Healthy and wholesome aquaculture is one of the thematic programs aimed to address the role of attaining sustainable aquaculture production through provision of protein needs for the growing human populace. Although research and development efforts on this aspect have already resulted in phenomenal growth of the sector in the last decades or so, there is still a need for further studies on this aspect since we are faced with challenges posed by ecological, economic, and climatic changes among others that we now encounter in our present situation. In order therefore to be assured of significant improvements and production sustainability for future generations, there is a need to consider working more on this healthy and wholesome aquaculture program. This Program aims to contribute to improvement of aquaculture production through innovations in nutrition and feeding and fish health management and in preserving the environmental integrity of aquaculture.

2. PROJECT

2.1 Objectives

(a) Investigate the efficacy of probiotics and rationalize the need and application of diagnostics that will ensure biosecurity within culture systems and keep out exotic pathogens, especially transboundary pathogens.

(b) Promote the wider use of conventional diagnostic as well as new methods especially for newly reported, emerging diseases.

(c) Find effective alternative safe drugs/chemicals (including natural products) to manage aquaculture diseases in lieu of the harmful chemicals and drugs which have been discouraged or banned for use due to quality and safety issues.

(d) Find different sources of fish meal substitutes and develop effective feed management schemes that incorporate sound management.

(e) Develop aquafeeds for selected species at specific growth stages especially for species or stages for which no artificial feed has been formulated.

(f) Promote better understanding of the concept of feed conversion ratio (FCR) and adequate nutrition and efficient feeding practices among fish farmers to promote fish health.

2.2 Outcomes and Expected Outputs

- The application of probiotic Bacillus species in larval rearing of Penaeus is established
- Field- tested immunostimulants for important marine species
- Identified causative agent for the two months mortality syndrome including ways to prevent

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1 Supplemental funds are also provided by other donors such as the Philippines’ Department of Science and Technology, USAID, JIRCAS, United Soybean Board (USA), ACIAR, Japan Fisheries Research Agency
or control disease occurrence/outbreak
- Bacterial and viral vaccines for the grow out of fish and crustaceans developed
- Zoonotic bacteria and parasites from cultured organisms identified
- Health status of wild shrimp stocks (primarily WSSV and other potential pathogens) updated
- Economics of producing mudcrab juveniles for soft-shell crab farming identified
- Fish health specialists from member countries trained on fish disease diagnostics (through Government of Japan trust funds)
- Effective feed management developed and different sources of fish meal substitutes identified
- Efficient diets developed for specific species at specific growth stages
- Netcage culture of freshwater prawn and tilapia in semi-commercial scale demonstrated

2.3 Project Description

The Program is critical in attaining significant improvements and sustaining aquaculture production in the face of many challenges posed by present and future ecological, economic, as well as, climatic changes. The strategies invoked in this Program will concentrate on improving the nutrition and feeding management practices to promote healthy farmed aquatic animals; disease diagnosis, control, monitoring and surveillance of aquatic animals; and environmental integrity, certification, and food safety. The optimization and sustainability of aquaculture production shall be based on Best Management and Good Aquaculture Practices to ensure the least impact on the environment.

3. PROGRESS OF ACTIVITIES IN THE YEAR 2015

Investigation on the efficacy of indigenous probiotics

An indigenous poly-β-hydroxybutyrate (PHB) containing bacterial cultures were incorporated in the feed and given to Shrimp PL1 and shrimp PL30 for 30 days and thereafter respectively challenged with Vibrio campbellii and exposed to a lethal dose of ammonia. Growth and survival were found higher for shrimps receiving the PHB accumulating bacteria as compared to shrimp receiving diets without bacterial additions before the bacterial challenge and ammonia exposure. Shrimp fed PHB accumulating bacteria showed a higher survival compared to non-treated shrimp after the bacterial challenge suggesting an increase in robustness for the shrimp. On the contrary, survival of shrimp exposed to lethal dose of ammonia did not significantly differ between PHB accumulating bacteria-fed shrimp and non-PHB treated shrimp. Current data clearly illustrate that indigenous bacilli capable of accumulating PHB can provide beneficial effects to P. monodon post-larvae during culture in conjunction with growth performance, survival and resistance against pathogenic infection and ammonia stress.

Another indigenous probiont (Bacillus subtilis G100R11) possessing antimicrobial and quorum-sensing activity against V. harveyi, a known pathogen in mudcrab hatcheries, was also isolated. Simulated hatchery runs showed that oral administration of B. subtilis could confer protection in mudcrab as evidenced by the successful production of crab instar with a survival rate of 1%, which is better than the commercial probiotic control with 0% survival. Confirmatory trials are ongoing.

Application of diagnostics that will ensure biosecurity within culture system

Two major oyster-producing areas located in Cabugao Bay, municipality of Panay (Western Visayas, Philippines), were investigated for their sanitary quality. The values obtained were above the microbiological standard set by EU for “Class A” production area. Lower V. parahaemolyticus counts (<3.0 MPN/g) and absence of V. cholerae were noted in all oysters examined. Because oyster production areas located in Barangay Cabugao examined in Year 1 were identified as “Class C” based on EU Shellfish Harvesting Area Classification Criteria, 3 sampling stations in the deeper portion of Cabugao Bay were chosen as potential stations for relaying experiment. Oysters that will be used for
the relaying and depuration experiments will be collected from the aforementioned oyster production areas.

The quantity and composition of *Aeromonas* spp. in the rearing water, sediment, gills and intestines of tilapia *Oreochromis niloticus* collected every 2 weeks from Day 30 to Day 120 after stocking for grow-out culture in 6 earthen brackish water ponds were also investigated. *Aeromonas* spp. are known opportunistic pathogens of tilapia. In terms of composition, a total of 3 species were identified with the dominance of *A. hydrophila* followed by *A. sobria* and *A. salmonicida* in all samples examined. Moreover, since the use of commercial antibiotics has been a common practice in tilapia hatchery and grow-out culture operations as prophylactic or therapeutic agent, the resistance of *Aeromonas* species isolated from the water, sediment, gills and intestines of tilapia to 10 antibiotics commonly used in tilapia culture was also investigated. More than 95% of *Aeromonas* species tested were resistant to amoxicillin followed by erythromycin (92%), neomycin (90%) and oxytetracycline (86%). Only 2% of the strains exhibited resistance to chloramphenicol and none to norfloxacin. The multiple antibiotic resistance indexing of *A. hydrophila* strains tested generally showed that they originated from high-risk sources.

*Promoting the wider use of conventional diagnostic as well as new methods especially for newly reported, emerging diseases*

Diseases affecting farmed and wild mud crab including predisposing, risk and protective factors, and possible prevention and control measures of the diseases were studied. External examination of mudcrab samples collected from the wild and grow-out ponds showed several abnormalities such as short abdominal flap; discoloration of the abdominal region; darker carapace, (necrotized) holes on carapace, claw and legs; presence of lumut/fouling organisms; and presence of barnacles *Balanus* sp. on the carapace. Internally, necrotic gills, black/brown gills, and discolored gonads were observed. Stalked barnacles, *Octolasmis* spp. were observed in the gills. Among the known viral diseases, only white spot syndrome virus (WSSV) and infectious hypodermal and haematopoirtic necrosis virus (IHHNV) were detected with WSSV solely implicated in mortality cases. Identified risk factors for WSSV include low temperature and presence of WSSV positive shrimp. A total of 1450 bacterial isolates were also recovered from the hemolymph. Pathogenicity tests of 20 representative bacteria isolated in pure form suggested that these opportunistic bacteria could only induce mortality when mudcrabs are stressed such as when they are newly molted.

Currently, there is lack of a comprehensive online information resource on shrimp pathogens with a centralized facility to store and archive isolates collected from outbreaks all over the country. To fill this need, a database of aquatic pathogens occurring in the country was recently instituted to ensure sustainability of the Philippine aquaculture industry. Samples from shrimp farms in different regions in the country were collected. A total of 1321 bacterial isolates were collected and biobanked. These samples came from 79 countrywide sites covering regions in Luzon, Visayas, and Mindanao. Of these, 27 sites were found to be disease-positive (WSSV, luminous vibriosis, IHHNV, MBV, and AHPND). To obtain meaningful information from the data generated, database software intended for web-based accessibility by users was developed. The biological data are accompanied by epidemiological information collected on site during sample collection. A Biobank Room dedicated for storing and archiving isolates that is compliant to at least level 2 biosafety and biosecurity standards has been recently established in SEAFDEC/AQD. OPSPIR is scheduled for launching in the last quarter of 2015.

In conjunction with the ongoing study on the development of shrimp pathogen diagnostic tools using nested PCR and lateral flow strip biosensors (LFSB) coupled with a mobile app and cloud-based information management, preliminary data revealed that the detection limit of LFSB kit is only at par with 1-step optimized WSSV PCR method. Refinements are currently being undertaken to improve the detection limit of LFSB kit, i.e. to be at par with the nested-step of the optimized WSSV PCR method.
Evaluating the effectiveness of natural products to manage aquaculture diseases in lieu of harmful chemicals

Infectious diseases of bacterial etiology are some of the constraints limiting mudcrab hatchery production. Lack of alternatives to antibiotics (whose use has been banned or discouraged due to food safety concerns) has hampered the production of adequate seeds to spur production in grow-out facilities. Two plants extracts (Terminalia catappa local name: Talisay and Nephelium lappaceum local name: Rambutan) were tested to have antimicrobial activity in vitro (agar disc diffusion method) and in vivo (oral administration followed by bacterial challenge). Simulated hatchery runs showed that oral administration of T. catappa successfully produced crab instar with a survival rate of 1.2% and 1.8% in trials 1 and 2 comparable to antibiotic control.

Finding different sources of fish meal substitutes and development of effective feed management schemes that incorporate sound management

Experiments revealed that fermented cowpea could replace 40% of fishmeal in milkfish feed. In another study on milkfish, the 50% fish meal replacement with defatted soybean meal and 40% for soy protein concentrate have been tried both in marine cages in Igang, Guimaras Province and brackish water pond in Dumangas, Iloilo. Results showed that the use of this SEAFDEC-USB diet gave a lower FCR (cages-1.6; ponds-1.8) compared to milkfish commercial feed (cages-2.3; ponds-2.6). The SEAFDEC-USB-fed stocks also attained a marketable size of >400 average body weight in less time. Distillers dried grain solubles (DDGS) could replace 45% of soybean meal in milkfish diet. This is being verified in milkfish culture in marine cages in Igang. Moreover, 10-15% inclusion of milkfish hydrolysate in grouper feed has been tested in a small scale experiment. In abalone, studies have shown that the fertilized Ulva pertusa can be considered as fish meal substitutes for juvenile feed, but not Sargassum sp.

Development of aquafeeds for selected species at specific growth stages especially for species or stages for which no artificial feed has been formulated

The study conducted in pompano found that taurine requirement for juveniles was around 1.5% at 46% protein. In another study on abalone, the refinement of diet formulation for the grow-out culture resulted in a water stable and highly digestible feed that significantly improved growth rate and shortened the culture period. The meat quality of the abalone fed the refined formulated feed, as determined through Hedonic scale taste testing, was comparable in terms of color, odor, taste, texture and general acceptability with those abalones fed seaweeds.

Promotion of better understanding of the concept of feed conversion ratio, and adequate nutrition and efficient feeding practices among fish farmers to promote fish health

This is being addressed through various studies. High yield was obtained in rearing of giant freshwater prawn with high substrate area equivalent to 120% of cage bottom and with a stocking density of 5 prawns/m². In another study on milkfish, the use of a feed where fish meal was partially replaced with soybean-based ingredients in grow-out culture both in marine cages and brackish water ponds gave a lower FCR of 2.1 compared to 3.4 FCR for commercial feed. For mud crabs, the spherical and tubular shape feeds facilitated feeding and resulted in higher feed consumption by the crabs. Asceites sp., squid meal, shrimp paste and spoiled fish flesh were also found as good attractants for mud crab feeds. In another experiment, mud crab crablets produced in the nursery can be used for subsequent production of 60-80g juveniles attaining high survival with stocking density of 0.5 individuals/m². Strategies to reduce cannibalism in mud crab nursery include the use of shelters. Frame ribbon nets were found to be the most suitable for the nursery with 60-70% survival at density of 50 individuals/m². For the white shrimp, Penaeus indicus, it was found that a stocking density of 10/m² for 60 -day grow-out culture period was economical.
Demonstration of semi-intensive grow-out culture of grouper using feeds formulated by SEAFDEC/AQD has been done in brackish water pond. Fish fed the SEAFDEC/AQD phase diet showed higher growth and survival rates and lower feed conversion ratio (FCR) compared to fish fed a commercial diet. The same results were obtained in the production of rabbitfish in semi-intensive farming using SEAFDEC/AQD formulated diet in brackish water pond.

4. PROPOSED FUTURE ACTIVITIES FOR THE YEAR 2016

4.1 Planning of the Project Activities

<table>
<thead>
<tr>
<th>Major program activity</th>
<th>Duration</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevention and mitigation of diseases in mud crab culture</td>
<td>2015-2018</td>
<td>New study funded by DOST-PCAARRD.</td>
</tr>
<tr>
<td>Quantitative and qualitative analyses of the bacterial microbiota implicated in persistent, emerging and re-emerging diseases of high-density net-caged tilapia cultured in lakes in the Philippines</td>
<td>2016-2017</td>
<td>Proposal for DOST-PCAARRD funding in preparation</td>
</tr>
<tr>
<td>Refinement of immunization regimen against VNN in high value marine fishes</td>
<td>2015-2019</td>
<td></td>
</tr>
<tr>
<td>Field application of the LFSB kit for WSSV detection</td>
<td>2016-2017</td>
<td>Phase 2 proposal for DOST-PCAARRD funding in preparation</td>
</tr>
<tr>
<td>Establishment of a Philippine shrimp pathogen bio-bank and online biosurveillance information resource (Phase 2)</td>
<td>2016-2017</td>
<td>Phase 2 proposal for DOST-PCAARRD funding in preparation</td>
</tr>
<tr>
<td>Efficacy of indigenous probionts and plant extracts against vibriosis in mud crab hatcheries</td>
<td>2016-2017</td>
<td>New proposal for DOST-PCAARRD funding in preparation</td>
</tr>
<tr>
<td>Demonstration of the viability of <em>P. indicus</em> culture in ponds using low-cost diet</td>
<td>2016-2017</td>
<td>Viability of <em>P. indicus</em> culture in ponds using low cost diet demonstrated</td>
</tr>
<tr>
<td>Refinement of existing grow-out diet Production cost and return analysis.-.</td>
<td>2016</td>
<td>Refinement of existing grow-out diet for <em>P. indicus</em></td>
</tr>
<tr>
<td>Improvement of feed production for grow-out mud crab (on-going)</td>
<td>2016</td>
<td>Feed production for grow-out mud crab improved</td>
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<tr>
<td>Improvement of feed formulation using SEAFDEC USB diet (on-going)</td>
<td>2016</td>
<td>Use of USB SEAFDEC diet in 10 x 10m in floating cages</td>
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<tr>
<td>Field testing of formulated diet containing DDGS as replacement to soybean meal</td>
<td>2016-2017</td>
<td>Use of soybean meal replacement with DDGS in Igang and ponds demonstrated Poultry by-product meal utilized as feed ingredient for milkfish diet</td>
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<tr>
<td>Utilization of poultry by-product meal in milkfish</td>
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<tr>
<td>Demonstration of economics of production using SEAFDEC diet</td>
<td>2016-2017</td>
<td>Economics of production for SEAFDEC diet for grouper demonstrated</td>
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<tr>
<td></td>
<td>2016-2017</td>
<td>Existing for diet formulation for pompano refined Nutritional requirements of pompano identified</td>
</tr>
<tr>
<td>Major program activity</td>
<td>Duration</td>
<td>Remarks</td>
</tr>
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<tr>
<td>Formulation of SEAFDEC diet for pompano</td>
<td></td>
<td>Nutritional requirements of pompano identified</td>
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<tr>
<td></td>
<td></td>
<td>Color quality of final product defined (golden)</td>
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<tr>
<td></td>
<td></td>
<td>(the golden color for the final product as required by consumers will be considered)</td>
</tr>
<tr>
<td>Formulation of SEAFDEC diet for seabass</td>
<td>2016-2017</td>
<td>Amino acid requirement of seabass juveniles identified</td>
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<tr>
<td>Testing of refined grow-out diet</td>
<td>2016-2017</td>
<td>Refined abalone grow-out diet tested in sea cages; Use of <em>Ulva</em> sp. In abalone diet</td>
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<tr>
<td>Feeding management strategies for silver therapon</td>
<td>2016-2017</td>
<td>Effective feeding management strategies for grow out silver therapon identified</td>
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<tr>
<td>Utilization of agricultural by-products as feed ingredient for tilapia</td>
<td>2016-2017</td>
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<tr>
<td>Feed development for <em>C. ignobilis</em></td>
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<tr>
<td>Culture of <em>Gracilaria</em> in ponds</td>
<td>2016-2017</td>
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4.2 Expected Outputs:

**Nutrition and Feed Development**

- Viability of *P. indicus* culture in ponds using low cost diet demonstrated
- Refinement of existing grow-out diet for *P. indicus*
- Feed production for grow-out mud crab improved
- Use of USB SEAFDEC diet in floating cages demonstrated
- Use of soybean meal replacement with DDGS in Igang and ponds demonstrated
- Poultry by-product meal utilized as feed ingredient
- Economics of production for SEAFDEC diet for grouper demonstrated
- Existing for diet formulation for pompano refined
- Nutritional requirements of pompano identified
- Amino acid requirement of seabass juveniles identified
- Refined abalone grow-out diet tested in sea cages
- Use of *Ulva* sp. In abalone diet
- Effective feeding management strategies for grow out silver therapon identified

**Fish Health**

Prevention and mitigation of diseases in mud crab culture

- Microbial load (WSSV, *Vibrio*) in water/soil/mudcrab that would result in infection/mortality quantified using qPCR;
- Environmental parameters that would affect infection/mortality of mud crab identified
- Epidemiological data generated

Quantitative and qualitative analyses of the bacterial microbiota implicated in persistent, emerging and re-emerging diseases of high-density net-caged tilapia cultured in lakes in the Philippines
  - Threshold level to bacterial load in water, sediment, gills and intestines of healthy tilapia reared in floating net cages in lakes established
  - Composition of bacterial microbiota in tilapia and environment generated
  - Zoonotic bacteria identified

Refinement of immunization regimen against VNN in high value marine fishes
  - Immunogenicity of inactivated vaccine to broodfish of high value marine fish verified
  - Kinetics of antibody production
  - Timing of booster vaccination
  - Vertical transmission of the virus aborted

Efficacy of indigenous probionts and plant extracts against vibriosis in mud crab hatcheries
  - Efficacy of the probionts and plant extracts verified

Field application of the LFSB kit for WSSV detection
  - Improved detection limit of LFSB kit, i.e. at par with nested-step of optimized PCR method.

Establishment of a Philippine shrimp pathogen bio-bank and online biosurveillance information resource (Phase 2)
  - Epidemiological information including genetic, phenotypic, ecological, spatial, and temporal characteristics of shrimp and fish pathogens generated uploaded in database