection and low-salinity stress. *Fish & Shellfish Immunology* 28: 121-127.
55. Van de Braak CB, Botterblom MH, Liu W, Taverne N, Van der Knaap WP, et al. (2002) The role of the haematopoietic tissue in haemocyte production and maturation in the black tiger shrimp (*Penaeus monodon*). *Fish & shellfish immunology* 12: 253-72.
56. Haug T, Kjuul A, Stensvåg K, Sandsdalen E, Styrvold O (2002) Antibacterial activity in four marine crustacean decapods. *Fish and Shellfish Immunology* 12: 371-85.
57. Sun YZ, Yang HL, Ma RL, Lin WY (2010) Probiotic applications of two dominant gut *Bacillus* strains with antagonistic activity improved the growth performance and immune responses of grouper *Epinephelus coioides*. *Fish and Shellfish Immunology* 29: 803-809.
58. Pirarat N, Pinpimai K, Endo M, Katagiri T, Ponpompisit A, et al. (2011) Modulation of intestinal morphology and immunity in Nile tilapia (*Oreochromis niloticus*) by *Lactobacillus rhamnosus* GG. *Research in Veterinary Science* 91: e92-e97.
59. Hai NV, Buller N, Fotedar R (2009b) The use of customised probiotics in the cultivation of western king prawns (*Penaeus latisulcatus* Kishinouye, 1896). *Fish Shellfish Immunology* 27: 100-104.
60. Tseng DY, Ho PL, Huang SY, Cheng SC, Shiu YL, et al. (2009) Enhancement of immunity and disease resistance in the white shrimp, *Litopenaeus vannamei*, by the probiotic, *Bacillus subtilis* E20. *Fish Shellfish Immunology* 26: 339-344.
61. Li K, Zheng T, Tian Y, Xi F, Yuan J, et al. (2007) Beneficial effects of *Bacillus licheniformis* on the intestinal microflora and immunity of the white shrimp, *Litopenaeus vannamei*. *Biotechnology Letters* 29: 525-30.
62. Liu KF, Chiu CH, Shiu YL, Cheng W, Liu CH (2010) Effects of the probiotic, *Bacillus subtilis* E20, on the survival, development, stress tolerance, and immune status of white shrimp, *Litopenaeus vannamei* larvae. *Fish Shellfish Immunology* 28: 837-844.
63. Rengpipat S, Rukpratanporn S, Piyatiratitivorakul S, Menasaveta P (2000) Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiotic bacterium (*Bacillus* S11). *Aquaculture* 191: 271-288.

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