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Association of microsatellite genetic diversity with growth related traits in the base population of African catfish, *Clarias gariepinus*, breeding program

Ind. Aqua. Journal Vol. 10 No. 1, 2015 p: 1-11

Genetic diversity at molecular level has been assumed to correlate with fitness related traits. However, accumulating evidences showed that the nature of that correlation has been variable. This study was aimed to explore the nature and possible mechanisms underlying that correlation by focusing on growth related traits in African catfish, *Clarias gariepinus* using microsatellite molecular markers. Fifty individual African catfish of 110 day-old were sampled and subjected to both morphological and molecular analyses. The standard length, total length and body weight as well as allelic scores of six microsatellite loci were measured on each individual. Indices of microsatellite diversity, namely individual multilocus heterozygosity (MLH) and mean microsatellite allelic distance (mean d2) for individual level, and mean observed heterozygosity (*Ho*) and single-locus heterozygosity (*ho*) for group level, were correlated to those traits using Pearson correlation coefficient (*r*). The Hardy-Weinberg and linkage disequilibrium were carried out to explore the possible mechanisms underlying correlation. The results showed that at individual level the MLH and mean d2 were weakly correlated with standard length, ($r=0.25$, $p<0.05$) and ($r=0.24$, $p<0.05$), respectively. At group level, *Ho* was correlated with both standard and total length ($r=0.99$, $p<0.05$) while *ho* identified two loci, *Cga*03 and *Cga*06 significantly contributed to the correlation. Combining all relevant information, present study identified associative overdominance, both local effect and general effect hypotheses might responsible for the observed correlations.

Keywords: microsatellite genetic diversity, multilocus heterozygosity, microsatellite allelic distance, observed heterozygosity, African catfish, *Clarias gariepinus*

UDC 639.64

Emma Suryati, Rosmiati, Andi Parenrengi, and Andi Tenriulo (*Research and Development Institute for Coastal Aquaculture, Maros*)

In vitro growth rate of *Kappaphycus alvarezii* micropropagule and embryo by enrichment medium with seaweed extract

Ind. Aqua. Journal Vol. 10 No. 1, 2015 p: 13-17

The development of micropropagule and embryo of seaweed depend on nutrient and fertilizer used. Seaweed has been reported contain hormone regulators such as auxine, cytokinine, gibbereline, and various minerals applied in stimulating the growth ocra plant and wheat culture. The objectives of this study were to determine the potential of *Kappaphycus alvarezii* extract and its optimal concentration in accelerating of *Kappaphycus alvarezii* micropropagule and embryo growth. Micropropagule and embryo produced through callus induction were planted into PES 1/20 liquid medium supplemented with seaweed extract at the concentrations of 0 (control), 25, 50, 75, and 100 ¼L in 10 mL of medium. The results showed that medium enrichment with 50 ¼L of seaweed extract had the highest survival rate and growth of thallus. In addition, this concentration was also resulted in a good performance of *K. alvarezii* thallus with the lighter color. The advantage of this study for seaweed cultivation in Indonesia, among others, seaweed can be used as fertilizer, especially in the maintenance of seaweed seed, so that cultivation can be better develop.

Keywords: callus induction, filamentous callus, micropropagule, embryo, *Kappaphycus alvarezii*

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Bambang Iswanto, Imron, Rommy Suprapto, and Huria Marnis (*Research Institute for Fish Breeding, Sukamandi, West Java*)

Embryonic and larval development of a red strain of the Egyptian African catfish (*Clarias gariepinus* Burchell, 1822)

Ind. Aqua. Journal Vol. 10 No. 1, 2015 p: 19-31

Egyptian African catfish is one of several African catfish (*Clarias gariepinus*) strains introduced to Indonesia. Several breeding activities using that strain in Sukamandi resulted in some individuals with redish-yellow body colour (a red strain). Biological informations related to aquacultural aspects of that red strain were still scarce. The present study aimed to elucidate the embryonic and larval developments of the red strain compared to those of the black (normal coloured) one, using the progenies obtained from artificial spawning of each red and black coloured brooders. Results of the present study revealed that embryonic developments of the red and black strains were similar. Their larval development were also quite similar, however, both larvae could be distinguished based on the pigmentation. The red strain larvae were golden-red-yellowish in colour, while the black strain larvae were greyish covered with dense melanophores. Total length of the red strain larvae tended to be lower than those of the black strain.

Keywords: embryo, larva, development, red strain, Egyptian African catfish (*Clarias gariepinus*)

UDC 639.512

Rosmiati, Andi Parenrengi, and Emma Suryati (*Research and Development Institute for Coastal Aquaculture, Maros*)

Marine sponge *Aaptos suberitoides*, it's potential source of natural antibacterial for controlling *Vibrio harveyi* on tiger shrimp (*Penaeus monodon*) culture

Ind. Aqua. Journal Vol. 10 No. 1, 2015 p: 33-40

The study aims toisolate and identify the natural antibacterial compounds potential from *Aaptos suberitoides* for *Vibrio harveyi* control on tiger shrimp (*Penaeus monodon*) culture. The agar diffusion method using paper discs was used to determine the antibacterial activity of extracts (diethyl ether (DEE), butanol (BUE) and aqueous (HOE) and compounds successfully isolated against *Vibrio harveyi*. Findings showed that the antibacterial activity was concentrated in BUE with the inhibition zone of 17.2 ± 0.1 mm. Meanwhile, two other extracts (DEE and HOE) did not exhibit any antibacterial activity against *V. harveyi*. From the active BUE, it was successfully isolated two compounds giving a strong anti-vibrio activity with the inhibition zone of 22 ± 0.1 mm. The IR, ^{1}H, ^{13}C, COSY, HMQC, HMBC, and MS spectrum analysis indicated that both active compounds identified as aaptamine (1) and 9-demethyloxyaaptamine (2). The Study suggested that marine sponge *A. suberitoides* may have potential compounds source for controlling of *V. harveyi* on tiger shrimp culture.

Keywords: *Aaptos suberitoides*, aaptamine, 9-demethylaaptamine, *Vibrio harveyi*, *Penaeus monodon*

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Vitas Atmadi Prakoso, Jojo Subagja, and Young Jin Chang (*Research and Development Institute for Freshwater Aquaculture, Bogor*)

Low water temperature and its effects on stress response of grey mullets *Mugil cephalus* acclimated in freshwater

Ind. Aqua. Journal Vol. 10 No. 1, 2015 p: 41-45

Grey mullet (*Mugil cephalus*) can adapt to saline and freshwater. Although belonged to euryhaline species, but information regarding their stress response on low temperature. Environmental disturbance such as low water temperature may effect their physiological condition. These information can be useful for aquaculture development of this species in freshwater. Therefore, the purpose of study was to investigate the effects of low water temperature on the stress response of grey mullets *Mugil cephalus* acclimated in freshwater. The blood samples of experimental fish (TL: 28.2 ± 1.1 cm, BW: 198.6 ± 25.9 g) were collected during winter season when the water temperature of controlled rearing system was stable at 25°C and uncontrolled rearing system slowly dropped until 12°C. Their stress response on both rearing systems was observed. The results showed that low temperature affected to lower the behavior activity and increase the stress response of grey mullets. The breath frequency of grey mullet regarding their opercular movement at 12°C was 74-97 breath/min., while at 25°C it was 95-114 breath/min. Hematocrit (Ht) and hemoglobin (Hb) were shown higher values of 43.5% and 9.5 g/dL, respectively at 25°C than 12°C (28.0% and 7.1 g/dL, respectively). The tendencies of cortisol and glucose level increased with the lowering temperature, showing higher value of 264.8 ng/mL and 35.5 mg/dL in 12°C than 5.5 ng/mL and 32.7 mg/dL in 25°C. The chemical properties of blood in grey mullets showed same tendency comparing between 12°C and 25°C, there was no significant different between each temperature, except for chloride ($P<0.05$). Chloride value was higher at 25°C, while other blood components such as osmolality, sodium, potassium, and magnesium were showing no significant differences. However, the results showed lower values at 12°C in every blood components, except for chloride. In conclusion, lowering water temperature to 12°C had impact as stressor to the behavior and stress response of grey mullets acclimated in freshwater.

Keywords: temperature, stress response, grey mullet, *Mugil cephalus*, freshwater

UDC 613.27

Asda Laining, Lideman, and Shunsuke Koshio (*Research and Development Institute for Coastal Aquaculture, Maros*)

Interaction between dietary mineral and phytase on biological performances of Japanese flounder, *Paralichthys olivaceus*. Part II. Mineral digestibility and vertebral mineral content

Ind. Aqua. Journal Vol. 10 No. 1, 2015 p: 47-55

Interactive effects between dietary inorganic phosphorus (IP) and phytase (P) on mineral digestibility and vertebral mineral content were investigated in a 30 days feeding trial followed by three weeks digestibility trial with Japanese flounder, *Paralichthys olivaceus*. Eight experimental diets were formulated based on two levels of dietary Ca at 0% and 0.2% combined with either 0% or 0.25% of dietary IP and either with 0 and 2,000 fytase unit (FTU)/kg of phytase in diet, respectively. Result indicated that digestibility of total phosphorus significantly increased by three dietary compounds where the highest was observed in fish fed diet contained 0.25% IP and 2,000 FTU phytase/kg and dietary Ca also included in diet. Significant interaction was only detected between dietary IP and P on this parameter. Supplementation of IP and Ca not phytase significantly improved Ca digestibility. Ca digestibility was very poor when dietary IP and Ca were not supplemented in diet even with when phytase supplemented in diet. There was significant interaction between dietary IP and Ca on Ca digestibility. Vertebral total phosphorus, Ca, and Mg content as well as Ca:P ratio were significantly enhanced by dietary IP and phytase. Dietary Ca has significant effect only on vertebral total phosphorus. Interaction between dietary IP and Ca was significantly found on vertebral Ca content and Ca:P ratio. No significant second-order interaction was observed among the three dietary mineral on overall parameters. Based on total phosphorus and Ca digestibility as well vertebral phosphorus content found in this study, dietary IP, Ca, and phytase at rate of 0.25%, 0.2%, and 2,000 FTU phytase/kg diet, respectively are needed to supplement in diet for a better mineral absorption and bone mineralization.

Keywords: mineral, phytase, digestibility, vertebrae, Japanese flounder

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Asda Laining, Rachman Syah, and Muslimin (*Research and Development Institute for Coastal Aquaculture, Maros*)

Potential use of organic mineral as mineral source for diet of juvenile vannamei shrimp, *Penaeus vannamei*

Ind. Aqua. Journal Vol. 10 No. 1, 2015 p: 57-63

The use of organic mineral (OM) has been recently introduced in aquaculture both as feed supplement and water quality improvement. A feeding experiment was conducted to evaluate a response dose of OM on growth, survival, and mineral content in whole the body and carapace of vannamei shrimp (*Penaeus vannamei*). Three diets were supplemented with different levels of organic mineral at 1 (OM1), 2 (OM2) and 4 (OM4) g/100 g diet. Positive control was a diet without OM inclusion but supplemented with commercial mineral mixture at level of 4 g/100 g diet. Juvenile vannamei shrimp with average initial body weight of 3.5 ± 0.1 g were stocked into 12 tanks with a capacity of 200 L. After 75 days feeding trial, highly significant weight gains was observed in shrimp fed OM at all levels compared to the positive control. However, no significant differences were found among dietary OM groups. The growth response was clearly shown by the same values of SGRs in the three OM supplemented groups (1.1%/d) and only differed significantly from positive control. Increasing of dietary OM significantly improved SR of shrimp where the highest was observed in group fed OM1 and the lowest was in control diet. Effect of dietary OM on whole body Ca and P were quite similar while whole body Ca and P content of OM1 group was slightly high and tended to decrease in two groups with higher level dietary OM. However, no significant differences among the treatment groups. A clear response of supplementing OM in diet was detected on whole body Zn content. Increase of dietary OM resulted in an increase of Zn content in whole body. The effect was clearly shown when diet contained 2 and 4% OM. Carapace Ca content was highly significant when diet contained 2% OM. Similar to whole body Zn content, there was also a linear trend of response dose of dietary OM on carapace Zn content which the highest was found in dietary OM4. Based on growth, survival rate, and Zn content in whole body and carapace, dietary OM at 1 g/100 g diet can replace mineral mixture as mineral source in diet of vannamei shrimp.

Keywords: organic mineral, carapace mineral, growth, vannamei shrimp

UDC 639.3.512

Arief Taslihan, Richard Callinan, Jenny-Ann Torribio, Bambang Sumiarto, and Kamiso Handoyo Nitimulyo (*Main Center for Brackishwater Aquaculture Development, Jepara)*

Cluster model for extensive giant tiger shrimp (*Penaeus monodon* Fab.) to prevent transmission of *white spot syndrome virus*

Ind. Aqua. Journal Vol. 10 No. 1, 2015 p: 65-70

White spot syndrome virus (WSSV) has become epidemic in Indonesia and affecting shrimp aquaculture interm of its production. *White spot syndrome virus* is transmitted from one to other ponds, through crustacean, included planktonic copepode as carrier for WSSV and through water from affected shrimp pond. A cluster model, consist of shrimp grow out ponds surrounded by non-shrimp pond as a role of biosecurity has been developed. The model aimed to prevent *white spot virus* transmission in extensive giant tiger shrimp pond. The study was conducted in two sites at Demak District, Central Java Province. As the treatment, a cluster consist of three shrimp ponds in site I, and two shrimp ponds in site II, each was surrounded by buffer ponds rearing only finfish. As the control, five extensive shrimp grow out ponds in site I and three shrimp grow out ponds in site II, with shrimp pond has neither applied biosecurity nor surrounded by non-shrimp pond as biosecurity as well considered as control ponds. The results found that treatment of cluster shrimp ponds surrounded by non-shrimp ponds could hold shrimp at duration of culture in the grow out pond (DOC) 105.6 ± 4.5 days significantly much longer than that of control that harvested at 60.9 ± 16.0 days due to WSSV outbreak. Survival rate in trial ponds was 77.6 ± 3.6%, significantly higher than that of control at 22.6 ± 15.8%. Shrimp production in treatment ponds has total production of 425.1 ± 146.6 kg/ha significantly higher than that of control that could only produced 54.5 ± 47.6 kg/ha. Implementation of Better Management Practices (BMP) by arranging shrimp ponds in cluster and surrounding by non-shrimp ponds proven effectively prevent WSSV transmission from traditional shrimp ponds in surrounding area.

Keywords: giant tiger prawn, extensive shrimp pond, shrimp pond biosecurity, cluster management shrimp pond

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Muharijadi Atmomarsono and Endang Susianingsih (*Research and Development Institute for Coastal Aquaculture, Maros*)

Effect of different probiotic bacteria on survival rate, growth, and production of whiteleg shrimp in traditional-plus technology
Ind. Aqua. Journal Vol. 10 No. 1, 2015 p: 71-79

Instead of culturing tiger shrimp that is frequently burdened by mass mortality, whiteleg shrimp (*Litopenaeus vannamei*) is then considered as an alternative commodity in Indonesian brackishwater ponds. To prevent the whiteleg shrimp from diseases, different probiotic bacteria were tested in completely randomized design experiment using nine 250-m^2 experimental ponds stocked with 10 PLs of whiteleg shrimp fry/m^2. Three treatments were applied, namely A) alternate use of probiotic bacteria RICA-1, RICA-2, RICA-3; B) alternate use of probiotic bacteria RICA-4, RICA-5, RICA-3, and C) control (without probiotic bacteria); each with three replications. After 11-week application, the results showed that the best survival rate of whiteleg shrimp was achieved by treatment B (98.83%) and the best production was achieved by treatment A (23.52 kg/250 m^2). However, there were no significant differences ($P>0.05$) among the three treatments tested for the shrimp survival rate. The whiteleg shrimp production in treatment A and B were signicantly better ($P<0.05$) than that in treatment C (control). These high shrimp production in treatment A and B were mainly caused by the capability of the applied probiotics in controlling some water quality variables and *Vibrio* numbers.

Keywords: probiotic bacteria, prevention, whiteleg shrimp, production

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Brata Pantjara, Muhammad Nur Syafaat, and Anang Hari Kristanto (*Research and Development Institute for Coastal Aquaculture, Maros*)

Effect of dinamical water quality on shrimp culture in the integrated multitropic aquaculture (IMTA)
Ind. Aqua. Journal Vol. 10 No. 1, 2015 p: 81-90

One of the technologies to improve the productivity of shrimp farms are environmentally friendly shrimp farming multitrophic integrated system known as Integrated Multitrophic Aquaculture (IMTA). The aims of the study were to observe the water quality dinamic on the integrated multitrophic aquaculture and the effect on the production. This study was used four plots which each of pond had 4,000 m^2 in sizing, located in experiment pond, at Research Institute for Coastal Aquaculture, Maros. The main commodities used were tiger and vannamei shrimp. In the A pond was cultivated the tiger prawn with density 12 ind./m^2, in B pond was tiger prawn with density 8 ind./m^2, C pond was vannamei with density 50 ind./m^2, and D pond; was vannamei with density 25 ind./m^2. Other commodities were red tilapia (*Oreochromis niloticus*). Each pond had stocking density 2,400 ind./plot which was divided into 5 hapas having a size of (6 m x 4 m x 1,2 m)/each, mangrove oysters (*Crassostrea iredalei* and *Saccostrea cucullata*) with density 7,500 ind./4,000 m^2 and seaweed (*Gracilaria verrucosa*) of 500 kg/ 4,000 m^2. The Observation of dinamic water quality in the pond was conducted every day i.e. temperature, dissolved oxygen, salinity, and measured pH, while the total organic matter total (TOM), total ammonia nitrogen (TAN), nitrite, nitrate, phosphate were taken every two weeks. The measurements methods of water quality in Laboratory was refered to APHA (2008); and Boyd (1990). During the study, absorption of N and P in seaweed were measured, the obtained plankton was identified and the ratio of carbon and nitrogen during the observation was also calculated. To determine the effect of dominant water quality on production was used the principal component analysis (PCA). The result showed that Water quality during the study was suitable for shrimp and red tilapia culture. The dominant water qualities which effected the shrimp production in IMTA system were total ammonia nitrogen (TAN), oxygen, total organic matter (TOM), phosphate, and salinity. The survival rate of the tiger shrimp in Intensive pond and semi Intensive pond was 50.68% and 59.28% respectively, while the survival rate of the vannamei shrimp in intensive and semi intensive was 71.26% and 68.06% respectively. The highest shrimp production in the cultivation of IMTA reached was 1,488 kg/pond (3,720 kg/ha) in C pond. The lowest Feed conversion ratio (FCR) was obtained in the D pond (0.89). The highest production of red tilapia in IMTA reached in C pond (426.65 kg/pond).

Keywords: water quality, shrimp culture, integrated multitrophic aquaculture

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Bambang Iswanto, Imron, Rommy Suprapto, and Huria Marnis (*Research Institute for Fish Breeding, Sukamandi*)

Morphological characterization of the African catfish (*Clarias gariepinus* Burchell, 1822) strains introduced to Indonesia

Ind. Aqua. Journal Vol. 10 No. 2, 2015 p: 91-99

African catfish (*Clarias gariepinus* Burchell, 1822) has become a great important species in Indonesian aquaculture. Several strains of the African catfish have been introduced to Indonesia for aquaculture purposes, initiated by Dumbo strain from Taiwan in 1985, followed by Paiton strain from Thailand in 1998, then Egypt strain from Egypt in 2007, Masamo strain from Thailand in 2010 and later Kenya strain from Kenya in 2011. Since its introductions, there were no reports yet on their characterization studies. The present study was conducted to morphologically characterize the strains of African catfish introduced to Indonesia, *i.e.* Dumbo, Paiton, Egypt, Masamo, and Kenya strains. Morphometric and meristic data obtained were analyzed using Principal Component Analysis. Results of the morphometric characterization in the present study revealed that Dumbo, Paiton, Masamo, and Kenya strains were indistinguishable, while morphometric characteristic of Egypt strain was more or less different from those of the other strains. On the other hand, results of the meristic characterization suggested that meristic characteristics of all strains of the introduced African catfish were not different from each other. Therefore, to keep the genetic purity of those introduced strains, they should be properly maintained in isolated places.

Keywords: morphometric, meristic, introduced strains, African catfish *Clarias gariepinus*

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Eni Kusrini, Riani Rahmawati, Siti Murniasih, Ruby Vidia Kusumah, and Anjang Bangun Prasetio (*Research and Development Institute for Ornamental Fish, Depok*)

Growth and colour performance of the crossbreed marble strain *Betta splendens* and *Betta imbellis*

Ind. Aqua. Journal Vol. 10 No. 2, 2015 p: 101-112

Betta ornamental fish of marble strain is a strain which is prefered by the hobbyists, because of its unique color, expensive price, and difficult to obtain the fish color strains. Therefore strain marble betta fish is still dominant for export quality for national and international contests. The aim of this study was to observe the growth and color performance of the crossedbreed marble strains of wild betta, *Betta imbellis* crossed with *Betta splendens*. The hybrids of *B. imbellis* (f) x *B. splendens* (m)) (D) had a specific length and weight rate, (1,113 ± 0.04%/day; 2,531 ± 0.14%/day; 26.61 ± 2.02 mm) which was better than that of the hybrids *B. imbellis* (m) x *B. splendens* (f) (C) (1,099 ± 0.02%/day; 2,244 ± 0.13%/day; 25.97 ± 1.33 mm; and 0.0027 ± 0.0003 mm). However, the survival rate of the C hybrids (42.19 ± 11.42%) was higher than those of D (33.67%-17.08%). Based on the obtained results of the color identification the hybrids had as many as 15 characters of color, homozygous strains of marble which became the target of as much as 1%.

Keywords: wild betta, marble, crossbreeding

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Imron, Bambang Iswanto, Huria Marnis, Rommy Suprapto, and Narita Syawalia Ridzwan (*Research Institute for Fish Breeding, Sukamandi*)

The dynamics of genetic variability in three generations of mass selection for fast growth in African catfish, *Clarias gariepinus* assessed by microsatellite markers

Ind. Aqua. Journal Vol. 10 No. 2, 2015 p: 113-123

Selective breeding aiming at improving the performance of economically important traits acts by exploiting population's phenotypic variance. Due to the relationship between phenotype and genotype, selection on phenotype may also affect the profile of genotype. This study was aimed to monitor the impact of three generations of mass selection for fast growth in African catfish, *Clarias gariepinus*, on genetic variability, assessed by microsatellite. A total of 350 fish representing four populations, namely a composite base population (G-0), selected lines of the first generation (G-1) to the third generation (G-3), were sampled. The samples were screened for their genetic diversity using five microsatellite loci1 namely cga01, cga02, cga03, cga05, and cga09. Several genetic parameters including number of allele (A), allelic richness (AR), observed (Ho) and expected (He) heterozygosity, and fixation index (Fis) were evaluated. The results showed that there was a slight increase in the value of diversity indices in the G-1 relative to the G-0 and to the other two generations. Among these parameters, the number of allele seemed to be the most sensitive parameter in detecting genetic changes. All populations experienced heterozygote deficit and positive fixation index indicating the phenomena of inbreeding. Overall, selection for growth for three generations in African catfish breeding program resulted in significant genetic differentiation between populations. Further, the level of genetic differentiation seemed to accumulate along with the number of generaton in breeding program. However, selection did not result in a decline in genetic diversity within population. A relatively short period of the program, along with the use a high number of broodstock (mating pairs) to produce each generation seems to be able to maintain the stability of genetic diversity of the population.

Keywords: genetic changes, *Clarias gariepinus*, microsatellite, mass selection

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Asep Permana, Alimuddin, Wartono Hadie, and Agus Priyadi (*Research Institute for Fish Breeding, Sukamandi*)

Growth response of clownloach (*Chromobotia macracanthus* bleeker 1852) juveniles immersed in water containing recombinant growth hormone

Ind. Aqua. Journal Vol. 10 No. 2, 2015 p: 125-130

The main problem in the culture of clownloach (*Chromobotia macracanthus*) is the slow growth rate, which takes about six months to reach its market size (two inches total body length). Slow growth eventually cause a long production time and increase the production costs. An alternative solution can be proposed in order to enhance the growth is by using recombinant growth hormone. The aim of this study was to determine the immersion dose of recombinant *Epinephelus lanceolatus* growth hormone (rElGH) which can generate the highest growth in clownloach. Larvae at seven day after hatching were hyperosmotic treated with NaCl 2.0% for one minute, then immersed for one hour in water containing 0.3% NaCl, 0.01% bovine serum albumin (BSA), and different doses of rElGH, namely: 0.12 (treatment A), 1.2 (B), 12 (C), and 120 mg/L (D). As control, fish were immersed in water without rElGH and NaCl (control-1), water containing 0.3% NaCl and 0.01% BSA (control-2), and 0.3% NaCl water (control-3). Each treatment was replicated three times. The results showed that clownloach juveniles in treatment B, C, and D had longer total body length ($P<0.05$) than control-1, while fish treatment A was the same as controls. Survival and body weight were similar in all treatments and controls ($P>0.05$). In addition, the percentage of large size juveniles increased approximately 5% in treatment B, almost the same as in the medium size, while the small size were decrease compared to the control-1. Thus, the best immersion dose of rElGH was 1.2 mg/L water.

Keywords: growth hormone, larvae, clownloach, immersion

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Irsyaphiani Insan, Endhay Kusnendar Kontara, and Raden Roro Sri Pudji Sinarni Dewi
Improved production of tiger shrimp (*Penaeus monodon*) through probiotics application
Ind. Aqua. Journal Vol. 10 No. 2, 2015 p: 131-136
The study was carried out in Brebes District, the North coast of Java. Tiger shrimp farming in Indonesia, particularly in this area faced some problems which caused by improper pond preparation, disease, and low seed quality. Probiotic was applied in pond to solve this problem. The aim of this study was to evaluate the production of tiger shrimp in ponds with probiotic applications. Six experimental ponds (each measuring 0.5 ha) were selected of which three were probiotic ponds and three were controlled. Tiger shrimp post-larvae (PL-30) were stocked at density of four shrimps/m^2. Tiger shrimps were reared for three months. Shrimps were fed by commercial pellet. In the first month, shrimp were fed about 7%-5% of the total biomass; in the second months, 3.5%-3% of the total biomass; and in the third month, 2.5%-2% of the total biomass. The treatments in this study were the application of probiotics with concentration of 3 mg/L that were given every five days and control (without probiotics). The results showed the rearing period was 92 ± 6 days in probiotic ponds and 76 ± 16 days in controlled pond. The shrimp in controlled pond should be harvest earlier caused by the high mortality. The average final weight was 16.2 ± 0.7 g in probiotic pond and 15.6 ± 1.9 g in controlled pond. The survival rate was 64.13 ± 12.63% in probiotic pond and 44.17 ± 14.15% in controlled pond. Production was 208 ± 46 kg/pond/cycle in probiotic pond and 123 ± 6 kg/pond/cycle in controlled pond. The result showed that probiotic plays an important role in maintaining water quality parameters and health management as well as increases the survival of shrimp.

Keywords: *Penaeus monodon*, productivity, probiotic

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Tb. Haeru Rahayu and Ketut Sugama
Identification of a local probiotic bacterium using 16s rRNA gene sequence that was used for field trial to enhanced whiteleg shrimp (*Litopenaeus vannamei)* survival
Ind. Aqua. Journal Vol. 10 No. 2, 2015 p: 137-142
The use of local probiotics in the culture of aquatic organisms is increasing with the demand for more environmental-friendly aquaculture practices. The local bacterium isolate considered as a probiotic was added into the water of whiteleg shrimp (*Litopenaeus vannamei*) culture in a field trial. Four rectangular plastic ponds (ca. 20 m x 30 m per pond) were used for 100 days experimentation for six consecutive crops in two years experiment. Survival, harvest size, feed conversion ratio (FCR) and *Vibrio* bacterial count was compared with those of shrimp receiving and none of local isolate. Identification based on 16S rRNA gene sequence shown those isolate was *Bacillus pumilus* strain DURCK14 with 99% homology. Water shrimp pond added a local isolate had significantly higher survival at about 10.0% to 11.7% than shrimp without added the isolate ($p<0.05$), and better FCR, but no significant different in shrimp harvest size. *Vibrio* bacterial was undetected by total plate count. Moreover, it shown better projected yields on an annual basis (three crops per year).

Keywords: shrimp pond, *Litopenaeus vannamei*, local probiotic, *Bacillus pumilus*, *Vibrio*, survival rate, harvest size, FCR

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Vitas Atmadi Prakoso, Ki Tae Kim, Byung Hwa Min, Rudhy Gustiano, and Young Jin Chang (*Institute for Freshwater Aquaculture Research and Development, Bogor*)

Effects of salinity on oxygen consumption and blood properties of young grey mullets *Mugil cephalus*

Ind. Aqua. Journal Vol. 10 No. 2, 2015 p: 143-153

Oxygen consumption (OC) is one of important factors in aquaculture activities, as the oxygen is a vital condition for all the organisms living in the water and having an aerobic type of respiration. OC is the preferred method for measuring and reporting the metabolic rate in fish. The aims of this study were to evaluate the effects of salinity on OC and blood properties of grey mullets. Five experimental groups were conducted to measure OC and blood properties of grey mullets *Mugil cephalus* (BW: 187.9 ± 45.8 g) according to salinity (30®0 psu, 0®30 psu) changes; SDS: fish reared in seawater (SW, 30 psu) directly shifted to SW, SGF: SW fish gradually shifted to freshwater (FW, 0 psu), SDF: SW fish directly shifted to FW, FDF: FW fish directly shifted to FW, and FDS: FW fish directly shifted to SW. The result showed that OC tended to decrease in the groups of SW fish shifted to FW showing 194.5 mg O_2/kg/h at 25°C in SDS to 82.4 mg O_2/kg/h at 15°C in SGF. On the contrary, OC increased in the groups of FW fish shifted to SW showing 80.5 mg O_2/kg/h at 15°C in FDF to 184.0 mg O_2/kg/h at 25°C in FDS. Cortisol levels at the end of experiments were rapidly increased with the lowering salinities in SW fish shifted to FW showing 20.6 ng/mL in SDS to 316.2 ng/mL in SDF, while those were decreased with the increasing salinities in FW fish shifted to SW showing 40.2 ng/mL in FDF to 10.3 ng/mL in FDS. However, glucose levels showed no significant differences among all experimental groups. Based on the information from this study, aquaculture of grey mullet might be applied or developed in freshwater due to its osmotic adaptation ability.

Keywords: grey mullet, salinity, oxygen consumption, blood properties

UDC 639.3.09

Lila Gardenia, Isti Koesharyani, and Tatik Mufidah

Immunogenicity assessment for determination of the most potential isolates of koiherpes virus

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Common carp and koi (*Cyprinus carpio*) are the main consumption fish commodity and ornamental fish in Indonesia. Diseases due to koiherpes virus (KHV) infection had caused a huge loss in both common carp and koi culture industries world-wide. This study was generated to select the most potential candidate from out of three koiherpes virus isolates based on their serological performances for vaccine development to control Koiherpes virus disease in koi. Virus collection, isolation, and propagation in cell culture have been conducted from common and koi samples originated from Banjarmasin, South Kalimantan; Takalar, South Sulawesi; Cirata Lake-Cianjur, West Java; Depok, West Java; Ciseeng, Bogor, West Java; and Purwokerto, Central Java. Determination of the causative agent of the disease was done by PCR (Polymerase Chain Reaction) assay and inoculation of filtrate homogenates from infected fish into cell culture. There are three koiherpes virus isolates (BJMN-1, BJMN-2, and SKBM) which are being successfully isolated, cultured and propagated in Koi Fin (KF-1) cell line. Calculation of tissue culture infection dose from each isolates were $10^{4.55}$ $TCID_{50}$/mL, $10^{4.72}$ $TCID_{50}$/mL, and $10^{3.28}$ $TCID_{50}$/mL. Immunoassay of the three isolates was conducted by Indirect-ELISA method. Polyclonal antibody was made by injecting each isolate to the experiment animals (rat/*Rattus norwegicus* strain Sprague Dawley). It was shown that all of three isolates have high level of immunogenicity, as seen from the absorbance values at 490 nm wavelength. BJMN-1 isolates have a higher potential for it's ability to cross-react with the other isolates and provided the highest absorbance values. In future, all isolates can be used as KHV vaccine candidates to prevent KHV infection.

Keywords: KHV, immunogenic, ELISA assay

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UDC 639.3.09
Ketut Mahardika and Indah Mastuti (*Institute for Mariculture Research and Development, Gondol*)
The effects of crude recombinant viral protein vaccines against grouper sleepy disease iridovirus (GSDIV) on humpback grouper (*Cromileptes altivelis*)
Ind. Aqua. Journal Vol. 10 No. 2, 2015 p: 163-172
Infection of *Megalocytivirus* cause serious mass mortality in marine fish in South East Asian countries. The aim of this study was to produce recombinant of GSDIV capsid protein and its protection to humpback grouper *Cromileptes altivelis* against grouper sleepy disease iridovirus (GSDIV). A major capsid protein (MCP) was selected for use as a crude subunit vaccines. This gene target (MCP) was inserted to the protein expression system vector of pET SUMO and cloned in cells bacteria *Escherichia coli* strain BL-21. The MCP was succeded to be induced using 1 mM of IPTG. Results of protein analysis using MALDI TOF-TOF indicated that the MCP has measurement of 49.566 kDa with PI index of 6.00, and contained 453 amino acids. BLAST homology analysis exhibited that the amino acid sequence of the MCP showed high similarity with MCP of Red Sea Bream Iridovirus (RSIV). *E. coli* expressing MCP protein was inactivated using 0.03% formalin overnight and washed using PBS. The inactivated *E. coli* as a crude subunit vaccine was then injected intramuscularly to humpback grouper juveniles. Subsequently, the juveniles were challenged tested with GSDIV. The juveniles vaccinated with the MCP recombinant bacteria showed significantly higher survival rates than control those vaccinated with PBS. Thus, the MCP fusion protein is considered as a potential vaccine against GSDIV infections in grouper.

Keywords: humpback grouper, GSDIV, protein recombinant, vaccine

UDC 639.3: 577.4
Andi Indra Jaya Asaad, Erna Ratnawati, and Akhmad Mustafa (*Research Institute for Coastal Aquaculture, Maros*)
The use of path analysis in the determination of environmental factor effects on the total production of aquaculture ponds in pasuruan, East Java Province
Ind. Aqua. Journal Vol. 10 No. 2, 2015 p: 173-182
Environmental factors in the form of soil and water quality are the important factors of aquaculture pond productivity, including total production (tiger shrimps, *Penaeus monodon*, and milkfish, *Chanos chanos*) in Pasuruan, East Java Province. The objective of this study was to analyze the direct or indirect effects of soil and water quality on the total production of ponds in Pasuruan using a path analysis application. Data were collected in the pond areas around Pasuruan Regency including Nguling, Lekok, Rejoso, Keraton, and Bangil Sub-Districts as well as Pasuruan City. Soil quality was determined as a free variable and exogen; water quality as mediate variable, suspended, and endogen; as well as milkfish production as suspended variable and endogen. Environmental characteristics were illustrated using descriptive statistics, while environment factor effects on total production were analyzed using path analysis. The results of path analysis show that from the 12 analyzed soil quality variables, only two variables were affected in the total production of pond (tiger shrimps and milkfish) namely: contents of soil organic carbon and soil phosphate. While based on 11 water quality variables, two variables (water salinity and water iron) were affected the total production of ponds in Pasuruan Regency. The direct effects of soil organic carbon and phosphate on the total production were 0.314 and -0.600, respectively. Water salinity and water iron gave direct effects on total production amounting to -0.678 and 0.358 respectively. It is also found that two soil variables which were affected in the total production, did not indicate the effect towards water quality in ponds. Further implication of this research is put more attention for these variables into pond's management in order to gain more production. Technical application could be appropriate for pond preparation and frequently water changing during grow out.

Keywords: path analysis, environment, aquaculture pond, Pasuruan Regency

Author Index

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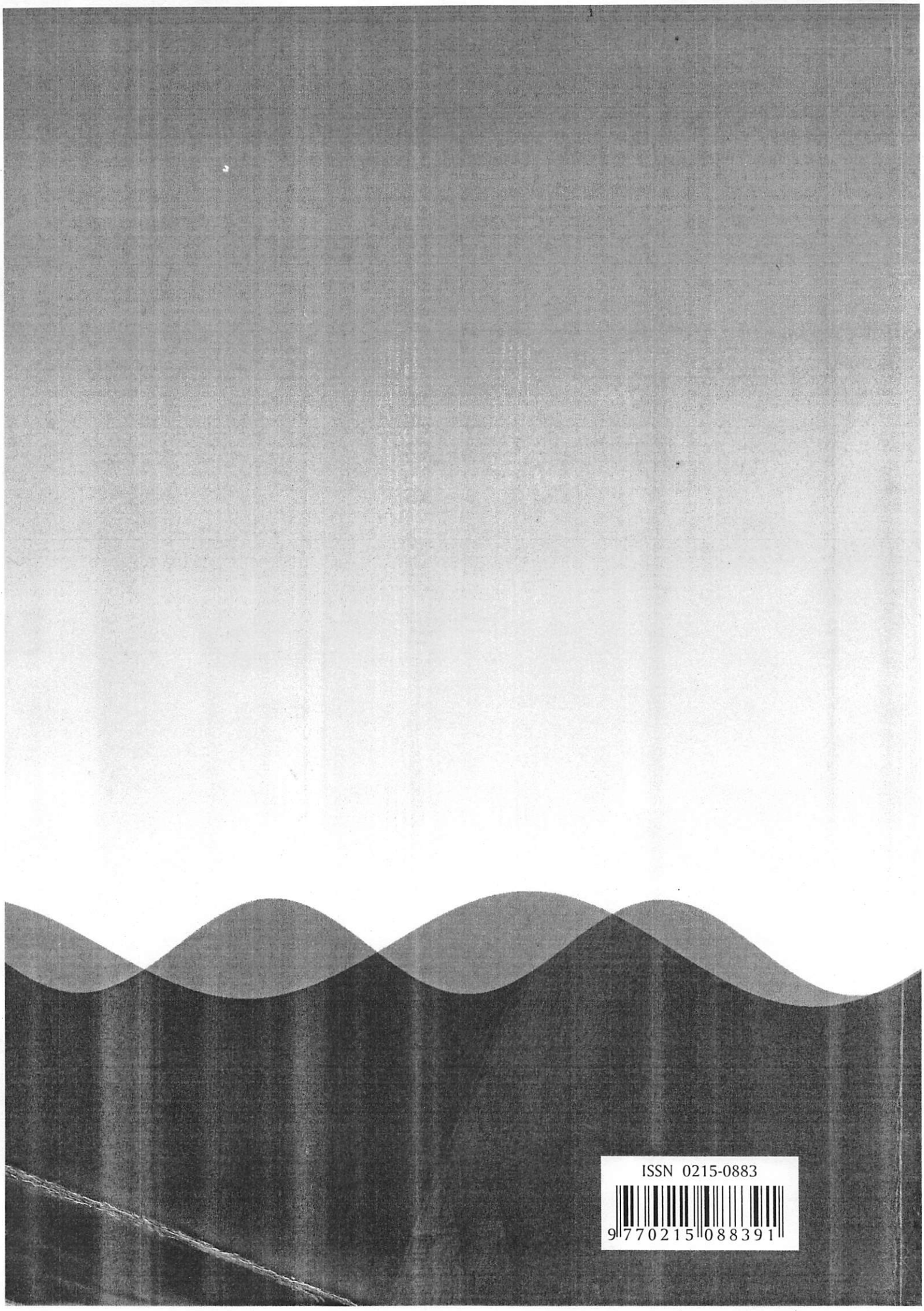
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