# Table of Contents

(Volume 10)

**Introduction** ........................................................................... iv

**Low-Head Saltwater Recirculating Aquaculture Systems Utilized for Juvenile Red Drum Production**
T.J. Pfeiffer, P.S. Wills................................................................. 1

**Effect of Phytase on Growth and Phosphorus Utilization in Japanese Flounder (*Paralichthys olivaceus*)**
P.K. Sarker, H. Hosokawa......................................................... 25

**Growth, Production and Economic Considerations for Commercial Production of Marketable Sizes of Spotted Babylon, *Babylonia areolata*, Using a Pilot Abandoned Marine Shrimp Hatchery and Recirculating Culture System**
N. Chaitanawisuti, S. Kritsanapuntu, W. Santhaweesuk......................... 43

**An Engineering Analysis of the Stoichiometry of Autotrophic, Heterotrophic Bacterial Control of Ammonia-Nitrogen in Zero-Exchange Marine Shrimp Production Systems**
J.M. Ebeling, M.B. Timmons, J.J. Bisogni ....................... 63

**Book Review - Anaesthetic and Sedative Techniques for Aquatic Animals, Third Edition**
Reviewed by S.A. Smith........................................................... 91

**Subscriptions** ................................................................................. 93

**Instructions for Authors** ....................................................... 95
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ABSTRACT
The USDA Agricultural Research Service and the Harbor Branch Oceanographic Institute - Florida Atlantic University (HBOI-FAU) Center for Aquaculture and Stock Enhancement are collaborating to evaluate low-head recirculating aquaculture system (RAS) designs for inland low salinity aquaculture production of marine finfish. As part of this project, the systems described were utilized to intensively produce red drum (*Sciaenops ocellatus*) juveniles that would be part of the Florida Fish and Wildlife Conservation Commission’s (FWC) Saltwater Hatchery Network Initiative. The design and performance data collected from these systems will be utilized in the engineering and determination of design costs for a statewide public-private saltwater hatchery network. The current low-head RAS design that was evaluated for the Phase I (25 mm to 60 mm standard length, SL) through Phase II (60 mm to > 100 mm SL) production of red drum is described and presented in this manuscript along with performance, design, and cost data.
red drum juveniles included a nine-tank system and a ten-tank system. Tank diameters were 1.5 m with a water depth of approximately 1.0 m. Mechanical and biological filtration mechanisms included polygeyser filters, sand filters, moving bed torrus filters, and filter pads. For the Phase II to Phase III (100 to 180 mm SL) production, the red drum juveniles were cultured in four larger-scale replicated RAS low-head systems. Mechanical and biological filtration mechanisms in these systems included moving bed torrus filters, long-flow pathway moving media bed filters, and rotary micron screen drum filters, along with supplemental liquid oxygen addition. The systems presented indicate that intensive inland culture of marine species for commercial aquaculture production or stock enhancement purposes is possible even under the technical constraints of low-head system operation.

**INTRODUCTION**

The red drum, *Sciaenops ocellatus*, (also known as redfish) is an important commercial and recreational fish species in the Gulf of Mexico and the Atlantic Ocean (Sandifer *et al.* 1993). This species is highly valued by both fishermen and consumers. In Florida, sport fishing is a significant tourist activity with a total of 885,000 anglers coming to the state, ranking Florida the number one freshwater and marine fishing destination in the United States (ASA 2006). Its expanded popularity has decreased wild stocks and resulted in restrictions on commercial harvests. During the late 1980s, red drum populations were declining drastically. This decline was halted by eliminating commercial harvesting and applying very restrictive regulations on the recreational harvest (Murphy 2006, FWC 2008). Despite these fishery restrictions, red drum stocks have not recovered to the point where harvest restrictions can be relaxed, an expressed desire of many red drum anglers. Consequently, red drum has become an important species for commercial aquaculture production and for cultivation by state agencies for stock enhancement efforts. To address concerns related to overexploited natural stocks, the Florida Fish and Wildlife Commission (FWC) is working with partners in the public and private sector to develop an expanded ability to produce saltwater fish for stocking. Florida’s marine fishery resources, based on direct recreational fishery expenditures and wholesale value received by the commercial fishery, are valued at close to two billion dollars annually. This resource translates to as much as eight billion dollars annually.
through industry related jobs (Babieri 2008). Because of the strong recreational fisheries interest, the Florida FWC is expected to protect and enhance the marine fishery resources for Florida's residents, tourists, and future generations.

Expansion of Florida’s marine hatchery production will assist conservation and restoration of declining fisheries and stimulate economic growth. FWC has operated a marine hatchery at Port Manatee, FL since 1988. During this time the FWC has raised and released millions of fish, with more than 4 million red drum released statewide. The vision for the FWC Saltwater Hatchery Program is to have a network of marine hatcheries directed towards development of reliable hatchery technology for mass multi-species production of fingerlings using recirculating aquaculture technology, and to integrate fish stocking efforts with habitat enhancement.

As part of this vision, the Center for Aquaculture and Stock Enhancement at Harbor Branch Oceanographic Institute - Florida Atlantic University (HBOI-FAU), in cooperation with the Engineering Unit of the Sustainable Marine Aquaculture Systems project of the USDA Agricultural Research Service, are collaborating with Florida FWC to develop indoor fingerling grow-out systems to intensively produce red drum juveniles. The design and performance data collected from these systems will be utilized to design the recirculating aquaculture systems that FWC plans in the new hatcheries/ecocenters throughout the state of Florida. Establishment and testing of recirculating aquaculture technologies using resources under specific climatic and culture conditions is a significant approach for maximizing water reuse and enhancing marine fingerling production for stock enhancement throughout the state of Florida. The design of the Phase I (25 mm to 60 mm SL juvenile) through Phase II (60 mm to 130 mm SL juvenile) production recirculating aquaculture systems included a nine-tank system and a ten-tank system. For the Phase II to Phase III (130 mm to 180 mm SL juvenile) production cycle, the red drum juveniles were cultured in larger replicated 4-tank RAS low-head systems. System design, operation, and water quality conditions were presented for each of the multi-tank systems.
MATERIALS AND METHODS

Phase I to Phase II Systems

Low-head propeller pump system design (Figure 1)

The system consisted of ten round polyethylene tanks with a diameter of 1.52 m and a depth of 0.86 m for a total tank volume of 1.55 m$^3$ (Model no. TP440A, Aquatic Eco-Systems, Apopka, FL, USA). A 5.1 cm diameter bulk head fitting (Slip x FIPT) was installed in the center of each tank for drainage and connection to an external standpipe (5.1 cm in diameter) that controlled water height in the tank. The ten tanks were set up in two rows of five tanks with the outflow from each external standpipe connected to a 10.2 cm drain manifold for each row of tanks. The water from the drain line for each set of five tanks gravity-flowed into a 0.61 m diameter Wave Vortex filter (265 liters) (W. Lim Corporation, San Diego, CA, USA). Outflow from the two system vortex filters entered a 15.2 cm diameter PVC pipe manifold that drained into a rectangular 3.2 m$^3$ fiberglass sump (1.2 m wide x 2.4 m long x 1.1 m deep).

Water from the sump was returned to the tanks and transferred through the water treatment unit by a 1 hp submersible propeller pump (3 Phase, 220 V, 60 Hz; Model no. 125AB2.75, Tsurumi Manufacturing Co., Ltd, Japan). The low-head propeller pump supplied approximately 910 Lpm at figure 1. The low-head recirculating aquaculture system design for Phase I to II (25 to 60 mm) juvenile red drum production. The system uses a low-head propeller pump for water movement.

1. Sump with propeller pump, float valves for salt and freshwater input, degassing barrels with bioball media, and return lines from the filters and tanks; 2. UV sterilizer; 3. Polygeyser; 4. Moving-media bed biofilter; 5. Swirl separators; and 6. Tanks with external standpipes and return water inflow.
1.8 m of head. The water treatment unit consisted of a open polygeyser filter unit filled with approximately 0.6 m$^3$ of EN plastic floating media (International Filter Solutions, Marion, TX, USA) and a 0.71 m$^3$ moving bead biofilter with floating plastic Kaldness™ K1 structured media (Evolution Aqua, Lancashire, United Kingdom). The polygeyser and LSB filters (Clearwater Low-space Bioreactor, Aquatic Eco-Systems, Apopka, FL, USA) were placed in series and the water flow through the filters was on a continuous loop from and back to the sump at a flow rate of approximately 378.5 Lpm. Return water flow to the tanks was roughly 454 Lpm to provide each tank with a return flow rate of 38-45 Lpm. Thus, the tank turnover time was 0.6 hr or 1.6 tank turnovers per hour. The water flowed through a 10 bulb, 550-watt UV sterilizer (Model no. UV300-2, Aquatic Ecosystems, Apopka, FL, USA) before returning to the tanks. Any excess water flow from the pump (75 to 80 Lpm) flowed to a packed column unit filled with bio-ball polypropylene media material. Adequate oxygen concentration in the tanks was maintained by a continuous flow of liquid oxygen (7 Lpm) into a 0.2 m long, medium pore stone diffuser located in each tank and in the sump.

**System maintenance**

The tank center drains, the swirl separators, the polygeyser filter, and sump were purged to remove any settled solids. On a weekly basis, the center and external standpipes of the tanks were plunged with a scrub brush to remove any accumulated solids and minimize biofilm buildup that would hinder flow out of the tanks and into the drain manifold. The drain line was cleaned on an as needed basis with a rotary spray nozzle and pressure washer unit to minimize biofilm collection. The tank and sump sidewalls were brushed approximately every week. Settled solids accumulated on top of the polygeyser filter were vacuumed off as needed. Total system maintenance took approximately 10-15 hours weekly.

**Hybrid low energy recirculation system design (Figure 2)**

The hybrid system consisted of nine separate modules that incorporated a double drain fish culture tank (Waterline Ltd., Charlottetown, Prince Edward Island, Canada) paired to a torrus moving bed biofilter. The nine fiberglass tanks were 1.5 m in diameter and 0.9 m in depth for a total tank volume of 1.6 m$^3$. The double drain of each tank had a central sump 0.25 m in diameter and 9.1 to 15.2 cm deep. A 2.5 cm diameter drain line with
a ball valve from the center sump was used to purge the accumulated solids from the sump. A slotted 5.1 cm diameter standpipe was located in the center of the tank and the 0.95 cm wide slots were located in the upper portion of the standpipe. The center standpipe fit into a bulkhead at the bottom of the sump that was plumbed to the 7.6 cm diameter approach pipe of the torrus biofilter. Water from the tank was airlifted into the biofilter through the approach pipe by blowing air into the bottom of the pipe via a 1.9 cm diameter opening. Air for the Phase I to Phase II systems was supplied by a 3.5 hp, 3-phase regenerative blower (Model no. HRB-502, Republic Sales, Dallas, TX, USA). The torrus filters were filled with 0.11 m$^3$ of floating plastic Kaldness™ K1 structured media. The airlifted water flow through the filters with gravity flow back to the tanks was maintained at approximately 60 Lpm, providing a turnover time of the tank of roughly 0.44 hours or 27 minutes.

A secondary “polishing loop” was included in the system design for fine particulate filtration, oxygen supplementation, and UV sterilization. A 5.1 cm diameter bulkhead fitting was placed in the tanks for surface water removal and tank water height regulation. Surface water from the tanks drained into a 7.6 cm diameter return manifold, which was plumbed to the rectangular 3.2 m$^3$ fiberglass sump (1.2 m wide x 2.4 m long x 1.1 m deep). Plastic extruded netting, 0.6 to 1.3 cm mesh size, wrapped around the surface drain pipe was used to prevent fish mortalities or media from flowing into the drain manifold. Water from the sump was continuously recirculated through a 0.11 m$^3$ polygeyser filter and a 0.13 m$^2$ sand filter.
Low-Head Saltwater RAS Utilized for Juvenile Red Drum Production

(Model no. TA35, Aquatic Ecosystems, Apopka, FL, USA) via a 0.75 hp centrifugal pump (Model no. JP1, Aquatic Ecosystems, Apopka, FL, USA). Flow through each filter unit was approximately 110 Lpm. Water outflow from the polygeyser filter drops through a degas tower with four distribution plates that had either coarse or medium matala matting on top of the plate to remove fine particles before returning to the sump. Water from the sump passed through an 80-watt UV sterilizer (Model no. AST-80-2, Emperor Aquatics, San Diego, CA, USA), and a 170-liter oxygen injection speece cone (Waterline Ltd., Charlottetown, P.E.I., Canada) on the return to the tanks. Return water flow into each tank was controlled by a 2.5 cm ball valve and was approximately 35 Lpm, providing a turnover of the tank water of 45 minutes.

System maintenance
The polygeyser filter, torrus filters, sand filter, and sump were purged or backwashed daily for removal of accumulated and settled solids. The polygeyser was set to automatically backwash approximately every 4-6 hours by release of air in the air charge chamber of the filter. The matala filter pads were replaced daily with clean rinsed pads. On a weekly basis, the tank center standpipe and drain pipes were plunged with a scrub brush to remove any accumulated solids and minimize pipe biofilm buildup that would hinder flow out of the tanks and into the drain manifold. The drain line was cleaned as needed with a rotary spray nozzle and pressure washer unit. The tank and sump sidewalls were brushed weekly. Total system maintenance took approximately 10-15 hours weekly.

Phase II to Phase III Systems

Low-head propeller pump system design (Figure 3)
The system consisted of four dual drain, round fiberglass tanks 3.1 m in diameter and 1.1 m in depth for a total tank volume of approximately 7.8 m$^3$. A sump 0.38 m in diameter by 0.25 m deep was in the center of each tank. The sump was covered by a 7.6 cm diameter slotted standpipe with a PVC bottom plate allowing approximately a 0.6 cm gap around the sump. The plate also had radial 0.95 cm slots for water and solids to enter. The standpipe was fitted into a 7.6 cm diameter bulkhead at the bottom of the sump that was connected to the approach pipe of the tank side filter and provided mid-column water flow to the side filter. The side
Figure 3. The low-head recirculating aquaculture system design for Phase II to III (60 to >180 mm) juvenile red drum production. A low-head propeller pump for water movement from the sump to the tank and cross-counter flow oxygenator, air lift for water movement between the tank and paired moving bed torrus filters, and air for media movement in the sump.

1. Long flow pathway moving bed reactor with cross-flow oxygenator, float valves, and propeller pump; 2. Incoming salt and freshwater lines with float valves and water meters; 3. UV sterilizer; 4. Torrus filters with 0.37 m$^3$ of MB3 floating plastic media; 5. Three meter diameter tanks w/ center sump and sidebox drain; 6. Diverter box; and 7. Sixty micron screen rotary drum filter.

tank filter was a Wave Vortex filter (0.64 m$^3$; W. Lim Corporation, San Diego, CA, USA) filled with 0.37 m$^3$ of MB3™ floating plastic media (WaterTek MB3 Moving Bed Media, WMT, Baton Rouge, LA, USA). The media was continuously moving by a 0.23 m diameter air disc diffuser located under the media bed. Water in the approach pipe to the filters was airlifted to the surface of the filter by using air that flowed into a 1.9 cm diameter hole located near the bottom of the pipe. Air flow was 0.14 m$^3$/min and provided a water flow through the filters around 130 to 135 Lpm. The air lifted water flow was distributed across the top of the moving media bed of the filter bed and returned back to the tank by gravity. A 7.6 cm diameter PVC pipe with ball valve was plumbed into the tank sump to purge the accumulated solids from the sump. A 0.1 m$^3$ tank sidebox (0.3 m wide x 0.6 m long x 0.6 m deep) with a 7.6 cm diameter opening at the bottom was used for surface water removal from the tanks into a 15.2 cm diameter drain manifold. Surface water out of the sidebox flows to the system drum filter (Model 801, WMT, Baton Rouge, LA, USA). A 40 μm screen was used on the drum filter that was in line before the 11.3 m$^3$ sump (3.0 m x 3.0 m x 1.2 m deep). The custom fabricated sump was divided into five compartments, four of which held media (1.1 m$^3$ of MB3™ media) and were aerated to keep the media moving. A remote
drive regenerative air blower (3 Phase, 220V, 60 Hz; Sweetwater Model no. S51, Aquatic Eco-Systems, Apopka, FL, USA) supplied air to the six medium pore air diffusers located in each of the four compartments to provide an air flow of approximately 25 m³/h for media movement. Water flowed through the four rectangular compartments (0.8 m x 2.4 m) before reaching the last compartment (0.6 m x 3.0 m). A 2 hp propeller pump (3 Phase, 220 V, 60 Hz; Model no. 125AB2.75, Tsurumi Manufacturing Co., Ltd, Japan) returned the water to the tanks. The propeller pump provided approximately 1500 Lpm against a total dynamic head of 2.4 m. An in-line programmable paddle wheel flow meter (Midwest Instrument & Controls Corporation, Rice Lake, WI, USA) monitored the total water flow returning to the tanks. An 8-bulb, 520 watt commercial size UV sterilizer (Model no. COM6520-Std, Emperor Aquatics, Inc., Pottstown, PA, USA) was used to disinfect all the return water to the tanks. A side-stream flow on the return line to the tanks supplied a low-head counter cross-flow (LHCCF) oxygenator with approximately 570-760 Lpm of water and the remaining flow returned to the tanks (760-910 Lpm). Liquid oxygen (LOX) flowed into the LHCCF oxygenator at 5-10 Lpm per unit. Each LHCCF tower was 0.6 m wide x 1.8 m high x 0.6 m deep. Water flow into the top of each tower was controlled by a 7.6 cm ball valve and flowed through four distribution plates before returning to the sump. Each 0.6 m x 0.6 m distribution plate had forty 0.95 cm holes for water dispersion. Liquid oxygen was injected into the tower at the bottom and passed through the plates in a zigzag counter flow pattern to the water flow. LOX volume into the towers was controlled by a flow meter with an adjustable valve. Additional LOX was added to the tanks using 30.5 cm ultra-fine bubble diffusers (Model no. AS303, Aquatic Ecosystems, Apopka, FL, USA) and controlled with 0 to 0.14 m³/min acrylic flow meters.

System maintenance

The side tank moving bed filters were purged daily and the tank sumps were purged twice daily. System drain lines from the side box to the drum filter were cleaned with the pressurized rotary nozzle as needed. Return lines from the side filters were cleaned out twice weekly and more often if the gravity flow back into the tanks was observed to be restricted. Tank side boxes and the drum filter diverter box were scrubbed twice weekly for biofilm removal. Foam buildup in the moving bed biofilter/sump was removed daily. Tank scrubbings were conducted as needed and
coordinated to minimize fish feeding disturbances. Maintenance for all four low-head systems was approximately 10-15 hours weekly.

**Filter performance analysis**

The volumetric total ammonia nitrogen conversion rate (VTR) was used as the principal indicator for evaluation of the filter performance. The VTR was obtained using the following equation:

\[
VTR = K_C \times (\text{TAN}_{\text{IN}} - \text{TAN}_{\text{OUT}}) \times \frac{Q_F}{V_{\text{Media}}}
\]

Where VTR is the g TAN removed per m\(^3\) of filter media per day; \(Q_F\) is the flow rate through the filter (Lpm); \(K_C\) is the unit conversion factor of 1.44; \(\text{TAN}_{\text{IN}}\) and \(\text{TAN}_{\text{OUT}}\) are the influent and effluent total ammonia concentration in mg/L, and \(V_{\text{Media}}\) is the volume of the filter media in m\(^3\).

**Water quality analysis**

Water quality in the systems was monitored daily. Measurements of pH, salinity, and temperature were taken from the sump or diverter boxes of the systems and the dissolved oxygen was measured in each individual tank. Measurements were done with a hand-held meter (YSI 556 MPS, Yellow Springs, OH, USA). Alkalinity of the systems was measured daily with a HACH test kit (Loveland, CO, USA) and maintained within the range of 150-200 mg/L CaCO\(_3\) through the addition of sodium bicarbonate. Filter inlet and outlet water samples were collected for TAN and nitrite determination. Samples were analyzed immediately after collection using the HACH DR-2800 portable spectrophotometer and Method 8038 (Nessler method) for total ammonia determination and Method 8507 (Diazotization method) for nitrite determination. Flow rates were measured with an Ultrasonic Flow meter (PortaFlow SE model, Greyline Instruments, Messena, NY, USA) or by a bucket and stopwatch determination. Total suspended solids analysis of weekly water samples collected from the system sumps or diverter boxes were conducted in triplicate according to Standard Methods (APHA 1998).
RESULTS

Phase I to Phase II Systems

Low-head propeller pump system performance

This system has been in continuous operation with varying numbers and biomass loads since November of 2007. During that time the number of fish in each tank ranged from over 3500 per tank at initial stocking with a mean size of 4.0 g to a minimum of approximately 500 fish per tank with a mean weight of over 60 g. Fish biomass in the tanks ranged from 8.5 kg/m$^3$ at initial stocking to a peak of 62.3 kg/m$^3$ before reducing biomass by grading and transferring the larger fish to other larger systems. Daily feed rates per tank have been greater than 1.0 kg of feed per day (45% CP). Ambient water temperature has been at 24.6°C ± 1.3°C and system salinity, maintained by float valve control, in a range between 10 and 13 ppt. Total ammonia nitrogen ranged between 0.14 and 1.09 mg/L and nitrite-nitrogen was between 0.047 to 0.321 mg/L. System pH and alkalinity ranged between 7.0 and 8.0 and from 14 to 264 mg/L CaCO$_3$, respectively. Alkalinity in the system was maintained through the addition of sodium bicarbonate at approximately 0.25 kg bicarbonate per kg of feed. The average total suspended solids in the system were 8.6 + 4.2 mg/L. The minimum weekly measured TSS concentration value was 2.9 mg/L and the maximum weekly measured value was 18.5 mg/L. The maximum TSS value most likely corresponded to a period when the tanks or drain lines were being cleaned. The average daily system makeup water percentage was 7.1%. Water quality and system metrics are provided in Table 1 for this system during a 117 day production run for the Phase I to Phase II red drum juveniles.

The volumetric nitrification rate (VTR) of the polygeyser filter in the system averaged 77.4 + 37.2 g TAN / m$^3$ media-day during the production trial. The average VTR for the low space moving bed bioreactor was 38.9 + 29.3 g TAN / m$^3$ media-day. Graphs of the volumetric TAN conversion rates for the polygeyser and moving bead filters at varying influent TAN concentrations are presented in Figures 4 and 5, respectively.
This system has been in operation with varying numbers and biomass loads since December of 2007. The number of fish in each tank has been as high as 3700 per tank at initial stocking with a mean size of 4.0 g and has varied depending on the grading needs. Four months after the initial stocking in December of 2007 the average number of fish per tank was approximately 500 with an average individual weight greater than 60 g. Fish biomass in the tanks ranged from 4.3 kg/m$^3$ at initial stocking to a peak of 54.1 kg/m$^3$ before grading and removal of the larger size fish. Daily feed rates per tank have been greater than 1.0 kg of feed per day (45% CP). The ambient water temperature of the system was 23.5°C ± 1.4°C. The range was larger because the system blower for airlift utilization was located outside and the outdoor air temperature had greater fluctuation than the indoor temperature. Salinity was between 6.4 and 14.2 ppt and was controlled by setting the flow of water coming through the make-up water float valves. Total ammonia nitrogen ranged

![Table 1. System and water quality metrics for the ten-tank low-head propeller pump recirculating aquaculture system used to culture red drum juveniles from Phase I to Phase II.](image)

**Hybrid low energy recirculation system performance**

This system has been in operation with varying numbers and biomass loads since December of 2007. The number of fish in each tank has been as high as 3700 per tank at initial stocking with a mean size of 4.0 g and has varied depending on the grading needs. Four months after the initial stocking in December of 2007 the average number of fish per tank was approximately 500 with an average individual weight greater than 60 g. Fish biomass in the tanks ranged from 4.3 kg/m$^3$ at initial stocking to a peak of 54.1 kg/m$^3$ before grading and removal of the larger size fish. Daily feed rates per tank have been greater than 1.0 kg of feed per day (45% CP). The ambient water temperature of the system was 23.5°C ± 1.4°C. The range was larger because the system blower for airlift utilization was located outside and the outdoor air temperature had greater fluctuation than the indoor temperature. Salinity was between 6.4 and 14.2 ppt and was controlled by setting the flow of water coming through the make-up water float valves. Total ammonia nitrogen ranged
Low-Head Saltwater RAS Utilized for Juvenile Red Drum Production

Figure 4. Volumetric nitrification rate (VTR) for a polygeyser filter with 0.57 m$^3$ of EN media utilized in the low-head recirculating aquaculture system for Phase I to Phase II (25 to 60 mm) red drum fingerling production. (N=94)

Figure 5. Volumetric nitrification rate (VTR) for a moving bead biofilter with 0.71 m$^3$ of floating plastic Kaldness K1 media utilized in the low-head recirculating aquaculture system for Phase I to II (25 to 60 mm) red drum fingerling production. (N=94)
Figure 6. Volumetric nitrification rate (VTR) for a polygeyser filter with 0.11 m$^3$ of crimped floating plastic media utilized in the low-energy hybrid recirculating aquaculture system for Phase I to II (25 to 60 mm) red drum fingerling production. (N=56)

Figure 7. Volumetric nitrification rate (VTR) for the moving bed torrus filters with 0.11 m$^3$ of Kaldness media utilized in the low-energy hybrid recirculating aquaculture system for (Phase I to II mm) red drum fingerling production. (N=419)
between 0.2 and 1.4 mg/L and nitrite-nitrogen was in the 0.020 to 1.590 mg/L range. System pH was usually in the 7.1 to 7.7 range as the air input to the torrus filters and degas towers helped keep the CO$_2$ concentration to a minimum in the system. Alkalinity was maintained at approximately 218 mg/L CaCO$_3$ by daily sodium bicarbonate additions. The average total suspended solids in the system was 10.9 ± 4.9 mg/L. The minimum weekly measured TSS concentration value was 5.4 mg/L and the maximum weekly measured value was 24.3 mg/L. The maximum TSS value corresponded to a period when the tanks or drain lines were being cleaned. The average daily system makeup water percentage was 8.3%. The average volumetric nitrification rate for the 0.11 m$^3$ polygeyser filter was 181.1 ± 88.9 g TAN /m$^3$ media-day. The average VTR for the side tank moving bed torrus filters of the system was 56.5 ± 36.7 g TAN / m$^3$ media-day. Graphs of the VTRs for the polygeyser and torrus filters with a range of influent TAN concentrations are presented in Figure 6 and Figure 7, respectively. Metrics for the system and the water quality during the production run are presented in Table 2.

<table>
<thead>
<tr>
<th>System metrics</th>
<th></th>
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<tbody>
<tr>
<td>Maximum fish density in culture tank (kg/m$^3$)</td>
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<tr>
<td>Mean turnover time:</td>
<td></td>
</tr>
<tr>
<td>Culture tank</td>
<td>0.75 h</td>
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<tr>
<td>System volume through filtration units</td>
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<tr>
<td>Mean system exchange rate (% volume per day)</td>
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<tr>
<td>Mean VTR for Polygeyser filter (g TAN / m$^3$-media-day)</td>
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<tr>
<td>Mean VTR for LSB (g TAN/m$^3$ media-day)</td>
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<table>
<thead>
<tr>
<th>Water quality metrics</th>
<th>Avg ± SD</th>
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<tr>
<td>Temperature (°C)</td>
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<tr>
<td>Salinity (ppt)</td>
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<td>Dissolved oxygen (mg/L)</td>
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<tr>
<td>pH</td>
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<tr>
<td>Alkalinity (mg/L CaCO$_3$)</td>
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<tr>
<td>Total Ammonia Nitrogen, TAN (mg/L)</td>
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<tr>
<td>Nitrite Nitrogen, NO$_2$-N (mg/L)</td>
<td>0.415 ± 0.279</td>
</tr>
</tbody>
</table>

Table 2. System and water quality metrics for the nine-tank hybrid low energy recirculation aquaculture system for culturing red drum juveniles from Phase I to Phase II.
Phase II to Phase III Systems

**Low-head propeller pump system performance**

The first of four of these systems (System A) was completed and all four tanks stocked with Phase II red drum juveniles in early March 2008. The second system (System D) was stocked with juveniles in late March 2008 and construction of a third system was completed and stocked with juveniles in June 2008. All three systems were stocked with 250 kg of red drum juveniles, weighing over 100 g each, in each tank. System A had a maximum biomass of 42.6 kg/m$^3$, System C maximum biomass was 50.8 kg/m$^3$, and System D had a maximum biomass of 48.3 kg/m$^3$ during the production runs. The water temperature was between 26 and 29°C for the three systems and was dependent on the outside air blowers that supply air for the moving bead and torrus filters. Salinity of the system was maintained between 11 and 13 ppt. Total ammonia nitrogen was under 1.5 mg/L and nitrite-nitrogen under 0.5 mg/L. System pH was above 7.0 as the air input to the moving beds and torrus filters minimizes CO$_2$ buildup in the system. Alkalinity was maintained over 250 mg/L CaCO$_3$ by daily dosing with sodium bicarbonate. The average total suspended solids concentrations in System A and System D were 5.2 ± 1.8 mg/L and 7.5 ± 1.4 mg/L respectively. The minimum weekly measured TSS concentration value was 1.9 mg/L and the maximum weekly measured value was 11.2 mg/L. The maximum TSS value corresponded to a period when the tanks or drain lines were being cleaned. The percent of daily makeup water for the systems in operation ranged from 7.2 ± 4.3% to 12.1 ± 7.3%. The amount of makeup water was dependent on the number of fish in the system and the number of tanks in each system with fish.

The average volumetric nitrification rate of the long flow pathway moving bead biofilter for System A was 59.5 g TAN/m$^3$ media-day (SD = 23.7) with a maximum rate of 152.9 g TAN/m$^3$ media-day. The average volumetric nitrification rate of the biofilter for System D was 62.2 g TAN/m$^3$ media-day (SD = 22.2) with a maximum rate of 123.7 g TAN/m$^3$ media-day. Volumetric nitrification rates for the side torrus filters on System A and D were 48.2 ± 27.4 and 87.0 ± 22.0 g TAN/m$^3$ media-day, respectively. The maximum VTR for the torrus filters on System A was 103.7 g TAN/m$^3$ media-day with an influent TAN concentration of 0.93 mg/L. The maximum VTR for System D torrus filter was 125.9 g TAN/m$^3$ media-day when the influent TAN concentration was 1.25 mg/L. The torrus filters on both systems showed low VTRs for a range of influent...
<table>
<thead>
<tr>
<th>System metrics</th>
<th>Units</th>
<th>System A</th>
<th>System C</th>
<th>System D</th>
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<tr>
<td>Culture period</td>
<td>days</td>
<td>142</td>
<td>57</td>
<td>127</td>
</tr>
<tr>
<td>Maximum fish density in tank</td>
<td>kg/m³</td>
<td>42.6</td>
<td>50.8</td>
<td>48.3</td>
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<td>Mean turnover time:</td>
<td></td>
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<td></td>
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<tr>
<td>Culture tank</td>
<td>Hour</td>
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<td></td>
<td></td>
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<tr>
<td>System volume through filtration units</td>
<td>Hour</td>
<td></td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Mean system exchange rate</td>
<td>% vol/day</td>
<td>9.8 ± 8.0</td>
<td>7.2 ± 4.3</td>
<td>12.1 ± 7.3</td>
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<tr>
<td>Mean VTR for Long path moving bed biofilter</td>
<td>g TAN/m³-media-d</td>
<td>59.5 ± 23.7</td>
<td>62.2 ± 22.2</td>
<td></td>
</tr>
<tr>
<td>Mean VTR for Torrus moving bed biofilter</td>
<td>g TAN/m³-media-d</td>
<td>48.2 ± 24.4</td>
<td>87.0 ± 22.0</td>
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<table>
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<th>Water quality metrics</th>
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<td>Temperature</td>
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<tr>
<td>Salinity</td>
<td>ppt</td>
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<tr>
<td>Dissolved oxygen</td>
<td>mg/L</td>
</tr>
<tr>
<td>pH</td>
<td></td>
</tr>
<tr>
<td>Alkalinity</td>
<td>mg/L CaCO₃</td>
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<tr>
<td>Total Ammonia Nitrogen, TAN</td>
<td>mg/L</td>
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<tr>
<td>Nitrite Nitrogen, NO₂-N</td>
<td>mg/L</td>
</tr>
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</table>

Table 3. System and water quality metrics for the low–head recirculation aquaculture system for culturing red drum juveniles from Phase II to Phase III.
Figure 8. Volumetric nitrification rate (VTR) for a long flow pathway moving bed biofilter with 4.5 m$^3$ of MB$^3$ floating plastic media at varying TAN influent concentrations that was utilized in a low-head recirculating aquaculture system for Phase II to III (60 to > 180 mm) red drum fingerling production. Gray triangles represent the VTRs for System A biofilter and black diamonds represent the VTRs for System D biofilter.

Figure 9. Volumetric nitrification rate (VTR) for the torrus filters with 0.37 m$^3$ of MB$^3$ floating plastic media utilized in the low-head recirculating aquaculture system for Phase II to III (60 mm to > 180 mm) red drum fingerling production. The volumetric nitrification rates for each torrus filter were determined for varying concentrations of influent total ammonia nitrogen (TAN) concentration. Red triangles represent System A torrus filter VTRs and black diamonds represent System D torrus filter VTRs.
TAN concentrations between 0.40 to 0.93 mg/L. Volumetric nitrification rates for the biofilters in System C were not collected during this trial period. The system and water quality metrics for the three low-head systems in operation are presented in Table 3. A graph of the VTR for various influent TAN concentrations for the long flow pathway moving bead biofilter of System A and D are presented in Figure 8. The VTR for various influent TAN concentrations for the side filters of System A and D are presented in Figure 9.

DISCUSSION

It should be emphasized that the volumetric nitrification rates (VTR) for the biofilters of the various systems presented do not represent complete nitrification rates to nitrate but only ammonia oxidation rates. These rates are useful for designing systems and allow one to determine the effective volume of biofilter media required to maintain a desired ammonia concentration dependent on the remaining engineering and management of the system. The observed VTR numbers for the biofilters were on the low end of the performance range. Biofilter nitrification rates are influenced by the organic load, the dissolved oxygen concentration of the filter water, the influent TAN concentration, temperature, the pH and alkalinity, and the previous history of the biofilm (Zhu and Chen 2001, Ebeling and Wheaton 2006, Michaud et al. 2006, Rusten et al. 2006). The low nitrification performance of the filters however, can be attributed to the salinity of the system. Nitrification rates of biofilters in seawater systems are generally lower than in freshwater systems (Otten and Rossenthal 1979, Nijof and Bovendeur 1990, Rusten et al. 2006).

The measured VTR values for the 0.71 m³ moving bed biofilter of the Phase I-II system were 30-40 percent of values reported for freshwater aquaculture applications. The 0.11 m³ moving bed torrus filters showed slightly better results but the VTRs varied wildly. This variation can be a result of the different feed rates to each of the tanks because of the different stocking densities or fish size, different water flow rates through the filters, and different aeration rates for media movement. In a moving bed reactor the ideal biofilm is thin and evenly spread over the media surface as substrate penetration (ammonia metabolites) is usually less than 100 µm. Thus, aeration of the filter media is of importance to maintain a thin biofilm on the media by shear forces, allowing diffusion transport
of dissolved oxygen and ammonia ions to the nitrifying bacteria layered in the media biofilm. The low nitrification rates observed may have been a result of the aggressive aeration of the media in the moving bed biofilters in addition to the saltwater environment. Aggressive aeration of the media results in over shearing of the media biofilm and limits the protective media surface area required for adequate nitrifying bacterial growth. In the long flow moving bed biofilter the filling fraction of the media in the reactor was 70%. Lower filling fractions in the range of 40 to 60% are recommended and as a result the media may have been substrate (ammonia) limited. Future studies are planned to evaluate an appropriate filling fraction of media in the long flow pathway reactor.

Nitrification of submerged plastic media biofilters for aquaculture applications has been thoroughly studied (Malone et al. 1993, De Los Reyes and Lawson 1996, Malone et al. 1999, Malone and Beecher 2000, Pfeiffer and Malone 2006). However, there is little information regarding nitrification performance of the polygeyser filters, especially in marine or brackish water aquaculture applications. The polygeyser is a submerged plastic media biofilter where the air chamber of the filter allows for frequent media air scrubbing backwashes each day. Increasing the backwashes reduces the back pressure of the filter due to particle entrapment and enhances the nitrification abilities of the filter media (Golz et al. 1999). The 0.57 m³ polygeyser filter was set to backwash every 6 hours and the 0.11 m³ polygeyser was set to backwash every 4 hours. Both units were primarily used as solids capture devices rather than biofiltration units. The polygeyser units provided higher nitrification rates than the moving bed biofilters, but the VTRs were still significantly lower than freshwater submerged media nitrification rates at comparable influent TAN concentrations (Ebeling and Wheaton 2006). The variability in the smaller polygeyser nitrification rate was most likely due to the variability of the sampling event with timing of the filter backwashing.

The Phase I to II systems implemented swirl separators, filter pads, sand filters, in-tank sump purging, and polygeyser filtration in an effort to reduce and minimize the solids load in the systems. The Phase II to III systems utilized 40 micron rotary drum filters as the primary solids removal mechanism. These mechanical methods were employed in an effort to reduce the solids load on the system, but an observed accumulation of fine particles in the system culture water was still
observed, which limited increased density loads and production from these systems. Future design consideration and evaluation should include the use of foam fractionation equipment with ozone to reduce the concentration of fine particles accumulating in the systems.

Our investigation and evaluation is an attempt to determine the usefulness of filtration equipment, both mechanical and biological, for low-head recirculating aquaculture systems used for inland culture of marine species. The systems presented indicate that inland culture of marine species for commercial aquaculture production or stock enhancement purposes is possible even under the technical constraints presented. The percent survival of red drum juveniles from Phase I to III in these systems was over 70%, the food conversion ratio was 1.02, and no diseases were detectable during the production run. The goal is to improve the efficiency of the low-head system design and reduce the energy, water, and supplemental oxygen usage of these systems, while increasing the culture capacity the system can effectively sustain.

ACKNOWLEDGEMENTS

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Effect of Phytase on Growth and Phosphorus Utilization in Japanese Flounder (*Paralichthys olivaceus*)

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Keywords: Japanese flounder, *Paralichthys olivaceus*, phytase, phosphorus, weight gain, feed conversion ratio, digestibility, retention

ABSTRACT

An experiment was conducted to investigate the effect of phytase supplementation on weight gain, phosphorus and protein digestibility and retention in Japanese flounder (*Paralichthys olivaceus*) fed a soybean meal (SBM)-containing diet. Six levels of phytase-supplemented diets containing 0, 150, 300, 450, 900 and 1500 FTU (phytase unit)/100g diet were assigned to triplicate tanks and fed to Japanese flounder (20 fish/tank, initial average weight 151.4 g) for 40 days. The increase of soluble phosphorus and decrease of phytic acid remained relatively constant for all levels receiving the 300 FTU diet and greater. Significantly (*P* < 0.05) greater weight gain and higher feed conversion ratios (FCR) were observed in fish fed diets supplemented at 300 FTU or greater compared to the control (0 FTU) diet. Significantly (*P* < 0.05) improved apparent protein and phosphorus digestibility, as well as serum calcium concentration were found in fish fed the 300 FTU diet. All
diets supplemented at 300 FTU or greater also showed a significantly ($P<0.05$) improved concentration of bone calcium and zinc. The 150 FTU inclusion level showed only better protein and phosphorus retention, bone phosphorus and magnesium than the control (0 FTU) diet. Therefore, this study indicated that supplementation of phytase is effective and that the 300 FTU/100g SBM-containing diet resulted in the maximum release of soluble phosphorus, and as a consequence improved weight gain, FCR, bone minerals, phosphorus and protein digestibility and retention in Japanese flounder.

INTRODUCTION

Soybean meal (SBM) is an important dietary protein source for cultured Japanese flounder (Paralichthys olivaceus). After oil extraction, SBM is used as a protein source in animal feeds due to its well-balanced amino acid profile and relatively high crude protein level (Cheng and Hardy 2003). Soybean meal is widely used in fish nutrition research, as well as for commercial fish feed purposes (Robinson et al. 1985, Hughes 1988, Riche and Brown 1996). However, it is also reported that SBM is rich in phytic acid, which reduces the availability of nutrients such as protein and minerals. Phytic acid has been shown to have a strong anti-nutritive effect due to its tendency to form insoluble complexes with di- and trivalent minerals, rendering these minerals unavailable to fish (Kerovuo 2000). Approximately two-thirds of the total phosphorus (P) in various plant ingredients is present as phytic acid (Ketola 1994). It is known that fish cannot utilize phosphorus bound in the phytic acid complex molecule because fish lack the phytase enzyme needed to hydrolyze bound phytic acid phosphorus to an available form of phosphorus. On the contrary, commercial phytase can hydrolyze the phytate complex bond producing a simpler form of P, thereby increasing the bioavailability of mineral elements (Persson et al. 1998). This holds promise for reduced phosphorus in effluents and reduced phosphorus pollution. Use of commercial phytase has been reported to improve phytate P bioavailability in poultry, swine, and fish, which should decrease the amount of phosphorus excreted (Nelson et al. 1971, Simons et al. 1990, Crowell et al. 1993, Rodehutscord et al. 1995, Schafer et al. 1995, Jackson et al. 1996, Lanari et al. 1998, Veilma et al. 1998, Veilma et al. 2000). The increased nutrient utilization due to the addition of phytase in the diet has yet to be widely studied in fish. Therefore, the present study was conducted to examine the effects
Effects of phytase addition on P utilization in Japanese flounder

of phytase supplementation on weight gain, phosphorus and protein digestibility and retention in Japanese flounder fed a diet containing soybean meal.

MATERIALS AND METHODS

Experimental diets

Six diets with common base ingredients were prepared in this study (Table 1). The six diets were: (1) Control diet with no added phytase (0 FTU/100g diet) and (2-6) five phytase-treated diets incorporating a dry enzyme powder (Natuphos™, BASF Corporation, Parsippany, NJ, USA) at incremental concentrations of 150, 300, 450, 900 and 1500 FTU/100g. The phytase premix was found to contain 10,000 U of phytase activity/g, with 1 FTU (phytase unit) of phytase activity defined as the amount of enzyme that liberates 1 µmol of inorganic phosphorus/min from 5.1 mmol/L of sodium phytate at a pH of 5.5 and a water bath temperature of 37°C (Hughes and Soares 1998). The diet materials were mixed in a Hobart mixer, moistened and then formed into granules 7.5 mm in diameter using a laboratory pellet mill. A portion of each of the six experimental diets was removed for chemical analysis which included protein, lipid, ash, total P, soluble P, and phytic acid content analysis. Three storage times were maintained (0 h, 40 h, and 60 h) at room temperature (26.4°C) to determine the optimal time for releasing the soluble P. Finally, the pellets were stored at –20°C until use.

Experimental design, fish rearing system, and feeding

Japanese flounder with a mean weight 151.4 ± 0.1g that had been maintained and acclimated at Akaoka Fish Center, Kochi University for 1 year, were used in this study. Three hundred sixty fish were randomly distributed in 18 flow-through fiber reinforced plastic (FRP) tanks (800 L holding capacity of 3 tanks/diet and 20 fish/tank). Filtered water (32 ppt salinity) was supplied to each tank at a flow rate of 10 L/min in a flow-through system located at this facility. An adequate level of oxygen in each system was maintained through artificial aeration throughout the experimental period. Even though this experiment was conducted in flow-through systems, the overall study is also relevant to recirculating aquaculture systems. Before the commencement of the feeding study, fish were gradually acclimated over three weeks to experimental conditions.
Table 1. Composition of experimental phytase-supplemented diets for Japanese flounder, Paralichthys olivaceus.

<table>
<thead>
<tr>
<th>Phytase (FTU/100g diet)</th>
<th>0</th>
<th>150</th>
<th>300</th>
<th>450</th>
<th>900</th>
<th>1500</th>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
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<tr>
<td>Brownfish meal</td>
<td>40</td>
<td>40</td>
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<td>40</td>
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<td>Soybean meal</td>
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<td>29</td>
<td>29</td>
<td>29</td>
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<td>29</td>
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<tr>
<td>Krill meal</td>
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<td>Blood meal</td>
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<td>5</td>
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<td>5</td>
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<tr>
<td>L-Lysine</td>
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<tr>
<td>Phytase</td>
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<td>0.03</td>
<td>0.045</td>
<td>0.09</td>
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<tr>
<td>α Cellulose</td>
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<td>2.37</td>
<td>2.355</td>
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Proximate composition (%)

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<td>6.8</td>
<td>6.7</td>
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<tr>
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<td>11.7</td>
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<td>8.7</td>
<td>8.9</td>
<td>9.3</td>
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<tr>
<td>Crude ash</td>
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<td>338</td>
<td>342</td>
<td>339</td>
<td>340</td>
<td>343</td>
</tr>
<tr>
<td>Energy (kcal/100g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>

1 Syowa Sangyo. Co. Ltd (Tokyo, Japan).
2 Contains (mg/100g): Thiamine HCl 2.2; Riboflavin 2.2; Pyrodoxin HCl 2.3; Nicotinic acid 9.6; Ca-pantothenate 7.2; Inositol 60.0; Biotin 0.14; Folic acid 2.4; Cholin chloride 300.0; Cyanocobalamin 0.04; Ascorbic acid 21.6; Vitamin A palmitate 1.1; α- Tocopherol 20; α- Cellulose 1571.22
3 Contains (mg/100g): KH₂PO₄ 412; Ca (H₂PO₄).2H₂O 618; Ca-lactate 282; Fe-fumaric acid 160; Trace minerals 50 α-Cellulose 478
and fed with a commercial diet (Marubeni Inc., Tokyo, Japan). Fish were held on a 12-h dark/12-h light photoperiod. Dissolved oxygen levels and water temperature were monitored daily with ranges of 5.20 - 6.89 mg/l and 20.5 - 24.3°C, respectively (TOA Electronic Ltd., Kobe, Japan). Fish were hand-fed to apparent satiation twice a day for a period of 40 days, and feed consumption was recorded at each feeding.

**Biological sampling and tissue collection**

Prior to the feeding trial, 10 fish were taken from the common tank, blood was collected for analyzing plasma mineral concentrations, and the fish humanely euthanized with MS-22 (Sigma Chemical Co., St. Louis, MO, USA). Of the 10 fish, five were finely ground for proximate analysis, while the remaining five fish were individually dissected to collect vertebrae for mineral concentration estimation. At the beginning of the experiment and at 10-day intervals during the trial, fish were counted and bulk-weighed after a 12-h fast. At the end of the feeding trial, after counting and weighing the fish, five fish were randomly selected from two tanks of fish fed each of the experimental diets for vertebrae collection and another five fish were collected for carcass analysis as previously described. The collected samples were immediately transferred to a freezer (-40°C) until analysis. After collecting vertebrae, soft tissue was carefully removed from the vertebral axis and the bones oven-dried at 110°C for 24 h, finely ground, and analyzed for mineral content.

**Analytical methods**

Blood was withdrawn through the caudal tail vessel using 2.5 ml heparinized syringes, then centrifuged for 5 min at 13750 x g. The resultant plasma was used to measure mineral contents of each fish. For proximate analysis and determination of whole-body P concentrations, fish were pooled and homogenized in a mincing machine. The phosphorus retention in flounder after 8 weeks of feeding was calculated as described previously (Papatryphon et al. 1999). The proximate analyses were determined according to the AOAC method (AOAC 1995). To determine phosphate (ascorbic acid method), ammonium molybdate and potassium antimonyl tartrate were made to react in an acid medium with orthophosphate to form a heteropoly acid- phosphomolybdic acid which was then reduced to intensely colored molybdenum blue by ascorbic acid. The plasma was analyzed for P (ammonium molybdate method)
and Zn (2-(5-bromo-2-pyridylazo)-5-(N-propyl—3-sulfopropylamino)phenol method) using a commercial diagnostic kit (Wako Pure Chemical Industries, Osaka, Japan). Chromic oxide was determined following the method of Furukawa and Tsukahara (1966). Diets were analyzed for phytic acid (myo-inositol 1,2,3,5/6- hexakis dihydrogen phosphate) by the method of Graf and Dintzis (1982).

**Digestibility study**

The effect of phytase on the digestibility of P and protein in SBM-containing diets was measured by an indirect method with chromic oxide ($\text{Cr}_2\text{O}_3$) as an inert reference substance. The use of an inert indicator, which passed unaffected by digestion through the alimentary tract, provided a convenient method of measuring digestibility. This method has been successfully applied to fish using $\text{Cr}_2\text{O}_3$ as the indicator (Furukawa and Tsukahara 1966). The marker (0.5%) was added to the feed for the digestibility study. After 40 days of the feeding trial, the remaining experimental fish were fed each diet to satiation at 8:00 PM and fecal samples were obtained by gentle manual stripping of the lower abdomen at 8:00 AM the next morning as recommended by Austreng (1978). Fecal samples were collected once a day for 2 weeks and were pooled for subsequent analyses and stored at −30°C. The diets and fecal samples were freeze-dried and were subjected to analyses for total P, crude protein and $\text{Cr}_2\text{O}_3$ content.

**Statistics**

Results were subjected to a one-way ANOVA (Statistica Release 6.0 StatSoft, Inc., Tulsa, OK, USA). Differences among treatments were compared by Tukey’s HSD (Honestly Significant Difference). Means were considered significant at $P<0.05$.

**RESULTS**

**Soluble P release pattern**

Comparatively little soluble P was released after 0-h storage time (Figure 1). The release of soluble P increased after 20-h storage time at room temperature (26.4°C), however, no additional release was observed after 40-h storage at room temperature. Moreover, the diets containing 300, 450, 900, and 1500 FTU released similar amounts of soluble P after
Effects of phytase addition on P utilization in Japanese flounder

Figure 1. Soluble P release patterns in control (0 FTU/100g) and phytase-supplemented diets (150, 300, 450, 900 and 1500 FTU/100g) held for various times (0 h, 20 h and 40 h) post-preparation at 26.4°C.

Figure 2. Phytic acid content in control (0 FTU/100g) and phytase-supplemented diets (150, 300, 450, 900 and 1500 FTU/100g).

storage at room temperature. However, the diets containing 0 and 150 FTU released significantly lower amounts of soluble P than the diets containing 300 FTUs or more.

The phytic acid content of the experimental diets varied between 3.1 and 5.8 mg/g, with the diet containing 0 FTU having the highest amount (5.8 mg/g) (Figure 2). The phytic acid content was reduced in the diets of 300, 450, 900 and 1500 FTU, where the values were 3.6, 3.8, 3.5 and 3.1 mg/g, respectively.
**Fish growth and feed performance**

Weight gain in fish fed the 150 FTU diet was not significantly different from the control diet (0 FTU). However, significantly greater weight gain was observed in the 300 FTU group and higher levels of phytase supplemented diets (Table 2). A significantly higher FCR (poorer conversion) was observed in the control dietary group (0 FTU) than in all other dietary treatments. Fish receiving a diet containing 300 FTU or greater exhibited a significantly better FCR than either the control (0 FTU) diet or the 150 FTU treatment. The 150 FTU treatment had a significantly better FCR than the control (0 FTU) diet, but was significantly lower than the 300 FTU or greater treatments.

**Proximate composition and phosphorus content in fish**

Moisture content in all the experimental groups was lower than in the initial fish, resulting in higher crude protein and crude lipid content (Table 3). However, at the end of the study, differences in proximate composition of the fish were not significant among the dietary treatments. Although the dietary treatments did not have a significant influence on the total P content, all groups receiving phytase in the diets showed higher total phosphorus concentrations than either the initial group or the control (0 FTU) group.

**Vertebrae mineral**

Although only the 900 FTU diet resulted in a significantly higher concentration of vertebrae P, all phytase-treated diets showed a higher vertebrae P concentration compared to the 0 FTU diet (Table 4). The vertebrae calcium (Ca) and zinc (Zn) were significantly higher for all diets containing phytase compared to the 0 FTU diet. The vertebrae Ca and Zn concentration were not significantly higher for the groups receiving 300 to 1500 FTU compared to the 150 FTU and 0 FTU groups, and there were no differences shown between the 300 and 1500 FTU groups. Vertebræ magnesium (Mg) concentrations were significantly higher for all phytase containing diets compared to the 0 FTU dietary group.
Table 2. Performance of Japanese flounder, Paralichthys olivaceus, fed six experimental phytase-supplemented diets.*

<table>
<thead>
<tr>
<th>Phytase (FTU/100g diet)</th>
<th>0</th>
<th>150</th>
<th>300</th>
<th>450</th>
<th>900</th>
<th>1500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary group</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Initial mean body wt. (g)</td>
<td>151.4 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>151.5 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>150.7 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>150.8 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>151.2 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>151.5 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final mean body wt. (g)</td>
<td>209.56 ± 9.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>220.0 ± 0.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>235.5 ± 1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>233.3 ± 2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>232.3 ± 5.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>237.0 ± 3.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean body wt. gain (%)</td>
<td>38.9 ± 5.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.2 ± 10.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>56.2 ± 6.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.7 ± 3.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.6 ± 12.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.4 ± 20.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>FCR</td>
<td>2.4 ± 0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.8 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Daily feeding rate (%)</td>
<td>1.72 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.73 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.69 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.74 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.71 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Feed efficiency (%)</td>
<td>147.1 ± 49.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>182.6 ± 41.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>198.8 ± 62.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>196.9 ± 20.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>197.7 ± 59.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>213.4 ± 74.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>100.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>97.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>97.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Values are means of triplicate tanks. Different superscripts within the same row are significantly different, P<0.05

Table 3. Whole body proximate composition and phosphorus content in Japanese flounder, Paralichthys olivaceus, fed in six experimental phytase-supplemented diets.*

<table>
<thead>
<tr>
<th>Phytase (FTU/100g diet)</th>
<th>Initial</th>
<th>0</th>
<th>150</th>
<th>300</th>
<th>450</th>
<th>900</th>
<th>1500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary group</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>75.2</td>
<td>74.2 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.3 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.5 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.8 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.9 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.6 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>16.7</td>
<td>17.8 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.5 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.6 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.3 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.5 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.4 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude lipid (%)</td>
<td>2.5</td>
<td>2.7 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.3 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude ash (%)</td>
<td>4.3</td>
<td>3.6 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.6 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.3 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total phosphorus (mg/g)</td>
<td>3.9</td>
<td>6.4 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.1 ± 4.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.3 ± 7.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.6 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.0 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.2 ± 4.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Mean values with standard deviation from triplicate tanks (except for initial sample) and pooled five fish for each. Different superscripts within the same row are significantly different, P<0.05
Serum mineral
The 300 FTU group showed a significantly improved serum Ca concentration over the control (0 FTU) dietary group (Table 5). There were no significant differences in serum P or Zn among the various dietary groups.

Apparent P and protein digestibility
The apparent P and protein digestibility of fish fed the different diets are shown in Table 6. All phytase-treated diets had significantly higher P digestibility values compared to the control (0 FTU) diet. Only the 300 FTU treatment showed significantly higher apparent protein digestibility than the 0 FTU treatment.

Apparent P and protein retention
Apparent retention of P and protein in Japanese flounder fed the different experimental diets are given in Table 7. Protein retention was significantly improved in fish fed any of the phytase-supplemented diets, and P retention was significantly higher in fish fed diets supplemented at the 150, 300, and 450 FTU levels compared to the control (0 FTU) level.

DISCUSSION
Soluble P release patterns showed that by using microbial phytase at the level of 300 FTU, the SBM-containing diets with a high content of phytic acid could be optimized after 20 h of storage time at room temperature (26.4°C). Several investigators have also reported that a significant amount of dephosphorylation of phytate occurred in a phytase-supplemented diet during diet preparation (Scafer et al. 1995, Branden and Carter 1999, Yan et al. 2002). The advantageous effects of phytase in SBM-containing diets were confirmed in this feeding experiment with improved growth performance, feed conversion, feed efficiency, P and protein digestibility and mineral utilization by Japanese flounder. It has been reported that dietary phytase improved growth and overall performance while reducing P excretion in rainbow trout, Oncorhynchus mykiss (Rodehutscord and Pfeiffer 1995, Sugiura et al. 2001), carp, Cyprinus carpio (Schafer et al. 1995) and striped bass, Morone saxatilis (Papatryphon et al. 1999). This study demonstrated that the addition of 150 FTU or more in SBM-containing diets improved the FCR of Japanese flounder compared to a
Table 4. Mineral contents of vertebrae of Japanese flounder, Paralichthys olivaceus, fed in six experimental phytase-supplemented diets.*

<table>
<thead>
<tr>
<th>Phytase (FTU/100g diet)</th>
<th>Initial</th>
<th>0</th>
<th>150</th>
<th>300</th>
<th>450</th>
<th>900</th>
<th>1500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorus (mg/g)</td>
<td>Initial</td>
<td>117.0</td>
<td>126.83 ± 3.0a</td>
<td>129.0 ± 3.5ab</td>
<td>128.3 ± 1.4ab</td>
<td>127.9 ± 0.7ab</td>
<td>134.2 ± 0.8ab</td>
</tr>
<tr>
<td>Calcium (mg/g)</td>
<td>Initial</td>
<td>175.8</td>
<td>188.9 ± 7.6a</td>
<td>193.6 ± 7.7b</td>
<td>198.5 ± 8.4c</td>
<td>198.3 ± 6.2c</td>
<td>200.6 ± 5.8c</td>
</tr>
<tr>
<td>Magnesium (mg/g)</td>
<td>Initial</td>
<td>3.8</td>
<td>3.81 ± 0.1a</td>
<td>3.95 ± 0.09b</td>
<td>3.97 ± 0.03b</td>
<td>4.0 ± 0.02b</td>
<td>3.96 ± 0.02b</td>
</tr>
<tr>
<td>Zinc (mg/g)</td>
<td>Initial</td>
<td>0.136</td>
<td>0.147 ± 0.009a</td>
<td>0.166 ± 0.004b</td>
<td>0.175 ± 0.004c</td>
<td>0.172 ± 0.004c</td>
<td>0.175 ± 0.006c</td>
</tr>
</tbody>
</table>

* Mean values with standard deviation from triplicate tanks (except for initial sample) and pooled five fish for each. Different superscripts within the same row are significantly different, \( P<0.05 \)

Table 5. Serum minerals in Japanese flounder, Paralichthys olivaceus, fed in six experimental phytase-supplemented diets.*

<table>
<thead>
<tr>
<th>Phytase (FTU/100g diet)</th>
<th>Initial</th>
<th>0</th>
<th>150</th>
<th>300</th>
<th>450</th>
<th>900</th>
<th>1500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (mg/100ml)</td>
<td>Initial</td>
<td>19.7 ± 4.0</td>
<td>16.6 ± 0.5a</td>
<td>17.8 ± 0.1ab</td>
<td>18.2 ± 1.0b</td>
<td>17.7 ± 0.4ab</td>
<td>17.6 ± 0.2ab</td>
</tr>
<tr>
<td>Phosphorus (mg/100ml)</td>
<td>Initial</td>
<td>18.9 ± 5.0</td>
<td>12.3 ± 1.5a</td>
<td>12.3 ± 0.4a</td>
<td>14.0 ± 1.4a</td>
<td>13.6 ± 1.5a</td>
<td>12.2 ± 0.9a</td>
</tr>
<tr>
<td>Zinc (µg/100ml)</td>
<td>Initial</td>
<td>43.07 ± 31.7</td>
<td>94.4 ± 50.9a</td>
<td>102.6 ± 53.8a</td>
<td>124.2 ± 45.0a</td>
<td>145.1 ± 22.5a</td>
<td>143.0 ± 19.4a</td>
</tr>
</tbody>
</table>

* Mean values with standard deviation for five fish per replication. Different superscripts within the same row are significantly different, \( P<0.05 \)
Table 6. Apparent phosphorus and protein digestibility of Japanese flounder, Paralichthys olivaceus, fed six experimental phytase-supplemented diets.*

<table>
<thead>
<tr>
<th>Phytase (FTU/100g diet)</th>
<th>0</th>
<th>150</th>
<th>300</th>
<th>450</th>
<th>900</th>
<th>1500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary group</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>33.6 ± 11.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.6 ± 2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.1 ± 5.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55.5 ± 11.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.2 ± 5.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51.3 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>78.4 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.5 ± 6.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>85.0 ± 2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.9 ± 2.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>82.0 ± 6.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>84.4 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Mean values with standard deviation for five fish per replication. Different superscripts within the same row are significantly different, $P<0.05$

Table 7. Apparent phosphorus and protein retention of Japanese flounder, Paralichthys olivaceus, fed six experimental phytase-supplemented diets.*

<table>
<thead>
<tr>
<th>Phytase (FTU/100g diet)</th>
<th>0</th>
<th>150</th>
<th>300</th>
<th>450</th>
<th>900</th>
<th>1500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary group</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>41.4 ± 33.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.7 ± 9.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.8 ± 18.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.6 ± 10.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>46.6 ± 6.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>44.2 ± 7.8&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>38.3 ± 2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.4 ± 6.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.7 ± 8.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45.4 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.0 ± 4.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.1 ± 2.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Mean values with standard deviation for five fish per replication. Different superscripts within the same row are significantly different, $P<0.05$
control diet with 0 FTU phytase addition. Inclusion of 300 FTU or more resulted in an additional FCR improvement over the 150 FTU inclusion rate. In this study, a significantly better FCR value was found in fish fed the 300 FTU diet. This suggests that growth reflects the FCR, which can be improved through the addition of 300 FTU in SBM-containing diet for Japanese flounder.

It is noteworthy that all levels of phytase addition showed significantly improved P digestibility over the control (0 FTU) diet. However, the higher dosages of phytase, such as 900 and 1500 FTU groups, did not further increase of P digestibility in the SBM-containing diet for Japanese flounder. Cheng and Hardy (2003) pointed out that when phytase was supplemented in extruded full-fat soybean meal, the apparent digestibility coefficient of total P and phytate P increased. Improved digestibility of P from pelleted diets containing SBM by supplementation with phytase has been demonstrated in both rainbow trout and common carp (Rodehutscord et al. 1995, Scafer et al. 1995). Sugiura et al. (1998) also reported that the digestibility of dietary P in rainbow trout was lower in SBM without supplementation of phytase.

Significantly higher protein digestibility values were also noted in fish fed the 300 FTU and 1500 FTU diets than the control (0 FTU) diet. Storebakken et al. (1998) demonstrated that phytase pretreatment of isolated soy protein resulted in improved protein digestibility in Atlantic salmon (Salmo salar). Lei et al. (1993) also reported that supplementation of phytase in the diet may improve bioavailability of protein and trace minerals. The results of the present study indicate that phytase in the diets improved P and protein retention. It is also reported that improved P retention was found with phytase (2000 FTU/kg diet) supplemented in soybean meal and canola meal diets in striped bass (Papatryphon 1999) and in Atlantic salmon (Sajjadi et al. 2004). In our study, phytase enhanced protein retention, suggesting that phytase reduces formation of phytate-protein complexes in the gut and causes an improvement in utilization.

In this study, flounder fed the 300 FTU diet showed a significantly higher serum Ca concentration. The diets containing 150, 450, 900 and 1500 FTU showed no significant difference of serum Ca compared to the fish fed the control (0 FTU) diet. Hughes and Soares (1998) reported that sea bass fed a diet containing phytase had a higher serum Ca content, which
is in agreement with our findings. However, no significant variation was observed in either serum P or Zn among the different dietary groups. The suitability of using serum P concentration as an indicator of P status seems questionable due to a combination of physiological and experimental factors. Skonberg et al. (1997) speculated that when rainbow trout are fed excess P, blood P does not respond to the excess P level.

Mineral bioavailability was increased with phytase supplementation as demonstrated through improved bone mineralization in this experiment. These results agree with other studies conducted concerning P bioavailability with carp, catfish (*Ictalurus punctatus*) and striped bass (Scafer *et al.* 1995, Jackson *et al.* 1996, Papatryphon *et al.* 1999). The vertebral Ca and Zn were significantly higher in the fish fed the 300 FTU diet than the control (0 FTU) diet. Vertebral P and Mg were markedly improved in all phytase-supplemented diets. Similar results appear to concur with these observations in striped bass (Hughes and Soares 1998).

**CONCLUSION**

This study indicates that in an SBM-containing diet, supplementation of phytase was effective and that 300 FTU/100g resulted in the maximum release of soluble P, and as a consequence improved the weight gain, FCR, bone mineral content, P and protein digestion and retention in Japanese flounder.

**ACKNOWLEDGEMENTS**

The scholarship received by Pallab Kumer Sarker from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MONBUKAGAKUSHO: MEXT) is gratefully acknowledged.
REFERENCES


Effects of phytase addition on P utilization in Japanese flounder


Effects of phytase addition on P utilization in Japanese flounder
Growth, Production and Economic Considerations for Commercial Production of Marketable Sizes of Spotted Babylon, *Babylonia areolata*, using a Pilot Abandoned Marine Shrimp Hatchery and Recirculating Culture System

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Keywords: Spotted babylon, *Babylonia areolata*, commercial production, growth, management, economics, Thailand.

ABSTRACT

This study was conducted to determine the feasibility for culture of spotted babylon juveniles (*Babylonia areolata*) to marketable sizes using an abandoned marine shrimp hatchery. It was reconstructed with a large-scale recirculating culture system of 4.0 x 24.5 x 0.4 m concrete rearing ponds. The growth, production and economic analysis for culture of spotted babylon was evaluated. The average growth rates of spotted babylon were 0.94 g / mo. Feed conversion ratio was 1.8 and the average final survival was 90.5%. At the end of the experiment, the average yield was 148 kg / pond. The total production for six rearing ponds was estimated at 884 kg. Based on the farm data, stocking data and harvest data used in this study, total cost per 6 month production cycle was $6,458.40 (USD). In 2007, at farm gate prices of $8.60/kg (USD) resulted in a gross return and net return per production cycle of $7,575.90 (USD)
Considerations for production of spotted babylon

and $1,117.50 (USD), respectively. The benefit cost ratio (BCR) showed a positive profit (1.17) and a payback period of 5.7 production cycles. The present study indicated that the use of an abandoned marine shrimp hatchery reconstructed to include a recirculating culture system was economically attractive for culture of juvenile *B. areolata* to marketable sizes.

**INTRODUCTION**

The spotted babylon, *Babylonia areolata* Link, 1807, (Figure 1) is now one of the most important marine gastropods for human consumption in Thailand, where the larger-sized specimens (>450 mm) are used for fried and steamed spotted babylon dishes in seafood restaurants. Spotted babylon belongs to Class Gastropoda, Order Neogastropoda, Family Buccinidae. It is abundant and widely inhabits littoral regions in the Gulf of Thailand, especially muddy sand areas not exceeding 10-20 m in depth. The price of spotted babylon ranges from 250 to 500 Baht per kilogram in seafood markets and restaurants, respectively. The spotted babylon fishery, a relatively small-scale fishery, is primarily carried out on natural beds in the Gulf of Thailand. Direct fishery of this species recently developed by means of baited-trap fishing carried out year round. The nature of this fishery is very similar to that of the sand crab (*Portunus pelagicus*) trap fishery. The spotted babylon fishery has provided an economic supplement to specialized small-scale fisheries for squid and sand crab. However, natural stocks have decreased drastically in recent years because of continuous exploitation in traditional fishing areas, and this has resulted in increased demand and higher prices. The spotted babylon has many biological attributes that make it suitable for profitable aquaculture and is considered a promising new candidate for the industry in Thailand. These attributes include fast growth, high survival rates, low FCR, and relatively simple culture techniques. Large-scale production of juveniles in hatcheries is considered to be technically feasible and these techniques can be transferred to industry. Farming of spotted babylon snail is still in early development in Thailand. The expansion of spotted babylon aquaculture has greatly increased the demand for juveniles. As a consequence, hatcheries need to produce large quantities of high quality eggs and larvae. There has been considerable interest in the commercial culture of spotted babylon in Thailand resulting from this growing demand, an expanding domestic market for seafood, and a catastrophic
Considerations for production of spotted babylon decline in natural spotted babylon populations in the Gulf of Thailand. From an aquaculture point of view, the spotted babylon has many biological attributes, production, and market characteristics necessary for a profitable aquaculture venture and it is considered a promising new candidate for land-based aquaculture in Thailand (Chaitanawisuti and Kritsanapuntu 1999). At present, the successful large-scale culture of spotted babylon juveniles to marketable sizes has been conducted in flow-through seawater systems in concrete / canvas ponds. However, this culture technique has substantial disadvantages for the culture purposes. Basically, the flow-through systems need a high flow rate of high quality seawater, limiting culture areas to those nearby the seashore, bringing seasonal problems related to water quality and pollution, and resulting in high operational costs. The production totals and low economic returns are not high enough to justify commercial operations (Chaitanawisutti, Kritsanapuntu and Natsukari 2002a,b).

Recirculating systems are mechanically sophisticated and biologically complex, and have been used for growing fish and shellfish for more than three decades. Interest in recirculating systems is due to their perceived advantages, including greatly reduced land and water requirements, high degree of environmental control allowing productive-cycle growth at optimum rates, the feasibility of locating culture areas far from the sea, and major improvements in water conservation and reuse (Losordo, Masser and Rakocy 1998; Masser, Rakocy and Losordo 1999). Research on recirculating systems may offer an alternative to pond aquaculture.
technology and represents a major leap in spotted babylon culture intensification and technology. Much of this progress is necessary to maximize profits by increasing production, lowering costs, and conserving water. This study may provide an opportunity to develop a sustainable aquaculture system for culture of spotted babylon juveniles to marketable sizes in large-scale recirculating culture systems in Thailand. In addition, a lack of economic data on the costs of production and expected economic returns has been a serious constraint to the successful development of spotted babylon aquaculture operations. A financial investment analysis brings together biological factors, production costs, and market price variables to make better decisions regarding culture methods, feasibility, and the overall potential for commercial operation of this enterprise. The objective of this study is to present the growth, production, and economic considerations for commercial production of juvenile spotted babylon, *Babylonia areolata*, to marketable sizes using an abandoned marine shrimp hatchery and recirculating culture system.

**MATERIALS AND METHODS**

**Pond design and construction**

This study was conducted at the pilot farm using an abandoned commercial marine shrimp (*Penaeus monodon*) hatchery at Samutsongkham Province, Thailand, where business operations had ceased seven years previously. The farm consisted of concrete floors and tile roofing in good condition, and was ready for use. The recirculating culture system used in this study consisted of rearing ponds and an integrated water treatment pond. Six concrete rearing ponds, each 98.0 m² (4.0 x 24.5 m) in size (0.4 m deep), were constructed. Ponds were arranged in a 2x3 array with common walls to reduce construction costs. The tank bottom was covered with a 2 cm layer of coarse sand (0.5-1.0 mean grain size) to serve as a substrate. A water treatment pond of 3,000 L capacity (3.0 x 10.0 x 1.0 m) was constructed, which contained limestone gravel and oyster shell fragments as biological filtration media, and seaweed (*Caulerpa lentillifera*) to provide macroalgal absorption. Water flowed from all rearing ponds through the water treatment pond via 2 hp water pumps operating at a constant flow rate of 300 L / h for 18 hours daily throughout the experimental period. The water was returned to the rearing ponds via water pumps at the same flow rate. A 3 hp blower was used to provide a high volume of uncontaminated air. Aeration was...
operating daily for 20 hours except during feeding and resting of the blower. Each rearing pond was continuously aerated by twenty air stones at 1.0 m intervals arranged in a 2 array. Temperature was maintained at $29 \pm 1.5^\circ$C. Water level in the ponds was maintained at 30 cm in depth and fresh water was added to make up losses due to water evaporation and water loss, maintaining a salinity of 29-30 ppt. The photoperiod was naturally 12-h dark/12-h light.

**Seawater preparation and management**

This study used artificial seawater for large-scale production of spotted babylon, in order to reduce costs related to construction of a seawater collection system and pipeline. The farm site was located far from the sea shore and salinity of natural water in the nearby canal was not more than 10 ppt. Prior to the start of culture, the artificial seawater was prepared by using brackish water of 10 ppt as the main component. Thereafter, highly saturated saline seawater obtained from a salt farm was added until culture water reached a salinity of 30 ppt. Seawater in each rearing pond was exchanged at 3 month intervals. When water exchanges were done for each pond, the substrate was cleaned by flushing it with a jet of water and sun dried for 6 h. Thereafter, the rearing ponds were refilled with new artificial seawater as mentioned above. Shell fragments and gravel were also rinsed in water to remove particulate matter, sun dried for 6 h, and returned to the water treatment ponds. Salinity was monitored daily to keep the variation within $\pm 2.0$ ppt through the addition of fresh water to correct for any increases in salinity due to water evaporation.

**Culture method**

Juvenile *B. areolata* was purchased from a private hatchery. Individuals from the same cohort were sorted by size to prevent possible growth retardation of small babylon when cultured with larger individuals. The spotted babylon juveniles had an average initial body weight of 0.13 g, averaging 7,490 snails per kilogram. Initial stocking density of spotted babylon juveniles was 300 individuals m$^{-2}$ (29,400 snails per pond). Spotted babylon were fed *ad libitum* with fresh trash fish once daily at 1000 h. Food was offered to the snails until they stopped feeding. Uneaten food was removed immediately, and air dried for a period of 10 min before weighing. The amount of food consumed was recorded daily for calculation of the feed conversion ratio (FCR). Size grading of snails in each treatment was not done throughout the culture period. No
Considerations for production of spotted babylon

chemical or antibiotic agents were used throughout the entire experiment. To determine growth performance, twenty percent of the snails from each pond were sampled randomly at 30 day intervals, and whole body weight was determined. Whole weight was measured after air drying for a period of 10 min before weighing. The snails were then returned to the tank. The number of dead individuals were recorded every 30 days. Average body weight gains and growth rates were calculated following the method of Chaitanawisuti and Kritsanapuntu (1999). Mortality, expressed as a percentage of the initial stocking density was calculated from the difference between the number of animals stocked vs. the number harvested. The spotted babylon juveniles were cultured to reach marketable sizes of 120-150 snails/ kg.

Economic evaluation

The components of the financial analysis were classified as part of the initial investment, annual ownership costs, and annual operating costs as follows:

Initial investment requirements for farm construction were evaluated. The investment requirements included land lease, construction of six 4.0 x 24.5 x 0.4 m rearing ponds, one water treatment pond of 3,000 L capacity, two water pumps, one air blower and a PVC pipeline for air and seawater systems.

Fixed costs per production cycle consisted of land, depreciation, and interest on investment. These costs are fixed and incurred in the short run regardless of whether the facilities are operated. Annual depreciation was estimated by the straight-line method based on the expected useful life of each item of equipment. Assets are assumed to have no residual value at the end of their useful life. Six culture ponds and one seawater treatment pond were assumed to have useful life of 5 years. The air blower and seawater pumps were assigned a useful life of 3 years. The life expectancies of equipment were 3 years. Interest rates for capital costs were based on 2007 bank loan rates (3.5% per year) for this type of business enterprise.

Operating costs per production cycle are incurred upon actual operation of the grow-out unit, and include repairs and maintenance, labor, feed, utilities and interest on operating capital. Costs for purchasing and transportation of spotted babylon juveniles are $0.01/juvenile (USD).
Spotted babylon are fed fresh trash fish at a cost of $0.13/kg (USD). The costs of repairs and maintenance were estimated based upon the actual expenses for the rearing ponds, water treatment pond, and operating equipment costs. Electricity is used for operating the various pumps and lighting units in the farm. The average charge was $0.03/kilowatt hour (USD). Labor requirements were based on the particular needs for each production cycle at the proposed farm. One laborer (full-time) was assigned for operation of the farm, at a cost of $142.90/month (USD). Interest charges for operating capital are based on 2007 bank loan rates (3.5% per year) for this type of business.

**Return analysis**

Net return and return on investment for grow-out production was computed at the selling price of market size spotted babylon at farm gate prices in 2007, approximately $8.57/kg (USD). Gross return was computed from total yield multiplied by the selling price. Net return was calculated from the gross return minus to the total cost per production cycle. Return to capital and management was calculated by subtracting total operation costs from the gross return. Return on investment was estimated by dividing return to capital and management by the initial investment. The payback period (in years) was calculated by investment cost divided by the net return (Fuller, Kelly and Smith 1992).

**RESULTS**

**Growth and production**

Growth, expressed as body weight and number of snails per kilogram of juvenile *B. areolata* cultured in large-scale recirculating culture systems over a period of 6 months is shown in Figure 2. Snails showed no signs of stress as exhibited by active movement, feeding, and protrusion of the siphon tube throughout the experiment. The mean (±SE) weight gains and increases in body weight of spotted babylon were 5.36 ± 0.42 g/snail and 0.94 ± 0.84 g mo⁻¹, respectively. The feed conversion ratio (FCR) was 1.8 and the average final survival was 90.5% (Table 1). At the end of the experiment, the snails reached an average size of 5.6 g / snail or 147 individuals / kg after a period of 6 months. The average yield of spotted babylon was 148 kg / pond and the overall production of the six rearing ponds was 884 kg (Table 1).
Water quality
Seawater monitoring indicated that water temperature, conductivity, salinity, pH, and dissolved oxygen changed gradually with no significant differences recorded throughout the experimental period ($P>0.05$) but there were significant differences ($P<0.05$) in alkalinity (50.5-120.0 mg/L) total suspended solid (25.3-74.5 mg/L), ammonia-nitrogen (0.002-0.950 mg/L), nitrite-nitrogen (0.007-0.225 mg/L), nitrate-nitrogen (0.050-28.644 mg/L) and phosphate-phosphorus (0.053-1.110 mg/L) (Table 2).

Financial analysis
Farm data (pond sizes and total pond area), stocking data (initial weight, stocking density), and harvest data (duration of culture, weight at harvest,
Considerations for production of spotted babylon

<table>
<thead>
<tr>
<th>Farm data</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rearing pond size (m)</td>
<td>4.0 x 24.5 x 0.4</td>
</tr>
<tr>
<td>Pond bottom area (m²)</td>
<td>98.0</td>
</tr>
<tr>
<td>Number of rearing ponds</td>
<td>6</td>
</tr>
<tr>
<td>Total culture areas (m²)</td>
<td>588.0</td>
</tr>
<tr>
<td>Water treatment pond (m)</td>
<td>3.0 x 10.0 x 1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grow out data</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (g/snail)</td>
<td>7,490</td>
</tr>
<tr>
<td>Initial sizes (snails/kg)</td>
<td>300</td>
</tr>
<tr>
<td>Stocking density (no per m²)</td>
<td>29,400</td>
</tr>
<tr>
<td>Number of snails per pond (individuals)</td>
<td>176,400</td>
</tr>
<tr>
<td>Total snails per crop (individuals)</td>
<td>0.01</td>
</tr>
<tr>
<td>Juvenile cost ($US/individual)</td>
<td>0.13</td>
</tr>
<tr>
<td>Duration of grow-out (mo/crop)</td>
<td>3,240</td>
</tr>
<tr>
<td>Feed cost ($US/kg)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Harvest data</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Final weight (g/snail)</td>
<td>5.4</td>
</tr>
<tr>
<td>Final sizes (individual/kg)</td>
<td>147</td>
</tr>
<tr>
<td>Growth rate (g/month)</td>
<td>0.9</td>
</tr>
<tr>
<td>Final survival (%)</td>
<td>90.5</td>
</tr>
<tr>
<td>Feed conversion ratio (FCR)</td>
<td>1.8</td>
</tr>
<tr>
<td>Average yield per pond (kg)</td>
<td>148</td>
</tr>
</tbody>
</table>

Table 1. Actual data used for culture of juvenile Babylonia areolata in a large-scale recirculating culture system.

Note: All cost estimations based on Thai Baht have been converted to US$, using 2007 currency exchange rates.

final survival, feed conversion ratio, and yield) are based on the actual data from the pilot farm. Parameters used for the economic analysis for culture of spotted babylon in large-scale recirculating culture system are summarized in Tables 3 through 8. The total investment required for construction of a culture area of 588 m² was estimated to be $6,371.40 (USD). Construction of rearing ponds and the seawater treatment pond was the largest cost component of the farm. These two components represented 77.1% of the total investment requirements for production of spotted babylon in this large-scale recirculating culture system (Table 3). Fixed cost per production cycle was estimated to be $1,004.90 (USD). The major fixed cost items were depreciation, repair and maintenance, and interest on investment, accounting for 73.0%, 15.8%, and 11.0% of total fixed costs, respectively (Tables 4 and 5). Operating costs per production
Considerations for production of spotted babylon

The costs for production of spotted babylon to marketable sizes in this farm design were estimated to be $7.30/kg (USD). The four major operating cost items were purchasing of juveniles, feed, labor, and electricity, representing 41.5%, 18.6%, 15.7%, and 12.6% of total operating costs, respectively (Table 6). Total costs per production cycle were estimated to be $6,458.40 (USD). The top five major total cost items were purchasing of juveniles, feed, labor, depreciation, and electricity, representing 35.1%, 15.8%, 13.3%, 11.4%, and 10.6% of total costs, respectively (Table 7).

Table 2. Water quality of seawater in recirculating and flow-through systems for culture of spotted babylon (Babylonia areolata).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Recirculating system</th>
<th>Flow-through system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water temperature (°C)</td>
<td>27.3 ± 0.5a</td>
<td>27.3 ± 0.5a</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>30.9 ± 0.5a (30.3-32.0)</td>
<td>30.8 ± 0.5a (29.8-31.9)</td>
</tr>
<tr>
<td>pH</td>
<td>7.78 ± 0.16a (7.59-8.30)</td>
<td>7.78 ± 0.16a (7.63-8.30)</td>
</tr>
<tr>
<td>Dissolved Oxygen (mg/L)</td>
<td>6.1 ± 0.6a (5.3-7.4)</td>
<td>6.2 ± 0.6a (5.2-7.5)</td>
</tr>
<tr>
<td>Alkalinity (mg/L)</td>
<td>72.5 ± 16.4ac (52.0-110.0)</td>
<td>112.7 ± 12.9ac (110.5-120.0)</td>
</tr>
<tr>
<td>Ammonia-nitrogen (mg-N/L)</td>
<td>1.36 ± 0.228a (0.006-0.950)</td>
<td>0.062 ± 0.063b (0.005-0.246)</td>
</tr>
<tr>
<td>Nitrite-nitrogen (mg-N/L)</td>
<td>0.062 ± 0.045a (0.007-0.225)</td>
<td>0.046 ± 0.028b (0.007-0.118)</td>
</tr>
<tr>
<td>Nitrate-nitrogen (mg-N/L)</td>
<td>10.661 ± 6.896a (0.050-19.097)</td>
<td>12.038 ± 8.418b (0.050-28.644)</td>
</tr>
<tr>
<td>Total dissolved nitrogen (mg-N/L)</td>
<td>12.275 ± 6.723a (2.019-22.109)</td>
<td>13.638 ± 8.032b (2.019-29.368)</td>
</tr>
<tr>
<td>Phosphate - phosphorus (mg-P/L)</td>
<td>0.543 ± 0.316b (0.053-0.997)</td>
<td>0.450 ± 0.265c (0.053-0.785)</td>
</tr>
<tr>
<td>Total dissolved phosphorus (mg-P/L)</td>
<td>0.749 ± 0.309bc (0.224-1.289)</td>
<td>0.631 ± 0.229c (0.224-0.949)</td>
</tr>
</tbody>
</table>

Note: Values are mean ± SD, numbers in parentheses are minimum and maximum.
### Considerations for production of spotted babylon

**Table 3. Estimated investment requirements for culture of juvenile Babylonia areolata in large-scale recirculating culture system.**

<table>
<thead>
<tr>
<th>Items</th>
<th>US$</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Six rearing concrete ponds (4.0x24.5x0.4 m)</td>
<td>4,285.7</td>
<td>67.3</td>
</tr>
<tr>
<td>One water treatment pond (3.0x10.0x1.0 m)</td>
<td>628.6</td>
<td>9.9</td>
</tr>
<tr>
<td>Two water pumps</td>
<td>400.0</td>
<td>6.3</td>
</tr>
<tr>
<td>One air blowers</td>
<td>342.8</td>
<td>5.4</td>
</tr>
<tr>
<td>Operating equipment</td>
<td>285.7</td>
<td>4.5</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>428.6</td>
<td>6.7</td>
</tr>
<tr>
<td><strong>Total investment</strong></td>
<td><strong>6,371.4</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

*Note: All cost estimations based on Thai Baht have been converted to US$.*

**Table 4. Estimated depreciation, interest charges, and repair and maintenance costs for culture of juvenile Babylonia areolata in a large-scale recirculating culture system.**

<table>
<thead>
<tr>
<th>Items</th>
<th>No. of units</th>
<th>Total cost of items (US$)</th>
<th>Estimated Life (year)</th>
<th>Annual depreciation (US$)</th>
<th>Annual interest charges¹ (US$)</th>
<th>Annual repairs / maintenance² (US$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rearing ponds</td>
<td>6</td>
<td>4,285.7</td>
<td>5</td>
<td>857.1</td>
<td>149.9</td>
<td>214.3</td>
</tr>
<tr>
<td>Water treatment pond</td>
<td>1</td>
<td>628.6</td>
<td>5</td>
<td>125.7</td>
<td>22.0</td>
<td>31.4</td>
</tr>
<tr>
<td>Seawater pumps</td>
<td>2</td>
<td>400.0</td>
<td>3</td>
<td>133.3</td>
<td>14.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Air blowers</td>
<td>1</td>
<td>342.8</td>
<td>3</td>
<td>114.3</td>
<td>11.9</td>
<td>17.1</td>
</tr>
<tr>
<td>Operating equipment</td>
<td>1</td>
<td>285.7</td>
<td>3</td>
<td>95.2</td>
<td>9.9</td>
<td>14.3</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>1</td>
<td>428.6</td>
<td>3</td>
<td>142.9</td>
<td>15.0</td>
<td>21.4</td>
</tr>
<tr>
<td><strong>Total cost per year</strong></td>
<td><strong>6,371.4</strong></td>
<td></td>
<td><strong>3</strong></td>
<td><strong>1,468.5</strong></td>
<td><strong>222.7</strong></td>
<td><strong>318.5</strong></td>
</tr>
</tbody>
</table>

*Note: All cost estimations based on Thai Baht have been converted to US$,
¹Annual interest charges for all items are estimated to be 3.5%, ²Annual repairs/maintenance for all items are estimated to be 5%.*

**Table 5. Estimated fixed costs for culture of juvenile Babylonia areolata in large-scale recirculating culture system.**

<table>
<thead>
<tr>
<th>Items</th>
<th>US$</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual depreciation</td>
<td>1,468.5</td>
<td>73.0</td>
</tr>
<tr>
<td>Annual interest charges</td>
<td>222.7</td>
<td>11.1</td>
</tr>
<tr>
<td>Annual repairs /maintenance</td>
<td>318.5</td>
<td>15.9</td>
</tr>
<tr>
<td><strong>Total fixed cost per annum</strong></td>
<td><strong>2,009.7</strong></td>
<td><strong>100</strong></td>
</tr>
<tr>
<td><strong>Total fixed cost per production cycle¹</strong></td>
<td><strong>1,004.9</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Fixed cost per kg²</strong></td>
<td><strong>1.14</strong></td>
<td></td>
</tr>
</tbody>
</table>

*Note: All cost estimations based on Thai Baht have been converted to US$,
¹One production cycle was 6 months, ²Yield per production cycle was 884 kg.*
Considerations for production of spotted babylon

Table 6. Estimated operating costs per production cycle for culture of juvenile Babylonia areolata in a large-scale recirculating culture system.

<table>
<thead>
<tr>
<th>Items</th>
<th>US$</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purchase of juveniles</td>
<td>2,268.0</td>
<td>41.6</td>
</tr>
<tr>
<td>Purchase of highly saturated seawater</td>
<td>154.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Electricity for water pump and air blowers</td>
<td>685.7</td>
<td>12.6</td>
</tr>
<tr>
<td>Feed</td>
<td>1,018.3</td>
<td>18.7</td>
</tr>
<tr>
<td>Hired labor (1 full time)</td>
<td>857.1</td>
<td>15.7</td>
</tr>
<tr>
<td>Repairs and maintenance</td>
<td>285.7</td>
<td>5.2</td>
</tr>
<tr>
<td>Interest on operating cost</td>
<td>184.4</td>
<td>3.4</td>
</tr>
<tr>
<td><strong>Operating cost per production cycle</strong></td>
<td>5,453.5</td>
<td>100</td>
</tr>
<tr>
<td><strong>Operating cost per kg</strong></td>
<td>6.17</td>
<td></td>
</tr>
</tbody>
</table>

Notes: All cost estimations based on Thai Baht have been converted to US$,

1 Selling price of juvenile spotted babylon was $US 0.01/individual,
2 Highly saturated seawater was $US3.7/ton,
3 Feed price was $US 0.13/kg, and total feed consumed of 32,400 kg,
4 Interest charges are based on 2007 bank loan rates (3.5% per year),
5 One production cycle was 6 months,
6 Yield per production cycle was 884 kg.

Table 7. Estimated total cost per production cycle for culture of juvenile Babylonia areolata in a large-scale recirculating culture system.

<table>
<thead>
<tr>
<th>Items</th>
<th>US$</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fixed costs per production cycle</strong></td>
<td>1,004.9</td>
<td>15.6</td>
</tr>
<tr>
<td>Depreciation</td>
<td>734.3</td>
<td>11.4</td>
</tr>
<tr>
<td>Interest</td>
<td>111.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Repairs and maintenance</td>
<td>159.3</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Operating costs per production cycle</strong></td>
<td>5,453.5</td>
<td>84.4</td>
</tr>
<tr>
<td>Purchasing for juveniles</td>
<td>2,268.0</td>
<td>35.1</td>
</tr>
<tr>
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<td>Feed</td>
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<td>Labor (1 full time)</td>
<td>857.1</td>
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<td>Repairs and maintenance</td>
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<td>Interests on operating cost</td>
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<tr>
<td><strong>Total cost per production cycle</strong></td>
<td>6,458.4</td>
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<tr>
<td><strong>Total cost per kg</strong></td>
<td>7.3</td>
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</table>

Notes: All cost estimations based on Thai Baht have been converted to US$,

1 One production cycle was 6 months,
2 Yield per production cycle was 884 kg.
Economic returns

The enterprise budgets based on the 2007 farm gate price of spotted babylon -- $8.60/kg (USD), resulted in a gross return and net return per production cycle of $7,575.90 (USD) and $1,117.50 (USD), respectively. Return to capital and management and return on investment were $2,122.40 (USD) and 0.3, respectively. The break-even production and break-even price were estimated to be 418.7 kg and $3,588.90 (USD), respectively. Benefit cost ratio (BCR) showed a positive profit (1.17) and a payback period of 5.7 production cycles (Table 8).

<table>
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<tr>
<th>Yield</th>
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<td>Yield per production cycle (kg)</td>
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<td>Operating costs (per production cycle)</td>
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<td>Gross return $^2$ (per production cycle)</td>
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<tr>
<td>Net returns (per production cycle)</td>
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<td>Return to capital and management $^4$</td>
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<td>Return on investment $^5$</td>
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<tr>
<td>Net returns (per kg)</td>
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<tr>
<td>Benefit-cost ratio (BCR)</td>
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<tr>
<td>Break-even production (kg)</td>
<td>418.7</td>
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<td>Break-even price</td>
<td>3,588.9</td>
</tr>
<tr>
<td>Payback period (production cycle) $^3$</td>
<td>5.7</td>
</tr>
</tbody>
</table>

Notes: All cost estimations based on Thai Baht have been converted to US$, $^1$ Whole operation of 6 rearing ponds of 4.0 x 24.5 x 0.4 m each, $^2$ Market price for spotted babylon in 2007 ($8.57 per kg), $^3$ One production cycle was 6 months, $^4$ Return to capital and management = Gross return – Total operation cost, $^5$ Return on investment = Return to capital and management / initial investment.
Considerations for production of spotted babylon

DISCUSSION

In this study, culture of spotted babylon juveniles (Babylonia areolata) to marketable sizes using a pilot abandoned marine shrimp hatchery reconstructed with large-scale recirculating system showed good results for growth, production, and economic returns. In this study, the average growth rates in body weight of spotted babylon were 0.94 g mo⁻¹ with final body weights are 5.66 g. Feed conversion ratio and final survival was 2.11 and 96.79%, respectively. At the end of the experiment, the snails reached an average size of 177 individuals / kg, and the average yield of spotted babylon was 148 kg / pond. Total production of six rearing ponds was 884 kg. In contrast to those raised in flow-through systems, Chaitanawisuti and Kritsanapuntu (1999) reported that average monthly growth rates of spotted babylon in a flow-through culture system consisting of concrete / canvas ponds was 1.4 g / mo. FCR and final survival were 1.6 and 95.8%, respectively. However, growth of the spotted babylon in the recirculating system was slightly slower than for those individuals raised in the flow-through system. The major issues leading to slow growth of spotted babylon in the recirculating system may be mineral depletion of the seawater used, particularly a shortage of calcium needed for shell formation, which caused shell abnormalities and slow growth. This problem was mainly characterized by observation of the following external shell morphology: shell color with dark brown spots gradually changed to pale brown, and the outer shell layer was partially removed (Figure 3). Shell abnormalities and slow growth may be due to insufficient calcium and other minerals in the recirculating system, because depletion of these elements required for shell building resulted in a loss of calcium from the shell to the outside medium to achieved equilibrium concentration of calcium between the blood and outside medium. Addition of these elements to the diet cannot compensate for their absence in the growing water, due to the low bioavailability of these feed additives for use in shell building. Calta (2000) reported that a number of aquatic mollusks are able to absorb most of their calcium directly from the surrounding water. Calcium is a very important element for fish and shellfish because it is necessary for a variety of functions such as bone and scale growth, shell building, muscle contraction, transmission of nerve impulses, intercellular signalling, hormone secretion, and buffering of osmotic and ionic changes. Hincks and Mackie (1997) reported that maximum growth of zebra mussel (Dreissena polymorpha)
Considerations for production of spotted babylon

Figure 3. Normal shell (above) and shell abnormality (below) in Babylonia areolata, characterized by change in shell color from dark brown spots to pale brown, and the partial removal of the outer shell layer.
occurred at calcium levels of 32 mg Ca / L, alkalinity of 65 mg CaCO$_3$ L$^{-1}$, and total hardness of 100 mg CaCO$_3$ / L. There was negative growth at calcium levels of less than 31 mg CaCO$_3$ / L, and positive growth of juvenile zebra mussels only occurred at a pH greater than 8.3. They also stated that mollusk shells are composed primarily of crystalline CaCO$_3$ (96.3% CaCO$_3$ and 0.34% MgCO$_3$ in zebra mussel) bound together in an organic matrix. Most of the calcium deposited in the shell (80%) is actively taken up from the seawater. Crystallization removes calcium and carbonate ions from the fluid and the reaction proceeds to add new shell layers. However, these reactions are reversible, and under certain conditions, calcium may be removed from the shell, which may explain the deterioration observed in some of the mussel shells. In addition, they suggested that normal calcium metabolism occurs at 10-12 mg/L. Below these levels the mussels lose calcium to the external medium. Presumably, low calcium had an impact on juvenile growth rates because there was not enough calcium provided for shell building.

Financial analysis showed that the initial investment requirement for reconstruction of this abandoned shrimp farm to add a recirculating system with a total culture area of 588 m$^2$ was $6,371.40 (USD). Construction of rearing ponds and the seawater treatment pond was the largest cost component of the farm, representing 77.13% of the total investment. Operating costs per production cycle were estimated to be $5,453.50 (USD). The four major operating cost items were purchasing of juveniles, feed, labor, and electricity. Total cost per production cycle was estimated to be $6,458.40 (USD). The top five major cost items were purchasing of juveniles, feed, labor, depreciation, and electricity. The cost of producing spotted babylon to marketable sizes in this farm design was $7.31/kg (USD). By contrast, Chaitanawisuti, Kritsanapuntu, and Natsukari (2002) reported that the cost of producing spotted babylon to marketable sizes in the flow-through culture system in Thailand was $5.96/kg (USD). For economic analysis, the enterprise budgets, based on the 2007 price of spotted babylon at the farm gate of $8.57/kg (USD), resulted in gross returns and net returns per production cycle of $7,575.90 (USD) and $1,117.50 (USD), respectively. The break-even production and break-even price were estimated to be 418.70 kg and $3,588.90 (USD),
Considerations for production of spotted babylon

respectively. Benefit cost ratio was 1.17 and payback period was 5.7 production cycles (2.9 years). This study presented a positive net return and a payback period of less than five years, which are often used as business investment criteria.

Under the basic assumptions used in this study [juvenile pricing of $0.02/juvenile (USD), feed pricing of $0.10/kg (USD), stocking density of 300 snails/m, and a sale price of $5.80/kg (USD)], there is an indication that an operation consisting of the proposed six 4.0 x 24.5 x 0.4 m rearing ponds is economically feasible under these conditions. The feasibility of producing spotted babylon to marketable sizes in this pilot abandoned shrimp farm operation should continue to be examined. Although the return is small, production with 96.8% survival and selling price of $5.80/kg (USD) is economically feasible under the assumptions employed. This study provides preliminary evidence for the biological feasibility of culturing the spotted babylon, *B. areolata*, in a large-scale recirculating system. Results of this work showed that juvenile spotted babylon could be successfully grown to marketable size in a recirculating system. In addition, many idled marine shrimp (*Penaeus monodon*) hatcheries are currently available in Thailand. This study may provide an opportunity to develop a sustainable aquaculture system for grow-out of spotted babylon juveniles to marketable sizes in abandoned marine shrimp hatcheries resulting in the best utilization of the many abandoned shrimp hatcheries in the coastal areas of Thailand. To achieve success, further study should be concentrated on refining farm design to reduce costs, management of seawater treatment, and addressing the problems of slower growth and reduced shell quality due to mineral depletion of the growing water.

**ACKNOWLEDGMENTS**

We thank the National Research Council of Thailand (NRCT), who provided funding for this research in fiscal years 2004-2007. I especially wish to express my sincere thanks to Professor Dr. Yutaka Natsukari, Faculty of Fisheries, Nagasaki University, Japan, for his supervision of this research and his revision of this manuscript.
REFERENCES


Considerations for production of spotted babylon


Considerations for production of spotted babylon
An Engineering Analysis of the Stoichiometry of Autotrophic, Heterotrophic Bacterial Control of Ammonia-Nitrogen in Zero-Exchange Marine Shrimp Production Systems

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Keywords: zero-exchange systems, autotrophic system, heterotrophic system, C/N ratio

ABSTRACT

After dissolved oxygen, ammonia-nitrogen buildup from the metabolism of feed is usually the limiting factor to increasing production levels in intensive aquaculture systems. Currently, large fixed-cell bioreactors are the primary strategy used to control inorganic nitrogen in intensive recirculating systems. This option utilizes chemosynthetic autotrophic bacteria, ammonia-oxidizing bacteria (AOB), and nitrite-oxidizing bacteria (NOB). Zero-exchange nitrification management systems have been developed based on heterotrophic bacteria and promoted for the intensive production of marine shrimp and tilapia. In these systems, the heterotrophic bacterial growth is stimulated through the addition
of an organic labile carbonaceous substrate. At high organic carbon to nitrogen (C/N) feed ratios, heterotrophic bacteria assimilate ammonia-nitrogen directly from the water, replacing the need for an external fixed film biofilter. As a result, build-up of suspended solids may become the second limiting factor after dissolved oxygen. This paper reviews two nitrogen conversion pathways used for the removal of ammonia-nitrogen in aquaculture systems; autotrophic bacterial conversion of ammonia-nitrogen to nitrate nitrogen, and heterotrophic bacterial conversion of ammonia-nitrogen directly to microbial biomass. The first part of this study reviews these two ammonia removal pathways, presents a set of balanced stoichiometric relationships, and discusses their impact on water quality. In addition, microbial growth energetics are used to characterize production of volatile and total suspended solids for autotrophic and heterotrophic systems. A critical verification of this work was that only a small fraction of the feed’s carbon content is readily available to the heterotrophic bacteria. For example, feed containing 35% protein (350 g/kg feed) has only 109 g/kg feed of labile carbon. In the paper’s second part, the results of a study on the impact C/N ratio on water quality is presented. In this experimental trial sufficient labile organic carbon in the form of sucrose (sugar) was added daily at 0%, 50%, and 100% of the system feeding rate to three prototype zero-exchange systems. The system was stocked with marine shrimp (Litopenaeus vannamei) at modest density (150 /m²) and water quality was measured daily. Significant differences were seen between the three strategies in the key water quality parameters of ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen, pH, and alkalinity. The control (0%) system exhibited water quality characteristics of a mixed autotrophic/heterotrophic system while the other two systems receiving supplemental organic carbon (50% and 100%) showed water quality characteristics of pure heterotrophic systems.

INTRODUCTION

The three pathways for the removal of ammonia-nitrogen in traditional aquaculture systems are: photoautotrophic (algae), autotrophic bacterial conversion from ammonia-nitrogen to nitrate nitrogen, and heterotrophic bacterial conversion from ammonia-nitrogen directly to microbial biomass, a more recent management method. Traditionally, pond aquaculture has used photoautotrophic algae-based systems (greenwater systems) to control inorganic nitrogen buildup. In intensive recirculating
aquaculture production systems, large fixed-cell bioreactors are routinely used that rely on the nitrification of ammonia-nitrogen to nitrate-nitrogen by ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) (Timmons and Ebeling 2007). In intensive recirculating systems, the growth of heterotrophic bacteria and the accumulation of organic carbon and nitrate are minimized intentionally through the rapid removal of solids from the system and through water exchange. It has been demonstrated that for zero-exchange pond production systems, the inorganic nitrogen build-up can be controlled by the manipulation of the organic carbon/nitrogen (C/N) ratio in such a way to promote the growth of heterotrophic bacteria (Avnimelech 1999, 2009). McIntosh (2001) demonstrated that heterotrophic bacteria assimilated the ammonia-nitrogen directly from the water column, producing cellular protein in a marine shrimp pond system. As an additional benefit, for some aquaculture species (marine shrimp and tilapia), this bacterial biomass can be an important source of feed protein, thus reducing the cost of production and improving the overall production economics (McIntosh 1999, Moss 2002).

In the last few years, research has demonstrated that low water exchange marine shrimp production systems can be technically feasible (Ebeling and LaFranchi 1990, Santos and Ebeling 1990). Large-scale pond production systems for marine shrimp have been demonstrated that are zero-exchange and are dominated by photoautotrophic algae (Hopkins et al. 1996, Avnimelech et al. 1994). Management of these systems has been improved by supplementing the shrimp feed with additional feeding of organic labile carbonaceous substrate to support and enhance microbial metabolism (Avnimelech 1999, 2009; McIntosh 1999). Several attempts have been made to develop technology for recirculating marine shrimp production systems at high densities (Weirich 2002, Otoshi 2003, Davis and Arnold 1998, Van Wyk 1999), although it should be noted that in addition to algae and bacterial biomass each of these also incorporated some form of fixed-film biofiltration.

In reviewing the literature on zero-exchange systems, there was usually no description of the pathways employed for ammonia removal and whether the removal was fundamentally photoautotrophic, autotrophic, or heterotrophic bacterial based, or in reality some mixture of the three. One exception was work done by Brune et al. (2003) who examined simplified
microbial growth fundamentals to analyze and compare conventional and heterotrophic techniques to the use of high rate photosynthetic systems. That paper presents a short review of two of these three pathways for the removal of ammonia-nitrogen and the results of a study conducted on the impact of C/N ratio on water quality. In these trials, supplemental carbon beyond that found in the feed in the form of sucrose (sugar) was added daily at 0%, 50%, and 100% of the shrimp feeding rate to three prototype zero-exchange systems. Every attempt was made to minimize photoautotrophic processes by shading the three systems with two layers of shade cloth (blocking 90% of the sunlight) and by high concentrations of total suspended solids (TSS). Although not measured at the time due to a limitation on resources, it was assumed that the role of photoautotrophic bacteria was minor in comparison to the heterotrophic and autotrophic bacteria populations. Thus, only the autotrophic and heterotrophic bacterial pathways were considered in the analysis.

Background: metabolic pathway for 1 kg feed (35% protein)

What follows is a short description of the metabolic pathway options for 1 kg of 35% protein feed and their impacts on water quality parameters. Ebeling et al. (2006) developed a set of stoichiometric relationships for the three pathways and discussed their impact on water quality. Based on these relationships, the fate of nitrogen can be determined for aquaculture systems without organic carbon supplementation and with varying degrees of added organic carbon.

Autotrophic/Heterotrophic bacteria – no carbon supplementation

If we examine a simple zero-exchange system with no supplemental organic carbon addition, the solids remain in the production tank and all of the organic carbon from decomposing feed and fecal matter is available to the heterotrophic bacteria (Figure 1). Normally in recirculating systems, uneaten feed and fecal matter containing organic carbon is quickly removed from the production system to prevent growth and build up of heterotrophic bacteria. In recirculating systems, heterotrophic bacteria are detrimental; in zero-exchange systems heterotrophic bacteria can be beneficial. Since the growth rate of heterotrophic bacteria is significantly higher than that of autotrophic bacteria (Table 1) it is assumed that the heterotrophic bacteria will initially dominate the metabolism of ammonia-nitrogen until the organic carbon source
Given: 1 kg of feed @ 35% protein

Ammonia-nitrogen production:

\[ 1 \text{ kg}_{\text{feed}} \times [0.35 \text{ g protein/kg feed} \times 0.16 \text{ g nitrogen/g protein} \times 0.90 \text{ excreted}] = 50.4 \text{ g NH}_3\text{-N} \]

Heterotrophic System: Organic Carbon from Feed

\[ 1 \text{ kg}_{\text{feed}} \times 0.36 \text{ kg BOD/kg feed} \times 0.40 \text{ kg VSS}_{\text{H}}/\text{kg BOD} = 144 \text{ g VSS}_{\text{H}} \]

\[ 0.124 \text{ g N}_{\text{H}}/\text{g VSS}_{\text{H}} \quad 0.531 \text{ g C}_{\text{H}}/\text{g VSS}_{\text{H}} \]

\[ 17.9 \text{ g N}_{\text{VSS}} + 76.5 \text{ g C}_{\text{VSS}} + 47.1 \text{ g C}_{\text{CO}_2} = 123.6 \text{ g C}_{\text{labile}} \]

\[ 108.2 \text{ g } C_{\text{feed}} = 15.3 \text{ g } C_{\text{alk}} \]

Excess Ammonia-nitrogen:

\[ 50.4 \text{ g NH}_3\text{-N} - 17.9 \text{ g N}_{\text{VSS}} = 32.5 \text{ g N}_A \]

Autotrophic System: Inorganic Carbon from Alkalinity

\[ 32.5 \text{ g N} \times 0.20 \text{ g VSS/g N} = 6.5 \text{ g VSS}_A \]

\[ 0.124 \text{ g N}_A/\text{g VSS}_A \quad 0.531 \text{ g C}_A/\text{g VSS}_A \]

\[ 0.80 \text{ g N}_{\text{VSS}} + 3.45 \text{ g C}_{\text{VSS}} + 55.8 \text{ g C}_{\text{alk}} \]

Figure 1. Zero-exchange system with no carbon supplementation, organic carbon for the heterotrophs from the feed and inorganic carbon for the autotrophs from alkalinity.
becomes the limiting factor. The remaining ammonia-nitrogen not assimilated by the heterotrophic bacteria will then be assimilated by the autotrophic bacteria using alkalinity as an inorganic carbon source.

For this analysis, marine shrimp are being grown. For every kg of feed at 35% protein, approximately 50.4 g of ammonia-nitrogen will be generated (Timmons and Ebeling 2007, Brune et al. 2003). This was estimated based on the chemical composition of protein (0.16 g nitrogen per g of protein) and that 90% of the nitrogen is being excreted by the shrimp, (Brune et al. 2003) or:

\[
1 \text{ kg feed} \times [0.35 \text{ g protein/g feed} \times 0.16 \text{ g N/g protein} \times 0.90 \text{ excreted}] = 50.4 \text{ g NH}_3\text{-N}
\]

By comparison, for finfish only 60 to 70% of the nitrogen is excreted into the water column. One of the difficulties in this analysis was determining the fraction of the organic carbon that was available to the heterotrophic bacteria. It is straightforward to measure the carbon content of feed (approximately 40 to 50%), but as will be shown later, only a fraction of the organic carbon not metabolized by the shrimp is available to the bacteria. Thus, an estimate was made of the organic carbon utilized by the bacteria by estimating the organic carbon sequestered in the volatile suspended solids (VSS) generated by the bacteria and their known carbon content. It has been shown that the biochemical oxygen demand (BOD) content of typical aquaculture feeds is approximately 60% of the dry weight and approximately 0.30 to 0.36 kg BOD per kg of feed is excreted into the water column (Zhu and Chen 2001, Brune et al. 2003). Using a yield fraction of 0.40 kg VSS_H (Heterotrophic) per kg BOD (Avnimelech 1999, Brune et al. 2003) and a BOD_excreted content of 0.36 kg per kg feed, suggests that a kg of feed should generate approximately 144 g of VSS_H. Since bacterial biomass contains 53.1% C and 12.3% N based on its stoichiometry (Ebeling et al. 2006), this heterotrophic microbial biomass would assimilate approximately 17.9 g nitrogen and 76.5 g of organic carbon. In addition in the research trials conducted by the author, the long-term ratio of VSS to TSS for an autotrophic/heterotrophic system was found to average about 0.72. Thus, approximately 200 g of heterotrophic bacterial TSS_H are produced for every kg of feed fed into a system.
Note that since only 36% of the nitrogen is assimilated into cell mass by the heterotrophic bacteria, the remaining nitrogen (32.5 g N) is thus available to the autotrophic bacterial population. Using a yield fraction of 0.20 g VSS\textsubscript{A}/g N (Table 1) produces 6.5 g VSS\textsubscript{A} from 1 kg of feed. Using the same C/N ratios listed previously yields 0.80 g of nitrogen and 3.45 g of carbon assimilated by the autotrophic microbial biomass from 1 kg of feed @ 35% protein. Thus only 0.80 g of nitrogen is incorporated into the autotrophic bacteria, and the remaining is excreted as nitrate-nitrogen. Using the same ratio of TSS to VSS listed previously, only 9.0 g of TSS\textsubscript{A} for every kg of feed is produced by the autotrophic bacteria. Combining the two forms of TSS yields a total of 209 g TSS produced per kg feed. It is interesting to note that only about 1.6% of the available nitrogen is actually contained in the autotrophic microbial biomass and about 36% in the heterotrophic microbial biomass. In addition, the mass of heterotrophic bacteria is more than twenty times the mass of the autotrophic bacteria produced.

It is somewhat more difficult to follow carbon consumption, since the carbon source can be either organic carbon from the feed (heterotrophic) or inorganic carbon from alkalinity (autotrophic). Using the stoichiometric relationships developed in Ebeling \textit{et al.} (2006), the total carbon consumed by the heterotrophic process is 123.5 g C, divided between organic carbon (108.2 g C\textsubscript{feed}) metabolized directly by the heterotrophic bacteria and the depletion of alkalinity, which provides the source of the remaining inorganic carbon consumed (15.3 g C\textsubscript{alkalinity}). All of the inorganic carbon consumed by the autotrophic bacteria (55.8 g C\textsubscript{alkalinity}) comes from alkalinity. Thus a total of 179.3 g of C per kg of feed is consumed by this pathway. This is divided between organic carbon (108.2 g C\textsubscript{feed}) and alkalinity carbon (71.1 g C\textsubscript{alkalinity}) or 293 g of alkalinity as CaCO\textsubscript{3}. Thus, if feed contains on average approximately 40% to 50% carbon, then only about 25% of that organic carbon is available to the heterotrophic bacteria as labile carbon. In addition, 220 g of oxygen are consumed and 363 g of carbon dioxide are produced.

The percent protein content of feed determines the ratio of autotrophic versus heterotrophic removal of ammonia-nitrogen. This is because of the direct relationship between protein content and quantity of ammonia-nitrogen that is generated and that only a fixed quantity of labile carbon is available from the feed. Using the same procedure as outlined previously, the ratio of autotrophic and heterotrophic removal was calculated for a
range of protein content in the feed (Figure 2). This figure shows that as the protein content of the feed increases, the percent removal of ammonia-nitrogen by the autotrophic pathway increases from complete removal by heterotrophic bacteria at 12.4% protein content to 75% removal of ammonia-nitrogen by the autotrophic pathway at 50% protein content.

**Heterotrophic bacteria – carbon supplementation**

Consider next a zero-exchange system where organic carbon is added to make up the difference between what is available from the feed and the total demand by the heterotrophic bacteria for complete conversion of all available nitrogen (Figure 3). From the above analysis, 32.5 g of nitrogen needs to be consumed by the additional heterotrophic bacteria from the supplemental organic carbon source. From Table 1, 8.07 g VSS$_H$ per g of N are produced, thus an additional 262 g VSS$_H$ are generated by the supplemental carbon. This additional VSS$_H$ requires 225 g of carbon, divided between organic carbon (197 g C$_S$(Substrate) metabolized by the heterotrophic bacteria and the depletion of inorganic carbon (28 g C$_{alkalinity}$). Thus the total VSS$_H$ generated is 406 g per kg feed. The research described later in this paper found a TSS to VSS ratio of 81%,

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*Figure 2. Percent removal of ammonia-nitrogen by heterotrophic or autotrophic processes as a function of % protein.*
which then suggests a total TSS\textsubscript{H} production of 500 g for every kg of feed. Thus a total of 349 g of C per kg of feed is consumed by this pathway, with the heterotrophic bacteria metabolizing all available organic carbon from the feed (109 g C\textsubscript{feed}) and the supplemental organic carbon (197 g C\textsubscript{S}) added to the system. In this case sucrose (C\textsubscript{12}H\textsubscript{22}O\textsubscript{11}) at 42% carbon was used requiring 470 g sucrose per kg feed. Concurrently, inorganic carbon as alkalinity was depleted (43.3 g C\textsubscript{alkalinity}) or 180 g of alkalinity as CaCO\textsubscript{3}. In addition 220 g of oxygen are consumed and 486 g of carbon

**Heterotrophic System: Organic Carbon from Feed**

\[
1 \text{ kg}_{\text{feed}} \times 0.36 \text{ kg BOD/kg feed} \times 0.40 \text{ kg VSS}_{\text{H}}/\text{kg BOD} = 144 \text{ g VSS}_{\text{H}}
\]
\[
0.124 \text{ g N}_{\text{H}}/\text{g VSS}_{\text{H}} \quad \text{0.531 g C}_{\text{H}}/\text{g VSS}_{\text{H}}
\]
\[
17.9 \text{ g N}_{\text{VSS}} \quad 76.5 \text{ g C}_{\text{VSS}}
\]
\[+ 47.1 \text{ g C}_{\text{CO}_2} = 123.6 \text{ g C}_{\text{labile}}
\]
\[
108.2 \text{ g C}_{\text{feed}} \quad 15.3 \text{ g C}_{\text{alk}}
\]

**Excess Ammonia-nitrogen:**

\[
50.4 \text{ g NH}_3-N - 17.9 \text{ g N}_{\text{VSS}} = 32.5 \text{ g N}_A
\]

**Heterotrophic System: Supplemental Organic Carbon**

\[
32.5 \text{ g N} \times 8.07 \text{ g VSS}_{\text{H}}/\text{g N}
\]
\[
0.124 \text{ g N}_{\text{H}}/\text{g VSS}_{\text{H}} \quad 0.531 \text{ g C}_{\text{H}}/\text{g VSS}_{\text{H}}
\]
\[
32.5 \text{ g N}_{\text{VSS}} \quad 139 \text{ g C}_{\text{VSS}}
\]
\[+ 85.5 \text{ g C}_{\text{CO}_2} = 224.5 \text{ g C}_{\text{labile}}
\]
\[
196.7 \text{ g C}_s \quad 27.8 \text{ g C}_{\text{alkalinity}}
\]

Carbohydrate is 40% Carbon ⇒ 492 g carbs

*Figure 3. Zero-exchange system with supplemental carbon addition of approximately 50% carbohydrate addition for 35% protein feed yielding a C/N ratio of approximately 13.0.*
Table 1. Comparison of autotrophic and heterotrophic bacterial in terms of production and consumption based on the stoichiometry (modified from Ebeling et al. 2006).

<table>
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<tr>
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<th>Heterotrophic</th>
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<tr>
<td><strong>C/N Ratio:</strong></td>
<td>4.28 g C/g N</td>
<td>4.28 g C/g N</td>
</tr>
<tr>
<td><strong>Yield (Y)</strong></td>
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<td></td>
</tr>
<tr>
<td>g VSS/g BOD*</td>
<td>0.16 g VSS_A / g BOD (0.1 – 0.3)</td>
<td>0.4 g VSS_H / g BOD (0.4 – 0.8)</td>
</tr>
<tr>
<td>g VSS/g N:</td>
<td>0.20 g VSS_A / g N</td>
<td>8.07 g VSS_H / g N</td>
</tr>
<tr>
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<td>0.12 g VSS_A / g C</td>
<td>1.33 g VSS_H / g C_s</td>
</tr>
<tr>
<td>g VSS/g sucrose:</td>
<td>----</td>
<td>0.56 g VSS_H / g sucrose</td>
</tr>
<tr>
<td><strong>Consumption</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g O_2/g N:</td>
<td>4.18 g O_2 / g N</td>
<td>4.71 g O_2 / g N</td>
</tr>
<tr>
<td>g C/g N:</td>
<td>1.69 g C / g N</td>
<td>6.07 g C_s / g N</td>
</tr>
<tr>
<td>g Alk (CaCO_3)/g N:</td>
<td>7.05 g Alk/ g N</td>
<td>3.57 g Alk/ g N</td>
</tr>
<tr>
<td><strong>Production</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g CO_2/g N:</td>
<td>5.85 g CO_2 / g N</td>
<td>9.65 g CO_2 / g N</td>
</tr>
<tr>
<td>g NO_3-N/g N:</td>
<td>0.976 g NO_3-N/g N</td>
<td>——</td>
</tr>
<tr>
<td><strong>Kinetic Rates</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>μ, specific growth rate</td>
<td>1 day^-1</td>
<td>5 day^-1</td>
</tr>
<tr>
<td>(range)</td>
<td>(0.4 – 2.0)</td>
<td>(2 – 10)</td>
</tr>
<tr>
<td>k_d, endogenous respiration (range)</td>
<td>0.05 day^-1</td>
<td>0.05 day^-1</td>
</tr>
<tr>
<td>(range)</td>
<td>(0.03 – 0.06)</td>
<td>(0.025 – 0.075)</td>
</tr>
</tbody>
</table>

C_s is carbon in substrate, i.e. carbohydrates or labile carbon in feed.

dioxide are produced, while 237 g of oxygen (50.4 g NH₃-N x 4.71 g oxygen per g of nitrogen produced) are consumed and 486 g of carbon dioxide are produced.

**MATERIALS AND METHODS**

The two pathways for nitrogen removal are very different in terms of substrate utilization, bacterial biomass generated, and by-products produced. The difficulty in practical application is that both may be present to some degree depending upon the availability of inorganic and organic carbon. The ability to control the C/N ratio by feed formulation, solids removal, or addition of organic carbon allows the aquaculture producer to manage what type of system is used. To examine this potential, a study was conducted where supplemental organic carbon in the form of the carbohydrate (sucrose) was added daily at 0%, 50% and 100% of the shrimp feed rate to three prototype zero-exchange systems. These systems had been operated for several months as marine shrimp juvenile production systems and all had well-developed and stable bacterial communities. The three systems were stocked with 675 *Litopenaeus vannamei* marine shrimp at a density of 150/m² with an initial average weight of 3.60 g.

**Juvenile Production System**

The juvenile production system (Figure 4) consisted of rectangular fiberglass tanks, measuring 1.22 m x 3.66 m x 0.76 m (4 ft x 12 ft and 30 in). Water depth was maintained at 61 cm (24 in) with an outside standpipe. Outside standpipes, 5 cm (2 in) in diameter were used to manage water removal and control water depth. A 7.6 cm (3 in) PVC drain line pipe was used to remove water or to harvest shrimp in bulk. In addition, a 1/4 in PVC mesh screen was placed at the discharge from the tanks. Tanks were initially covered with 1/4 in PVC mesh tops, but shade cloth was added within the first week to help reduce stress on the juvenile shrimp and limit growth of photoautotrophic algae.

Two titanium, 1.8 kW, 240 VAC bayonet style heaters were mounted in each tank to maintain system temperature at approximately 30 ± 2°C. Aeration in the tanks was provided by four 5 x 30 cm (2x12 in) air stones and two 3.66 m (12 ft) lengths of aeration hose on each side of the bottom of each tank. The aeration hose provided good mixing by creating two
counter-rotating cells along the long axis of the tank. Additional air stones were used when needed to maintain dissolved oxygen levels above target levels of 4.0 mg/L. Two automatic vibratory feeders hung above the tanks dispensed feed every 2 hours from 8 am to 10 pm. Fresh water was added as needed to make up for evaporation and other minor losses. A clarifier (Figure 4) was used to harvest suspended solids from the tank when the TSS approached 450 mg/L. Figure 5 shows the weekly average weight of a sample of approximately 50 to 100 animals. Over the first four weeks of

![Figure 4. Three juvenile shrimp production tanks showing automatic feeders and solids management clarifier.](image)

![Figure 5. The mean weekly weights of the marine shrimp showing an average growth rate of 0.90 g/week.](image)
growout, survival averaged 90% in the three tanks with an average feed conversion ratio (FCR) of 1.8. During this phase of research, the shrimp were seen primarily as ‘food processors’ for conversion of the feed to either small organic particles or fecal matter.

**Water quality analysis**

Dissolved oxygen, temperature, and salinity were measured daily between the hours of 0800 to 0900 h. At the same time, grab samples were taken and filtered through 8 - 12 µm filter paper (506-59 filter paper, Hach Company, Loveland, CO, USA) with the filtrate then used to determine dissolved constituent concentrations, TAN, nitrite-nitrogen, nitrate-nitrogen, pH, and alkalinity. In addition, daily samples were also analyzed for TSS and VSS. Weekly samples were analyzed for total organic carbon and total nitrogen. Standard methods were routinely used and, where appropriate, primary standards were analyzed along with the samples for quality assurance (Table 2).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method / Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO / Temperature</td>
<td>Hach Model 58 Dissolved Oxygen Meter</td>
</tr>
<tr>
<td>Salinity / Conductivity</td>
<td>Hach Model 33 S-C-T Meter</td>
</tr>
<tr>
<td>Nitrogen – Ammonia*</td>
<td>Hach Method 8038 Nessler Method</td>
</tr>
<tr>
<td></td>
<td>0 – 2.50 mg/L NH₃-N</td>
</tr>
<tr>
<td>Nitrogen –Nitrite*</td>
<td>Hach Method 8507 Diazotization Method</td>
</tr>
<tr>
<td></td>
<td>0 – 0.300 mg/L NO₂-N</td>
</tr>
<tr>
<td>Nitrogen -Nitrate</td>
<td>Hach Method 8039 Cadmium Reduction Method</td>
</tr>
<tr>
<td></td>
<td>0.0 – 30.0 mg/L NO₃-N</td>
</tr>
<tr>
<td>Total Organic Carbon</td>
<td>Hach Method 10173 Direct Method 15 to 150 mg/L as C</td>
</tr>
<tr>
<td>Total Nitrogen</td>
<td>Hach Method 10071 Persulfate Digestion Method</td>
</tr>
<tr>
<td></td>
<td>0 to 25.0 mg/L - N</td>
</tr>
<tr>
<td>Alkalinity*</td>
<td>Standard Methods 2320B as CaCO₃</td>
</tr>
<tr>
<td>Total Suspended Solids</td>
<td>Standard Methods 2540D</td>
</tr>
<tr>
<td>Total Volatile Solids</td>
<td>Standard Methods 2540E</td>
</tr>
</tbody>
</table>

*US-EPA approved for reporting, +Adapted from Standard Methods for the Examination of Water and Wastewater (APHA, 1998)

Table 2. Laboratory methods used for analysis via titration and Hach DR/2500 colorimeter.
RESULTS

Water quality

Water quality data for the three treatments over the research period is presented in Table 3. Overall water quality in all three systems was maintained within the range for optimal shrimp growth and survival. Note the substantial difference in nitrate-nitrogen (54.7 versus 7.7 mg/L) and alkalinity (183 versus 328 mg/L) between the control system and the two systems receiving supplemental carbon. Figures 6 through 9 show the impact of the three treatments (control, sucrose at 50% and 100% of feed rate) on TAN, NO$_2$-N, NO$_3$-N, and alkalinity over the 10-week research period.

There was a substantial difference in nitrate-nitrogen, alkalinity, and pH for the three treatments. Since the control tank received no supplemental organic carbon, it should exhibit water quality that is a combination of a heterotrophic and autotrophic system. For example, Table 1 shows a lower mean pH for the control versus the two other treatments, which would be expected in an autotrophic system because of the alkalinity reduction due to H$^+$ production. The impact of the autotrophic bacteria is especially apparent in Figures 8 and 9, with the increase of nitrate-nitrogen and the rapid decline in alkalinity. The alkalinity became so low that sodium bicarbonate was added on day 58 to increase it above the minimum recommended level of 150 mg/L (Timmons and Ebeling 2007). In all three systems, TAN increased slowly over the research trial, but was never higher than 1.5 mg/L –N. For the control, nitrite-nitrogen was typically less than 0.1 mg/L, although it reached a maximum of 0.2 mg/L near the end of the 10 week research period.

Table 3. Average water quality for the three treatments over the study period.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DO (mg/L)</th>
<th>Temp (°C)</th>
<th>Salinity (ppt)</th>
<th>pH</th>
<th>TAN (mg/L)</th>
<th>NO$_2$-N (mg/L)</th>
<th>NO$_3$-N (mg/L)</th>
<th>Alkalinity (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.1</td>
<td>29.5</td>
<td>4.8</td>
<td>7.78</td>
<td>1.15</td>
<td>0.13</td>
<td>54.7</td>
<td>183</td>
</tr>
<tr>
<td>StDev:</td>
<td>0.4</td>
<td>0.5</td>
<td>0.4</td>
<td>0.20</td>
<td>1.06</td>
<td>0.16</td>
<td>29.0</td>
<td>49</td>
</tr>
<tr>
<td>50% of Feed</td>
<td>5.7</td>
<td>29.8</td>
<td>4.5</td>
<td>8.15</td>
<td>1.06</td>
<td>0.39</td>
<td>7.7</td>
<td>328</td>
</tr>
<tr>
<td>StDev:</td>
<td>0.9</td>
<td>0.9</td>
<td>0.4</td>
<td>0.14</td>
<td>0.26</td>
<td>1.02</td>
<td>3.3</td>
<td>22</td>
</tr>
<tr>
<td>100% of Feed</td>
<td>5.3</td>
<td>29.4</td>
<td>4.7</td>
<td>8.19</td>
<td>1.36</td>
<td>0.61</td>
<td>1.9</td>
<td>360</td>
</tr>
<tr>
<td>StDev:</td>
<td>1.5</td>
<td>0.2</td>
<td>0.2</td>
<td>0.18</td>
<td>0.81</td>
<td>1.13</td>
<td>0.8</td>
<td>24</td>
</tr>
</tbody>
</table>
Both treatments (50% and 100% of feed as carbohydrate) exhibited similar pH values. The pH decreased slightly during the initial start-up phase, then increased and finally remained constant throughout the trial around a pH of 8.3. The direct conversion of ammonia-nitrogen to bacterial biomass in these systems is demonstrated in Figure 8, where the nitrate-nitrogen concentrations are either very low or at barely detectable limits. The limited number of autotrophic bacteria implies that very small quantities of nitrite-nitrogen or nitrate-nitrogen is produced. The higher than expected nitrite-nitrogen concentrations in the 50% of feed as sucrose (Figure 7) might be explained by a limited population of autotrophic bacteria that are inhibited by the high carbon/nitrogen ratios in the system from completing the conversion of TAN to nitrate (Zhu and Chen 2001, Michaud et al. 2006). Near the end of the growout, the concentration of nitrite-nitrogen was significantly reduced, although it should be noted that at no time was the concentration high enough to have any significant impact on the marine shrimp juveniles. The fact that the alkalinity (Figure 9) increased and then remained constant during the growout trial is unexplained. Theoretically, alkalinity should be consumed by the heterotrophic bacteria, although at a much lower rate than for an autotrophic system. One explanation might be the recovery

![Graph showing TAN for three treatments (control, sucrose at 50% and 100% of feed rate) over the 10 week research period.](image)

*Figure 6. TAN for the three treatments (control, sucrose at 50% and 100% of feed rate) over the 10 week research period.*
Figure 7. Nitrite-nitrogen for the three treatments (control, sucrose at 50% and 100% of feed rate) over the 10 week research period.

Figure 8. Nitrate-nitrogen for the three treatments (control, sucrose at 50% and 100% of feed rate) over the 10 week research period.
of alkalinity during some limited denitrification that may have occurred. Denitrification might be occurring in the interior of the large floc particles, where oxygen would be limited and anoxic conditions would prevail, which would potentially cause denitrification.

**Mathematical model**

A simple model to predict VSS and TSS concentrations in the three systems was written using an EXCEL® spreadsheet (Microsoft Office, Redmond, WA, USA). The three systems were modeled as a mixed autotrophic/heterotrophic system (control) and as a pure heterotrophic system (50% and 100% of feed as sucrose). As was shown earlier, the amount of sucrose required to fulfill the carbon requirement to consume all of the ammonia-nitrogen produced by the feed is approximately 470 g sucrose / kg feed, or 47% of the feed as sucrose. As a result, the system supplemented with 50% of feed as sucrose should be a pure heterotrophic system, the system supplemented with 100% of feed as sucrose should be overdosed, and the effect on resulting TSS is unknown.
In the case of the control, the model:

- allocated the daily feed organic carbon to heterotrophic bacterial production,
- calculated $VSS_H$
  \[ VSS_H = \text{feed g/m}^3 \text{day} \times 0.36 \text{ g BOD/g feed} \times 0.40 \text{ g VSS}_H / \text{g BOD}, \]
- calculated amount of ammonia-nitrogen assimilated in the $VSS_H$
  \[ TAN_H = 0.123 \times VSS_H, \]
- subtracted $TAN_H$ from the daily $TAN_{feed}$ produced
  \[ TAN_{feed} = \text{feed g/m}^3 \text{day} \times (0.35 \times 0.16 \times 0.9), \]
- allocated excess ammonia-nitrogen to autotrophic bacterial consumption \[ TAN_A = TAN_{feed} - TAN_H, \]
- determined $VSS_A$ \[ VSS_A = TAN_A \times 0.20 \text{ g VSS}_A/\text{g N}, \]
- calculated total VSS and TSS.

In the case of 50% of feed as sucrose, the model:

- allocated the daily feed carbon to heterotrophic bacterial production,
- calculated $VSS_H$
  \[ VSS_H = \text{feed g/m}^3 \text{day} \times 0.36 \text{ g BOD/g feed} \times 0.40 \text{ g VSS}_H / \text{g BOD}, \]
- calculated amount of ammonia-nitrogen sequestered in the $VSS_H$
  \[ TAN_H = 0.123 \times VSS_H, \]
- subtracted from the daily $TAN_{feed}$ produced
  \[ TAN_{feed} = \text{feed g/m}^3 \text{day} \times (0.35 \times 0.16 \times 0.9), \]
- allocated excess ammonia-nitrogen to additional heterotrophic bacterial production \[ TAN_{H+} = TAN_{feed} - TAN_H, \]
- determined $VSS_{H+}$ \[ VSS_{H+} = 8.07 \text{ g VSS}_H/\text{g N} \times \text{g N}, \]
- calculated total VSS and TSS.

Finally in the case of 100% feed as sucrose, it was observed that significant quantities of TSS were produced in excess of the available nitrogen. Thus the assumption was made that somehow there was sufficient nitrogen in the water column to react with all of the available carbon from the sucrose.
In the case of 100% of feed as sucrose, the model:
- allocated the daily feed carbon to heterotrophic bacterial production,
- calculated $VSS_H$
  \[ VSS_H = \text{feed g/m}^3\text{ day} \times 0.36 \text{ g BOD/g feed} \times 0.40 \frac{g\ VSS_H}{g\ BOD}, \]
- assumed all of the sucrose carbon was converted into bacterial biomass \[ VSS_{H+} = \frac{g\ sucrose}{m^3\ day} \times 0.56 \frac{g\ VSS_H}{g\ sucrose}, \]
- calculated total VSS and TSS.

In each case, the TSS values were estimated based on the long term average of the measured ratio of TSS to VSS determined during the course of this research period for the heterotrophic system.

The results of these models are shown in Figures 10 through 12. Figure 10 shows excellent agreement between the model and the actual measured TSS concentrations. The saw-tooth nature of the TSS data reflects the periodic harvesting of bacterial biomass using a cone-bottom clarifier. The model was restarted after each harvest of biomass from the tank using the experimentally determined TSS value for the starting point. The control tank required solids culling approximately every three weeks in order to maintain tank TSS concentrations below 450 mg/L.

![Figure 10. Predicted and measured TSS concentration for an autotrophic / heterotrophic system without carbon supplementation with periodic harvesting of excess bacterial biomass.](image-url)
Analysis of bacterial control of ammonia-nitrogen in shrimp production systems

Figure 11. Predicted and measured TSS concentration for a heterotrophic system with carbon supplementation at 50% of feed rate as sucrose and periodic harvesting of excess bacterial biomass.

Figure 12. Predicted and measured TSS concentration for a heterotrophic system with excess carbon supplementation at 100% of feed rate as sucrose and periodic harvesting of excess bacterial biomass.
Figure 11 reflects what would occur if sufficient carbon supplementation was available to completely convert all metabolic ammonia-nitrogen to bacterial biomass. The model predictions and the observed data agree quite closely, although in some cycles the model tended to over predict TSS values as a solids harvesting event was about to occur. Due to the rapid production of biomass, the production system was culled of excess bacteria on average every ten days.

The results of excess carbon supplementation (100% of feed as carbohydrate), in this case twice what is stoichiometrically required, is shown in Figure 12. The assumption that there was sufficient nitrogen to react with all of the available carbon from the sucrose appears to be verified in this instance. The source of this nitrogen, which is beyond that provided by the shrimp feed, is unknown. One of the problems with excess carbon supplementation is the large quantity of bacterial biomass that is generated, requiring frequent (every five days) harvesting of excess biomass.

**Dissolved organic carbon and total nitrogen**

Figure 13 shows the dissolved organic carbon (DOC) concentration in the three treatments over the ten week research trial. As can be seen, there appears to be no major difference in the DOC between treatments and there was a consistent increase in the DOC over the growout period. This is probably the result of the gradual buildup in all the systems of humic substances, the ‘tea’ color seen in intensive recirculation systems that accumulates when ozone or UV is not used to remove it. Humic substances correspond to the non-biodegradable part of the dissolved organic carbon and are not available as a carbon source to the bacteria. Humic substances are hydrophobic dissolved organic matter produced by the auto-oxidation of polyunsaturated fatty acids released by fish feces, uneaten feed, and the lysis of dead bacteria.

Figure 14 shows the results of a mass balance on nitrogen for the autotrophic/heterotrophic system without carbon supplementation and with periodic harvesting of excess bacterial biomass. The amount of total nitrogen (Total Nitrogen - Model) was calculated using the VSS concentrations predicted by the previously presented model and assuming it contained 12.4% nitrogen based on the stoichiometry of bacterial biomass. Total Nitrogen - Experimental Data represents the
Figure 13. Dissolved organic carbon (DOC) concentrations for the three treatments (control, sucrose at 50% and 100% of feed rate) over the 10 week research period.

Figure 14. Mass balance on nitrogen for the autotrophic/heterotrophic system without carbon supplementation and with periodic harvesting of excess bacterial biomass.
sum of the nitrogen contained in the experimentally measured VSS plus experimentally measured concentrations of TAN, NO$_2$-N, and NO$_3$-N. The Measured Total Nitrogen is the sum of the nitrogen contained in the experimentally-measured VSS plus the experimentally-measured Total Nitrogen. Finally, the total nitrogen-feed is the estimated nitrogen content of the feed (35% protein), 0.0504 kg N/kg feed.

In Figure 14, the stair step nature of total nitrogen can be seen as bacterial biomass is removed from the system even as the cumulative total nitrogen from the feed steadily increases. The experimentally measured value for total nitrogen falls below the model for several possible reasons including the difficulty in measuring nitrate-nitrogen accurately with the analysis methods employed and the impact of denitrification, especially noticeable near the end of the research period. The use of total nitrogen appears to do a better estimation of the nitrogen and also shows a reduction near the end of the research period, most likely due to denitrification. Interestingly, over the growout period almost all the nitrogen remains in the system.
Figure 15 shows the impact of carbon supplementation at 50% of the feed as sucrose on the system with excess bacterial biomass and nitrogen being periodically removed from the system. Since this is a pure heterotrophic system, there is no nitrate-nitrogen created. Thus the system’s total nitrogen remains at very low levels, fluctuating within a very narrow range, even as the cumulative total nitrogen steadily increases. The system supplemented at 100% of feed as sucrose showed similar characteristics, except for a greater rate of increase in nitrogen per harvesting cycle and a need for more frequent culling of biomass.

CONCLUSIONS

The pathways for nitrogen removal are very different in terms of substrate utilization, bacterial biomass generated and by-products generated. Using simple stoichiometry for autotrophic and heterotrophic bacteria, it is possible to characterize and model the two pathways for nitrogen removal. The difficulty in practice is that each bacterial pathway may be present to some degree and the bacterial communities associated with each will compete for the same substrate, possibly resulting in dominance by one group over another. The ability to control the carbon to nitrogen ratio by feed formulation, solids removal, or addition of organic carbon allows the aquaculture producer to manage what type of system is created.

ACKNOWLEDGEMENTS

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Analysis of bacterial control of ammonia-nitrogen in shrimp production systems


Analysis of bacterial control of ammonia-nitrogen in shrimp production systems
BOOK REVIEW

Anaesthetic and Sedative Techniques for Aquatic Animals, Third Edition

L. Ross and B. Ross

Reviewed by:
Stephen A. Smith, DVM, PhD
Virginia-Maryland Regional College of Veterinary Medicine
Virginia Tech
Blacksburg, VA 24061 USA

The third edition of this book is a welcome revision of the expanding literature on anesthesia, sedation and euthanasia methods used in aquatic animals. Chapters cover such topics as defining and identifying stress in aquatic animals, an introductory overview of anesthesia principles, factors affecting anesthesia in aquatic animals, the use of anesthesia in the transportation of aquatic animals, and chapters on anesthesia of fish, amphibians and reptiles, and selected aquatic invertebrates.

The book provides a brief overview of international regulations with respect to the use, food safety, and environmental safety of these chemicals and techniques. The two major advancements of the book are the addition of a chapter on pain in fish and the revision of the chapters on anesthesia techniques in fish. Taking into consideration the expanding field of animal welfare, the authors include a new chapter summarizing the current literature on nociception in aquatic animals, and supports the conclusion of many researchers that even though aquatic animals may not perceive or experience pain in the same manner as higher vertebrates, the precautionary principles should be adopted when utilizing potentially stressful or painful techniques.
The revised chapters on individual anesthetic compounds categorized by route of administration (inhalation, oral, parenteral) have been completely reorganized and expanded to incorporate essential new information and updated references. Each anesthetic compound described has detailed information on the chemical nomenclature, structure, mode of action and recommended dosages. In addition, pertinent safety and toxicology information is included for each compound.

As in the past edition, the book includes a chapter on non-chemical methods of anesthesia such as hypothermia and electroanesthesia. Unfortunately, and most likely due to a paucity of available literature, this section is not revised significantly and still avoids discussing these techniques in terms of current animal welfare considerations. Another minor deficiency of the book is the use of photographs from the older edition. For instance, the authors could have added more up-to-date photographs of the anesthesia machine (Figure 8.6) and IM injection of fish (Figure 10.3), and several photographs depict handling fish and amphibians bare-handed without protective gloves (Figures 8.3 and 13.1). The authors should have also included the more current 2007 reference to the AVMA Guidelines on Euthanasia instead of the 2001 reference. Aside from these few shortcomings, the book is an easy-to-follow, well-written treatise on anesthesia of aquatic animals. Compared to the previous edition, the publisher has set this work in a much more acceptable layout and font for ease of reading. Thus, this book remains an essential resource for anyone working with aquatic animals including primarily fish, but also amphibians, reptiles and aquatic invertebrates and will serve as a practical guide for veterinarians, researchers, and personnel in fisheries, aquaculture, and aquarium settings.
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example: Times New Roman 12pt Italic BOLD

Times New Roman 10pt Italic BOLD

Subsection headings should be italicized in the manuscript. Spell out one-digit numbers, except when they are used with units of measure – e.g., six ponds, 7 days. Spell out any number that begins a sentence.
When two numbers occur sequentially in the text, one of the numbers must be spelled out – e.g., In 2007, fifty fish were stocked. Use commas in numbers of 1,000 or more. Use the 24-hour clock to describe time – e.g., 1300, not 1:00 p.m. Always place a zero to the left of a decimal point if the number is less than one; this includes probability values – e.g., P = 0.05, not P = .05. All units of measurement must be reported via the metric system. Parts per million or milligrams per liter should be reported as either “ppm” or “mg/l,” but not as “mg L⁻¹.”

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Commonly used aquaculture jargon can be used without defining the term – e.g., fry, fingerling. Less common jargon may be used, but terms should be defined the first time they are used. Jargon from fields outside of aquaculture should also be defined the first time they are used.

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Articles and notes should include a title, names of authors and their addresses, abstract, introduction, materials and methods, results, discussion (or a combined results and discussion), conclusion (if needed), acknowledgements (if any), references, figures, and tables, in that order.

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