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Belize Aquaculture, Ltd. successfully uses a zero-water exchange production strategy to reduce effluents, increase biosecurity, and generate high yields. Shrimp grown under heterotrophic conditions here have an excellent flavor and are well received in the marketplace. The discussion begun in the December 2000 issue of the Advocate on development of the heterotrophic systems in shrimp ponds at Belize Aquaculture is concluded here.

Organic Material and C:N Ratio

In addition to high stocking rates and adequate pond aeration, the final factor that must be considered in the development of a heterotrophic system is the amount of organic material being fed to a pond, and the carbon/nitrogen balance of that material. Bacteria feed on organic material, and in order to establish a healthy bacterial population, there must be sufficient organics in the water. Equally important is a balance of other nutrients, such as nitrogen, required by bacteria for growth.

In order to transition from an autotrophic to a heterotrophic community, it is necessary to add significantly higher amounts of organic material – with a balanced carbon/nitrogen ration – than the shrimp will consume. Initially, be-
fore we understood our system, we added feeds and organ-
ic material with an average carbon/nitrogen ratio of 11:1
(protein level of 30%) at Belize Aquaculture.

This resulted in a system that was out of balance, hav-
ing too much nitrogen for the amount of carbon present.
Pond water and bottoms became fouled, and sulfides were
often detected. Raising the carbon/nitrogen ratio to 16:1
(protein level of 22%) resulted in a very healthy het-
erotrophic community that appears to be more in balance.

A grain-based pellet with a high carbon/nitrogen ratio
of 20:1 (protein level of 18%) is used to counterbalance the
lower carbon/nitrogen ratios of the shrimp diets being fed.
These pellets are added at a rate of 250 kg/ha several days
before stocking, and at a rate of 220% of biomass for the
first week after stocking (representing 90% of the organic
input). This overfeeding continues until the shrimp grow
to 3 grams, at which time feed rates are determined by con-
sumption patterns established by monitoring feed trays.

**Molasses Boosts Initial Growth**

Recently, we found the use of molasses in ponds can
speed up the transition between the autotrophic and het-
erotrophic states. Applying 50 l/ha of sugar cane molasses
twice a week to recycled water (nitrogen level of 3 ppm)
lead to the development of bacterial flocs within the sec-
ond week of culture.

Initial shrimp growth rate increases in these ponds were
significant, reflecting juveniles that weighed 2.5 grams
after four weeks of growout. After the bacterial flocs are
established, however, there were no obvious advantages to
continuing the addition of molasses.

**Types of Bacterial Communities**

Not all heterotrophic communities are the same. Belize
Aquaculture has identified three distinct types of bacterial
communities: brown flocs, black flocs, and green flocs.
Good shrimp crops have been obtained with all three com-
munity types, but we prefer the brown flocs, as shrimp from
these ponds have shells with a shiny, healthy appearance
and negligible scarring.

Shrimp from ponds with black flocs always develop
black gills. It has been determined that black flocs have
much higher iron levels than do brown flocs (Rod McNeil,
personal communication). I believe black gills are the re-
sult of colonization in the gill tissue of shrimp by the same
bacteria that make up the black flocs. The bacteria deposit
iron on the shrimps’ gill lamellae.

Black flocs developed approximately 40% of the time
during Belize Aquaculture’s first two years of operation
(1998-1999), but they occurred in only 5% of the ponds in
2000. Brown flocs now predominate, and black flocs de-
veloped in only 5% of the ponds in 2000. By pumping
water from a pond with brown flocs into newly filled
ponds, some control seems to be possible in selecting the
type of bacteria community that will dominate the pond.

An interesting question is why bacteria so completely
dominate the ecology of the system. The pond water always

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**Most ponds are dominated by planktonic communities based on algae, which are termed autotrophs, because they are capable of synthesizing their own food using light energy. Heterotrophic organisms cannot synthesize their own food, and are dependent on complex organic substances.**

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Shrimp harvested from ponds with black flocs tend to develop black gills (circled above), presumably due to iron deposition.
has sufficient nutrients to grow phytoplankton, but about the only phytoplankton observable are a few phytolflagellates, which are known to be capable of both autotrophy and heterotrophy. There is sufficient sunlight, and ponds would typically have a transparency of 30 cm using a Secchi disk. A possible explanation is the production of lysozymes by the bacteria, which digest phytoplankton cells. This would also explain why the system has such a large capacity to handle feeding rates as high as 500-600 kg/ha/day.

**Water Chemistry Dynamics**

Plastic-lined ponds that do not exchange water develop some interesting water chemistry dynamics. After initial stocking, oxygen levels are typical of semi-intensive non-aerated ponds. As the shrimp biomass increases, and the system converts to heterotrophy, the diurnal difference in oxygen levels becomes less pronounced (Figure 1).

Instead of oxygen being produced by photosynthesis, more CO₂ is produced by both bacterial and shrimp respiration. Free CO₂ levels can rise to 15 ppm at the end of a cycle, and it may be CO₂ that eventually becomes the factor that limits shrimp growth.

Increases in CO₂ levels also result in a lowering of pond water pH to a range of 6.8-7.3. The Litopenaeus vannamei cultured at Belize Aquaculture do not seem to react adversely to this pH range. It is probable the lowered pH is beneficial, in that it reduces the level of toxic unionized ammonia.

Ammonia-nitrogen levels increase to 8-12 ppm before starting to decline around weeks six to eight. As would be expected, nitrite and nitrate levels begin to increase. Ammonia-nitrogen levels generally decline to less than 3 ppm after week eight of the cycle, but nitrite-nitrogen levels generally do not decline and usually range from 3 to 8 ppm. It appears the system is still limited by carbon relative to nitrogen. The addition of molasses, which further enriches the carbon relative to nitrogen, results in a further decline in both ammonia and nitrite levels, supporting the idea that the system is carbon-limited.

Alkalinity levels (Figure 2) can decline to less than 20 ppm if there is no supplementation of liming or carbonate materials. It is assumed the reduction in alkalinity is the result of nitrification processes that are taking place within the system.

Total phosphorus levels (Figure 3) increase linearly with time, and can reach levels higher than 25 ppm as phosphorus. When phytoplankton dominate the culture system, orthophosphate represents less than 20% of the total phosphorus. When the system becomes heterotrophic, orthophosphate can represent 80% of the total phosphorus. It appears the bacteria do not store phosphorus, as many algae and phytoplankton do.

Because the ponds are lined with high-density polyethylene plastic liners, and the water does not contact bottom clay or other ionic materials that can result in the absorption and removal of phosphorus from the water, phosphorus levels build up with time. However, so far no negative impacts on shrimp growth or health have been attributed to the high phosphorus levels.

Because there is no water exchange, the idea that pond water must have the appearance of thick “pea soup” was popularized. After the heterotrophic state and flocs are es-

**Figure 1.** Oxygen levels during a 20-week culture cycle show a diminishing difference between morning and afternoon values due to increasing dominance by bacteria.

**Figure 2.** Alkalinity drops steadily during a 20-week culture cycle if not prevented by the addition of lime.

**Figure 3.** Total phosphorous accumulation during a 20-week culture cycle in ponds filled with seawater or with recycled water from a previous production cycle.
established, however, typical Secchi transparency is between 25 and 35 cm.

**Management**

Management of intensive systems using zero water exchange can be easier than management of well-run semi-intensive farms. Heterotrophic systems are forgiving when it comes to feeding, and do not require constant monitoring of environmental parameters, or nutrient levels so that fertilization rates can be calculated daily. Heterotrophic systems also do not have a “Ph.D. requirement” to run successfully. Belize Aquaculture’s ponds are managed by a group of dedicated technicians that have high school equivalency only.

Although heterotrophic systems are forgiving in the area of pond management, there is a tradeoff in the area of machine maintenance. A dedicated team is needed to maintain aerators, generators, and electrical systems.

**Useful Chemicals**

In the development of the zero-water-exchange culture system, several chemicals have proven useful, including lime, silicate cake, and a product called Aquaculture Pond Stabilizer™ (PVS Technologies, Detroit, Michigan, USA). Hydrated lime or the equivalent is added at a rate of 100 kg/ha every 14 days, starting at week seven in the cycle. This dosage seems to maintain pond alkalinity in the range of 75-100 ppm.

Silicate cake, a by-product of the clay mining industry, is composed of 25-45% zeolite, 15-30% kaolin, and 5-15% sodium silicate. Although this product is not essential, we believe the small particles of the material act as an agent to initiate nucleation of the bacteria into flocs. Silicate cake is added during pond preparation at a rate of 250 kg/ha, and supplemented at 100 kg/ha every four weeks. There are certainly other materials that can be used to facilitate the nucleation of the bacteria into flocs.

Aquaculture Pond Stabilizer is a product that has been effective in neutralizing sulfides that build up in areas of sludge accumulation. Application is made with aerators on, so the material settles into areas where sludge is accumulating. There the material binds and precipitates the sulfide in a non-toxic form. Initial trials with the product have resulted in positive production trends.

**Conclusion**

Heterotrophic bacterial communities facilitate the production of high yields (~15 mt/ha/cycle) in ponds with zero water exchange. Factors that have helped improve the efficiency of this system include:

- seeding of newly filled ponds with brown floc
- early addition of molasses to advance floc development
- use of feed with high C:N to avoid nitrogen accumulation
- use of lime to maintain alkalinity
- use of silicate to nucleate flocs
- use of Aquaculture Pond Stabilizer to neutralize sulfides.