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The Impact of Seagrass Degradation on Macrobenthic Fauna in Seagrass Bed at Ao Kham, Trang Province

Duanghwang, P.¹ and Rodcharoen, E.^{1*}

¹ Aquatic Science and Innovative Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla, 90110

*Corresponding author: e-mail eknarin.r@psu.ac.th

Abstract

Seagrasses play a crucial role in coastal ecosystems, providing a habitat for aquatic animals. Currently, seagrasses in Trang province are experiencing degradation due to the impact of natural phenomena and human activities. This study aims to investigate macrobenthic fauna and environmental factors to assess the impact of seagrass degradation on macrobenthic fauna at Ao Kham, Trang province. Samples were collected in December 2023 from three areas: the *Enhalus acoroides* area, where the seagrass status was in degradation (% coverage = 5); the *Halophila ovalis* area, where the seagrass status was moderately fertile (% coverage = 35); and an area without seagrass. The macrobenthic fauna consisted of representatives from 3 phyla and 30 families, with an average abundance of 606 ± 60 individuals/m². The *Halophila ovalis* area exhibited the highest diversity (21 families, $H' = 3.7$), while the area without seagrass displayed greater diversity (18 families, $H' = 3.5$) compared to the *Enhalus acoroides* area (17 families, $H' = 3.4$). Moreover, the area without seagrass had a higher abundance (720 ± 80 individuals/m²) compared to both the *Enhalus acoroides* (581 ± 19 individuals/m²) and *Halophila ovalis* areas (517 ± 125 individuals/m²). Cluster analysis of the macrobenthic fauna revealed similarities in the range of 56.61% – 59.32%. Canonical correspondence analysis indicated that the percentage coverage of *Halophila ovalis* had the greatest effect on the distribution of macrobenthic fauna. It was observed that some families were exclusively found in the seagrass area. Therefore, if seagrasses experience degradation, this will directly affect the macrobenthic fauna.

Keywords: macrobenthic fauna, seagrass degradation, Ao Kham

Microbial Community During Shrimp Culture in Commercial Bacterial Seedling Pond and Heterotrophic Nitrifying Seedling Pond

Sangnoi, Y.^{1*}, Chankaew, S.¹ and O-Thong, S.²

¹ Aquatic Science and Innovative Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla, 90110

² Biofuel and Biocatalysis Innovation Research Unit, Nakhonsawan Campus, Mahidol University, Nakhonsawan 60130

*Corresponding author: yutthapong.s@psu.ac.th

Abstract

Halophilic heterotrophic nitrifying bacteria are vital in shrimp production systems as they convert toxic ammonia and nitrite into less harmful nitrate through nitrification. This process improves water quality, reduces nitrogen toxicity, and supports healthier shrimp growth and productivity. In this study, the water quality and the DNA sequencing was applied to monitor the microbial communities from water sample in the pond which used the heterotrophic nitrifying seedling pond (mixture of *Bacillus oceanisediminis* Ba9 and *B. vietnamensis* SF- 1) and commercial bacterial seedling pond. The result showed that the ammonium quantity was below 1.56 mg-N/L in the heterotrophic nitrifying seedling pond, significantly ($p < 0.05$) lower than the commercial bacterial seedling pond. The amounts of nitrite and nitrate in heterotrophic nitrifying seedling ponds were raised later in the culture process. This might have resulted from the nitrification process in which ammonia was transformed into nitrite and then nitrite into nitrate. The community structure of bacteria in both cultures of experiments, the dominance of the bacterial phylum found Proteobacteria, Bacteroidota, Actinobacteriota, and Firmicutes. Members of the phylum Firmicutes could find *Exiguobacterium* sp. and *Bacillus* sp. as the main groups throughout the culture. *Exiguobacterium* sp. was found in commercial seedling ponds, but *Bacillus* sp. was rarely found. In contrast with the heterotrophic bacterial seedling pond, *Bacillus* sp. was the dominant. Through transforming ammonia to nitrite followed by nitrate through the process of nitrification, it effectively decreases ammonium levels.

Keywords: heterotrophic nitrifying bacteria, *Bacillus* sp., community structure of bacteria

Eutrophication as Marine Pollution on the Songkhla Coast: An Investigation of Phytoplankton Dynamics and Water Quality

Roekngandee, P.¹ and Rodcharoen, E.^{1*}

¹ Aquatic Science and Innovative Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90110

*Corresponding author: e-mail eknarin.r@psu.ac.th

Abstract

Eutrophication is a form of marine pollution caused by excessive nutrient inputs, particularly nitrogen (N) and phosphorus (P), into coastal waters. Currently, there have been reports of a recurring eutrophication phenomenon along the Songkhla coast, especially prevalent after the rainy season. The objective of this study was to investigate the species composition and abundance of phytoplankton and to analyze the correlation between phytoplankton populations and water quality parameters during eutrophication events at the Sathing Phra coast, Songkhla province. Samples were collected from January to March in 2024. The results showed 108 species of phytoplankton, 39 genera and 3 divisions: Bacillariophyta (95 species), Cyanobacteria (1 species), and Myzozoa (12 species). In February, a notable eutrophication event was observed by a bloom of the dinoflagellate *Noctiluca scintillans*, which reached an abundance of $11,996 \pm 1,324$ cells/L, accounting for 34% of the total phytoplankton population. The ratio of dissolved inorganic nitrogen to dissolved inorganic phosphorus (DIN:DIP) was 8.2 during the eutrophication event, indicating nitrogen limiting factor controlling phytoplankton growth in the area. Correlation analysis revealed that *N. scintillans* exhibited a positive relationship with biological oxygen demand (BOD) and a negative relationship with pH. The BOD levels increased, while the pH levels decreased during the *N. scintillans* bloom. These findings highlight the significant impact of nutrient loading on coastal ecosystems and the importance of monitoring and managing nutrient inputs to mitigate eutrophication and its adverse effects on marine life and water quality.

Keywords: Eutrophication, *Noctiluca scintillans*, Nutrient Marine pollution

ขนาดของอาหารเปียกที่เหมาะสมสำหรับปลาหมึกหอม (*Sepioteuthis lessoniana*) ระยะวัยรุ่น

The Suitable Size of Moist Diet for Juvenile Bigfin Reef Squid (*Sepioteuthis lessoniana*)

ณัฐวุฒิ จันทร์เหล็ก¹, จิรพรรณ สัจจารักษ์¹, การุณ ทองประจุกแก้ว^{2*}, นันทน์ นันทพงษ์¹ และ ยูซุรุ อิเคดะ³

Chanlek, N. ¹, Satjarak, J. ¹, Thongprajukaew, K. ^{2*}, Nuntapong, N. ¹ and Ikeda, Y. ³

¹สาขาวิชาวาริชศาสตร์และนวัตกรรมจัดการ คณะทรัพยากรธรรมชาติ มหาวิทยาลัยสงขลานครินทร์ จ.สงขลา 90110

¹ Aquatic Science and Innovative Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla, 90110

² สาขาวิทยาศาสตร์สุขภาพและวิทยาศาสตร์ประยุกต์ คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ จ.สงขลา 90110

² Division of Health and Applied Sciences, Faculty of Science, Prince of Songkla University, Songkhla, 90110

³ ภาควิชาเคมี ชีววิทยา และวิทยาศาสตร์ทางทะเล คณะวิทยาศาสตร์ มหาวิทยาลัยวริวกิว จ.โอกินาวา 903-0213

³ Department of Chemistry, Biology and Marine Science, Faculty of Science, University of the Ryukyus, Okinawa, 903-0213

*Corresponding author: karun.t@psu.ac.th

บทคัดย่อ

ปลาหมึกหอม (*Sepioteuthis Lessoniana*) เป็นหนึ่งในปลาหมึกที่เป็นทรัพยากรประมงที่สำคัญของประเทศไทย ปลาหมึกชนิดนี้มีศักยภาพในการเพาะเลี้ยงเชิงพาณิชย์ เนื่องจากมีอัตราการเจริญเติบโตที่รวดเร็ว และตัวอ่อนแรกฟักมีขนาดใหญ่ อย่างไรก็ตาม การจัดการอาหารในระยะวัยรุ่นยังมีข้อจำกัด เนื่องจากยังไม่มีรายงานการศึกษาเกี่ยวกับอาหารสำเร็จรูป ดังนั้น การศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษาขนาดของอาหารเปียก (ความยาว 4, 5, 6, 7 และ 8 มิลลิเมตร) ที่ผลิตจากเนื้อกุ้งขาว (*Litopenaeus vannamei*) ต่อการเจริญเติบโตและการใช้ประโยชน์จากอาหาร โดยออกแบบการทดลองแบบสุ่มสมบูรณ์ แบ่งชุดการทดลองโดยใช้ลูกปลาหมึกหอมอายุ 10 วัน ชุดการทดลองละ 3 ซ้ำ (น้ำหนักเฉลี่ย 26 ± 1 มิลลิกรัม) ทดลองเป็นเวลา 2 สัปดาห์ เมื่อสิ้นสุดการทดลอง ลูกปลาหมึกที่ได้รับอาหารที่มีความยาว 4 มิลลิเมตร มีน้ำหนักสุดท้ายสูงสุด 423 ± 44 มิลลิกรัม มีน้ำหนักสุทธิเพิ่มขึ้น 387 ± 39 มิลลิกรัม และมีประสิทธิภาพการใช้โปรตีน 1.74 ± 0.39 สูงกว่าชุดการทดลองอื่น ๆ อย่างมีนัยสำคัญ ($P < 0.05$) ในขณะที่อัตราแลกเนื้อ (2.29 ± 0.10) ลดลงอย่างมีนัยสำคัญ ($P < 0.05$) อย่างไรก็ตาม การกินอาหาร (63.0 ± 4.0 มิลลิกรัม อาหาร/วัน) และอัตราการอด (ร้อยละ 33.3 ± 4.2) ไม่มีความแตกต่างกันในทุกชุดการทดลอง ($P > 0.05$) ข้อมูลเหล่านี้แสดงให้เห็นว่าการพัฒนาอาหารสำเร็จรูปที่มีขนาดเหมาะสมจะช่วยเพิ่มการเจริญเติบโตและประสิทธิภาพการให้อาหารของปลาหมึกหอมระยะวัยรุ่นได้

คำสำคัญ: อาหารสำเร็จรูป ระยะวัยรุ่น ขนาดอาหาร ปลาหมึก

Abstract

Bigfin reef squid (*Sepioteuthis lessoniana*) is one of squid that are valuable fishery resources in Thailand. This species is a candidate for culture in commercial scale due to rapid growth rate and large-size hatchling. Nevertheless, there is still a lack of feed management, particularly in the juvenile stage, and their diets have never been studied. Therefore, the suitable size (4-, 5-, 6-, 7-, and 8-mm length) of moist diet was observed on growth performance and feed utilization in the present study. A completely randomized design, comprising triplicate groups of 10-day-old juvenile squids (average weight 26 ± 1 mg), was formulated. The squids were fed by moist diet made from whiteleg shrimp (*Litopenaeus vannamei*) meat for two weeks. At the end of the trial, the squids receiving 4-mm diet length had significantly higher final body weight (423 ± 44 mg), weight gain (387 ± 39 mg), and protein efficiency ratio (1.74 ± 0.39) than the other diet lengths ($P < 0.05$), concurrently with a significantly decreased feed conversion ratio (2.29 ± 0.10). However, feed intake (63.0 ± 4.0 mg diet/day) and survival rate ($33.3 \pm 4.2\%$) did not differ across five alternative treatments ($P > 0.05$). These findings imply that the development of an artificial diet with the proper size could enhance the growth and feed efficiency of juvenile bigfin reef squid.

Keywords: artificial diet, juvenile stage, feed size, squid

Antibacterial Activities of Marine *Streptomyces* (SK3 Strain) Against Pathogenic *Vibrio* spp.

Kaowchomnan, R.¹, Suanyuk, N.² and Pedpradab, P.^{3*}

¹ Kidchakan Supamattaya Aquatic Animal Health Research Center, Aquatic Science and Innovative Management Division, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla, 90112 Thailand

² Department of Marine Science, Faculty of Sciences and Fishery Technology, Rajamangala University of Technology, Trang, 92150 Thailand

³ School of Allied Health Sciences, Walailak University, Nakhonsithammarat, 80160 Thailand

*Corresponding author: ppedpradab@gmail.com

Abstract

Isolation, and identification of a marine *Streptomyces* bacterial (strain SK3) from the marine sediment were performed using the integrated methods. The screening of chemical compounds from the bacteria extract was also experimentally tested against Anti-*Vibrio* spp. (*Vibrio harveyi*, *V. vulnificus* and *V. parahaemolyticus*). The marine sediment bacteria was isolated from an unidentified marine sediment, which the potential isolates of their bacteria was identified based on microscopic observation and 16S-rRNA gene analysis throughout the biochemical characterization. Chemical composition of crude extract was examined by LC-MS analysis. Anti-*Vibrio* activity against was finally determined by dual culture test. Results from this study revealed that the isolated colony of bacteria showed a reddish color colony as well as the fungal-like formation and embedded on media agar plate. It warranted that the isolated bacteria were identified to be a *streptomyces hiroshimensis*. Based on TLC and LC-MS analysis, the strain SK3 could produce chemical constituents with multiple active compounds including fatty acid, alkaloids, glycosides and steroids. Two major groups of active ingredients (phytosphingosine and alvimopan) from *streptomyces hiroshimensis* were detected. Anti-*Vibrio* spp. activity Interestingly, the MIC values varied between 0.101 and 0.310 mg/ml, while the MBC value between was between 0.610 - 1.250 mg/ml. Our observation suggests that the isolated strain bacteria from a marine sediment were potentially could inhibit *Vibrio*'s activity in vitro, which needs pursuing further observation.

Keywords: marine *Streptomyces*, Bioactive compounds, Pathogenic bacteria, 16s rDNA analysis

Prevention of Scuticociliatosis in Cultured Japanese whiting (*Sillago japonica*)

Masaharu, M.^{1*}, Toshiaki, F.², Taishi, S.¹, Ruri, F.¹, Yohei, M.¹, Tohru, M.³, Masato, A.¹, Kengo, O.¹, Toshiaki, I.¹, Shin-Ichi, K.⁴ and Tadashi, I.⁴

¹ Faculty of Life Science and Biotechnology, Fukuyama University, Hiroshima, 729-0292, Japan

² Institute for East China Sea Research, Nagasaki University, Nagasaki, 851-2213, Japan

³ Graduate School of Veterinary Science, Okayama University of Science, Ehime, 794-0085, Japan

⁴ Graduate School of Bioresources, Mie University, Mie, 514-8507, Japan

Abstract

The Japanese whiting (*Sillago japonica*) is distributed across Japan, Korea, China, and Taiwan. In Japan, it has long been cherished as a popular target for surf fishing and is an essential ingredient in tempura. Fukuyama University began the aquaculture of this species in 2015, successfully developing a complete aquaculture system in collaboration with local companies for production and distribution. However, from December 2019, mass mortality occurred among yearlings cultured, and *Miamiensis avidus*, the causative agent of scuticociliatosis, was isolated from the deceased fish. Since then, periodic outbreaks and mortalities have continued, causing significant damage to the aquaculture of this species. Therefore, this study investigated the outbreak conditions in aquaculture tanks.

For the 2018 group, daily mortality and water temperature were recorded from December 14, 2018, to May 7, 2019 (145 days), and for the 2023 group, from May 24, 2023, to June 27, 2024 (401 days). The dead fish were first observed for external and internal symptoms. Then, part of the pectoral fins' gills or diseased skin was excised, cultured in filtered sterile seawater at 20°C for 48 hours, and examined under a microscope to check for *M. avidus* infestation. Histopathological examinations were also conducted. Identification of *M. avidus* was performed through 18S rRNA gene analysis. Based on the results obtained, the daily mortality rate and cumulative mortality rate due to *M. avidus* infection were calculated. Additionally, the muscle, brain, heart, kidney, liver, intestine, and feces of the dead fish were individually sampled for PCR amplification of the gene encoding the ciliary protein using specific primers for *M. avidus* serotype I, to detect the presence of *M. avidus*.

In the 2018 group, the number of mortalities increased 72 days after the lowest annual water temperature of 11°C was recorded, reaching a peak daily mortality rate of 8.3% after 111 days, with a cumulative mortality rate of 99.2%. In the 2023 group, high mortality rates were observed twice: during an approximately one-month period after 62 days of rearing, when the water temperature exceeded 26°C and continued to rise until reaching a peak of 29.6°C, and again during another approximately one-month period after 350 days of rearing, when the water temperature began to exceed 17°C and continued to rise to 22°C. The cumulative mortality rate reached 38.3%. 18S rRNA gene analysis performed on seven dead fish revealed that the gene sequences detected in six specimens were identical and showed 100% homology to the known gene sequence of *M. avidus*. Pathological examinations

showed bleeding and congestion in the exposed connective tissue and muscle layers, with numerous infiltrating cells present. The affected tissue frequently contained oval-shaped parasites, approximately 25 x 20 μm in size, suspected to be scuticociliates, appearing with moderate to high frequency. Additionally, PCR tests conducted on various fish body parts revealed that *M. avidus* was detected in 100% of the affected muscle tissue, with high detection rates also observed in the heart and kidneys, while the brain had the lowest detection rate at 22.2%. Currently, we are using PCR to detect *M. avidus* in seawater and sediments collected from the tank of 2023 group and are comparing the onset of the disease with the dynamics of *M. avidus* in the environment to explore potential control measures.

Effect of Soybean Meal and Protease Enzyme Supplementation in Diets on Growth Performance, Digestibility and Histology of Pacific White Shrimp (*Litopenaeus vannamei*)

Hlongahlee, B. ¹, Tantikitti, C. ^{1*} and Chotikachinda, R. ²

¹ Kidchakan Supamattaya Aquatic Animal Health Research Center, Aquatic Science and Innovative Management Division, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla, 90110 Thailand

² Animal Nutrition and Health, DSM Nutritional Products (Thailand) Ltd., Huai Khwang, Bangkok, 10310 Thailand

*Corresponding author: chutima.t@psu.ac.th

Abstract

An experiment was conducted in a completely randomized design to evaluate increasing substitution levels of soybean meal (SBM) for fish meal (FM) protein at 21% and 26%, and protease enzyme (ProAct) supplementation at 0%, 0.02%, 0.04% and 0.06%, respectively, in Pacific white shrimp (*Litopenaeus vannamei*) diets. Eight experimental diets and a control diet (100% FM) were formulated containing 37% crude protein and 7% crude lipid. Diets were fed to quadruplicate groups of shrimp (3.92±0.33 g initial weight) four times daily to apparent satiation for 8 weeks. The results showed that the levels of SBM protein and protease enzyme supplementation had no effect on survival rate and feed intake ($p>0.05$). The shrimp fed with the control diet had significantly lower final weight and weight gain than those of the groups fed with experimental diets ($p<0.05$). The shrimp fed with diet containing 26% protein from SBM without protease enzyme supplementation had significantly higher average daily growth than those of the control group, but significantly lower than the other feeding groups ($p<0.05$). The specific growth rate was significantly lower in the control shrimp than those of the other groups ($p<0.05$). The feed conversion ratio was significantly higher in the shrimp fed with the control diet as compared to experimental diet fed groups ($p<0.05$). The moisture, crude protein and ash contents of the final shrimp carcass were not affected by the diets ($p>0.05$), but crude lipid was significantly higher than the control group ($p<0.05$). For nutrient utilization, the control group showed significantly lower protein efficiency ratio, protein retention efficiency, lipid efficiency ratio and lipid retention efficiency than those of the other groups ($p<0.05$). In case of digestibility, all experimental diets showed significantly higher digestibility coefficients than that of the control group ($p<0.05$). The histology of shrimp hepatopancreas fed with high level of SBM and supplemented with protease enzyme showed a normal structure. In conclusion, protease enzyme supplementation might help increase SBM substitution for FM up to 26% protein with good growth and good health.

Keywords: soybean meal, protease enzyme, digestibility, histology, Pacific white shrimp

Effect of Fermented Para Rubber Seed Kernel Meal for Fish Meal Substitution on Growth Performance in Diet of Red Claw Crayfish (*Cherax quadricarinatus*)

Chantakam, S.¹, Tantikitti, C.^{1*} and Maneerat, S.²

¹Aquatic Science and Innovative Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla, 90110 Thailand

²Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Songkhla, 90110 Thailand

*Corresponding author: chutima.t@psu.ac.th

Abstract

Red claw crayfish (*Cherax quadricarinatus*) is an interesting economic aquatic animal in many countries. Currently, there is no commercial crayfish diet available, some farmers use Pacific white shrimp diet in their cultivation. Moreover, the high fish meal cost has also increased the cost of diet. Therefore, finding an alternative ingredient to replace fish meal is needed. In this study, para rubber seed kernels were selected because it is an important economic crop. It can produce a lot of seeds per year, but only a small portion is utilized because it contains a high level of cyanide. Processed para rubber seed kernels by boiling help remove the toxic cyanide and subsequent fermentation by *Aspergillus oryzae* and *Saccharomyces cerevisiae*. In this study, five iso-nitrogenous and iso-lipidic diets were formulated using fermented para rubber seed kernel meal (FPRSM) to replace fish meal (FM) at 0%, 25%, 50%, 75%, and 100% diet, respectively. The diets were fed twice daily to red claw crayfish (initial weight: 1.23±0.02 g) in triplicate for 8 weeks. The results showed that the levels of FPRSM substitution had no effect on feed intake ($p>0.05$). The highest final weight (2.78±0.04 g/individual), weight gain (1.55±0.06 g/individual) and specific growth rate (1.36±0.04 %/day), were found in crayfish fed the control diet ($p<0.05$). The crayfish fed 25% FPRSM replacement diet had the highest survival rate of 91.67±14.43 ($p<0.05$). The lowest feed conversion ratio of 3.79±0.94 was found in crayfish fed the control diet ($p<0.05$). In conclusion, the replacement of FM with FPRSM at 25% can be used in the crayfish diet without affecting on growth performance.

Keywords: Fermented para rubber seed kernel meal, Fish meal substitution, Red claw crayfish