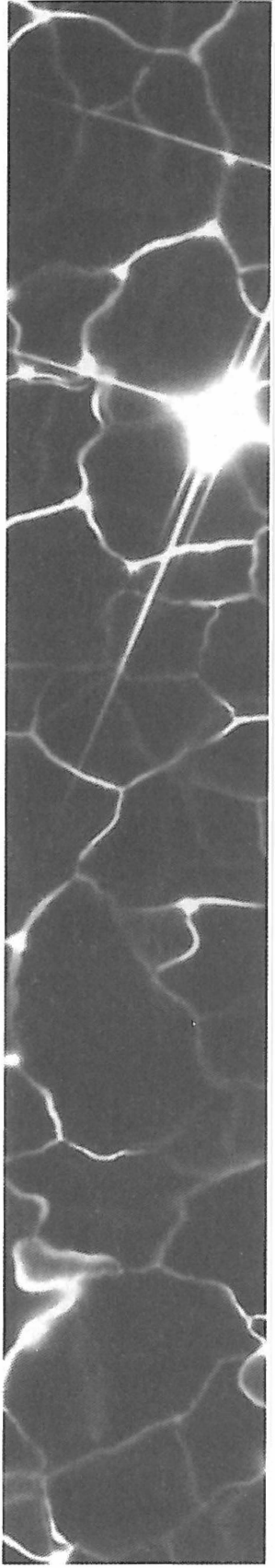
International Journal of Recirculating Aquaculture



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Using the Pond as a Biofilter: Review of Theory and Practice

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Keywords: fish, shrimp, suspension pond, water treatment, recirculating aquaculture, pond, biofilter

ABSTRACT

Intensive aquaculture systems are being used to efficiently produce fish and shrimp. However, an intrinsic problem of these systems is the rapid accumulation of feed residues, organic matter, and toxic inorganic nitrogen species. This cannot be avoided, since fish assimilate only 20-30% of feed nutrients. The rest is excreted and typically accumulates in the water. Often, the culture water is recycled through a series of special devices (mostly biofilters of different types), investing energy and maintenance to degrade the residues. The result is that in addition to the expense of purchasing feed, significant additional expenses are devoted to degrade and remove two-thirds of it.

There is a vital need to change this cycle. One example of an alternative approach is active suspension pond (ASP) systems where the water treatment is based upon developing and controlling heterotrophic bacteria within the culture component. Feed nutrients are recycled, doubling the utilization of protein and raising feed utilization. Other alternatives, mostly based upon the operation of a water treatment / feed recycling component besides the culture unit, are also relevant.

Active suspension ponds are being practiced and their numbers have increased dramatically during the last 10 years, most notably with shrimp culture. The purpose of this paper is to raise discussion on alternative routes to the classical recycling approach.

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INTRODUCTION

There is a natural desire to achieve higher and higher yields. However, getting listed in the *Guinness Book of World Records* is not the goal of an aquaculture business. The justification for intensification stems from specific culture, environmental and economic reasons. Several reasons for intensification, listed here, have different priorities under different conditions.

1. Environmental regulation prohibiting or limiting water use and disposal.
2. Biosecurity concerns limiting water intake.
3. Water scarcity or cost. Conventional aquaculture usually uses 2-10 m^3 water to produce 1 kg fish. In Israel, for example, water costs are rising to ~0.4/m^3 (US$), i.e., 0.8-4.0 $/Kg fish.
4. There is a demand for product quality control and transparency, which are otherwise difficult to achieve in intensive systems.
5. Feed utilization may be higher than in conventional systems.
6. In cases where production occurs close to a major market, space limitations are also of concern.
7. Intensification enables easier temperature control.
8. Intensification and automation may save labor costs.

However, intensification costs money, and is not always the recommended mode of development.

DISCUSSION

Development and Modes of Intensive Aquaculture Systems

The evolution of pond intensification can be better seen in perspective by looking at the whole spectrum of pond intensity, as given in Table 1.

Feed, generally, did not limit fish growth once fed ponds were introduced. The limiting factor in fed ponds was usually early-morning low oxygen conditions. With aeration, though partial and not aerating the whole pond area and volume, there is enough oxygen to support the fish, and it can usually be assumed that oxygen is not a limiting factor. The next limitation is the high rate of organic matter accumulation on the bottom of the pond,

development of anaerobic conditions and production of toxic metabolites (Avnimelech and Ritvo 2003), retarding further intensification. This was overcome by thoroughly mixing the pond and aerating it 24 hours/day, enabling growers to raise yields to levels of up to 100 kg m^{-3}.

Fish (and shrimp) can be grown at very high density in aerated – mixed ponds. However, with the increased biomass, water quality becomes the limiting factor due to the accumulation of toxic metabolites, the most notorious of which are ammonia and nitrite. To realize the potential of aerated – mixed ponds, water quality has to be controlled.

Three different approaches can be used to control water quality:

(a) Replace pond water with fresh water, usually at exchange rates of over five times a day. This option, though, is in conflict with environmental constraints, biosecurity needs, and water-scarcity issues.

Table 1. Levels of pond intensification: Schematic representation.

POND TYPE	HUMAN INTERVENTION	Approximated YIELDS (kg/ha*yr)	LIMITING FACTOR
Minimal feed	Feeding with grain, farm & home residues. Fertilizers	2,000	Limit of primary productivity. Food chain efficiency
Fed Ponds	Feeding by complete diet pellets	4,000	Night time oxygen deficiency
Night time aeration	Night time or emergency aerators ~1-5 hp/ha	10,000	Sludge accumulation. Anaerobic pond bottom
Intensive mixed –aerated ponds	24 h/day aeration (~ >20 hp/ha), constant and full mixing	20,000 – 100,000	Water quality control

(b) Recycle the water through an external unit ("biofilter") that treats and purifies the water.

(c) Treat water quality within a pond system, using algae in partitioned aquaculture ponds, (Brune *et al.* 2003) or bacterial communities (e.g. active suspension ponds, ASP).

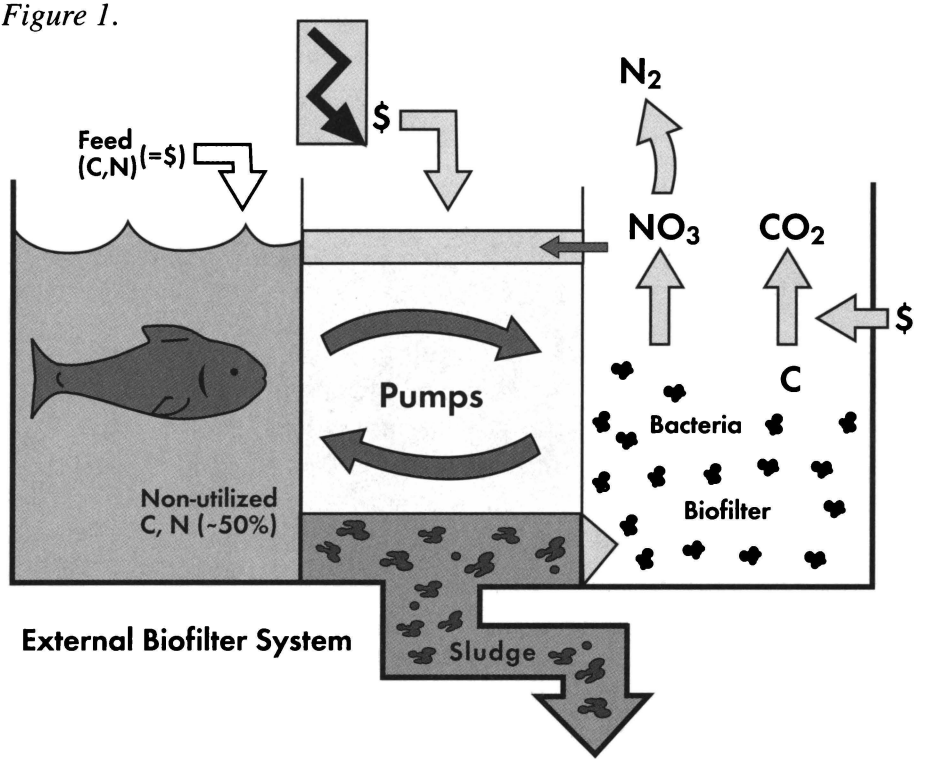
The use of external biofilters (schematically shown in Figure 1) has been practiced for years in hatcheries, nurseries, culturing of ornamental fish, and to some extent, in culturing of commodity fish. These systems are operative, well-tested, proven, and can be obtained commercially. However, they are quite costly, both in investment and in operation. As an example, we can compare wastewater treatment plants' required biofiltration capacity. Taking an average chemical oxygen demand (COD) in raw municipal wastewater as 600 mg/l and wastewater production of 300 l/capita x day, we get a COD release of 180g/capita x day. A town of 10,000 inhabitants has to treat 1800 kg COD/day. In an equivalent fish farm, about 20kg feed is given per ton of fish each day. About half of it is released to the water, i.e. 10 kg COD/ton x day. A fish farm holding 180 tons of fish emits about the same load as the 10,000-inhabitant town. Moreover, the standards and demands in fish water treatment are generally higher than in wastewater treatment. The latter releases treated water having more than 10 mg total ammonia nitrogen (TAN) per liter, while in fish farming, less than 1mg/l is standard (in Israel).

An additional basic feature of the "biofilter" approach is the rapid removal of feed residues. According to classical biofilter design parameters, one removes unused feed or feed residue as fast as possible, in contrast with the "in pond" method, which strives to recycle the non-utilized feed as much as possible.

Research efforts of the last decades were (and are) directed to lower the cost of biofilter systems, raise the efficiency of water treatment, oxygen introduction, and utilization of energy input. Efforts to maximize feed utilization and recycling have been meager. Yet, feed cost is the biggest component in the cost of producing fish in intensive systems.

Intrinsic features of intensive ponds are high aeration rate and thorough mixing. These features, obtained as existing features of the pond, are the ones that we find in almost all biotechnological industries as features maximizing the activity of microorganisms. An additional characteristic that encourages microbial dominance in intensive ponds is the

Figure 1.



accumulation of organic substrates in zero or limited exchange ponds. The organic residues mixed in the water serve as a growth substrate for bacteria, leading to a transition of the pond to a more and more heterotrophic system. Achieving high heterotrophic biomass and providing optimal conditions toward their activity is an intrinsic trait of intensive ponds.

The Nitrogen Syndrome

An intrinsic problem in intensive ponds is the nitrogen syndrome. Inorganic nitrogen accumulates in the pond due to several reasons. Fish metabolize proteins as an energy source (Hepher 1988), leading to the excretion of ammonia that accumulates in the pond. Moreover, while organic carbon in the pond is metabolized to CO_2 that leaves the pond to the atmosphere, the transformation of inorganic nitrogen is not effective in getting the nitrogen out of the system (unless intensive nitrification and subsequent denitrification take place). As a result, the C/N ratio continually narrows with intensification and time, with the result that toxic ammonia and nitrite levels may endanger fish growth and health.

The nitrogen syndrome can be controlled by utilizing the microbial system that exists in intensive ponds. A straightforward solution is to raise the C/N ratio, counteracting the nitrogen deterioration trend. Adding carbon-rich and nitrogen-poor feed, the following processes take place (Avnimelech 1998):

$$\text{Organic C} \rightarrow CO_2 + \text{Energy} + \text{C assimilated in microbial cells,} \quad (1)$$

where the ratio of C assimilated to the organic carbon metabolized is defined as the microbial efficiency (E).

For the creation of new proteinaceous cell material, microorganisms need to take up inorganic nitrogen (preferably ammonium). Adding carbonaceous material (CH) leads to the immobilization of inorganic nitrogen into the microbial protein pool (Equations 2 and 3).

$$\Delta C_{mic} = \Delta CH \times \%C \times E \quad (2)$$

$$\Delta N = \Delta C_{mic} / [C/N]_{mic} = \Delta CH \times \%C \times E / [C/N]_{mic} \quad (3)$$

where ΔCH is the amount of carbohydrate fed into the pond, ΔC_{mic} is the amount of carbon assimilated in microbial cells, %C is the percentage of carbon in the added feed, and $[C/N]_{mic}$ is C/N ratio in the microbial cells.

The amount of carbonaceous feed needed to remove one unit of inorganic nitrogen, ΔN, following Equation 3 (using approximate values of %C, E, and $[C/N]_{mic}$ as 0.5, 0.4 and 4, respectively) is:

$$\Delta CH = \Delta N/(0.5 \times 0.4 / 4) = \Delta N/0.05 \quad (4)$$

The equations given here, as well as others defining microbial kinetics and input-output data were used to model nitrogen transformation in active suspension ponds (Kochba *et al.* 1994). Nitrogen control using carbon addition is predictable and controllable. A more comprehensive modeling effort has been initiated by Bergeron *et al.* (2004), a model covering both carbon and nitrogen fluxes in ASP. Inorganic nitrogen in intensive ponds, through the manipulation of C/N ratio, is easily controlled, predictable, and inexpensive as cheap carbohydrates can be used.

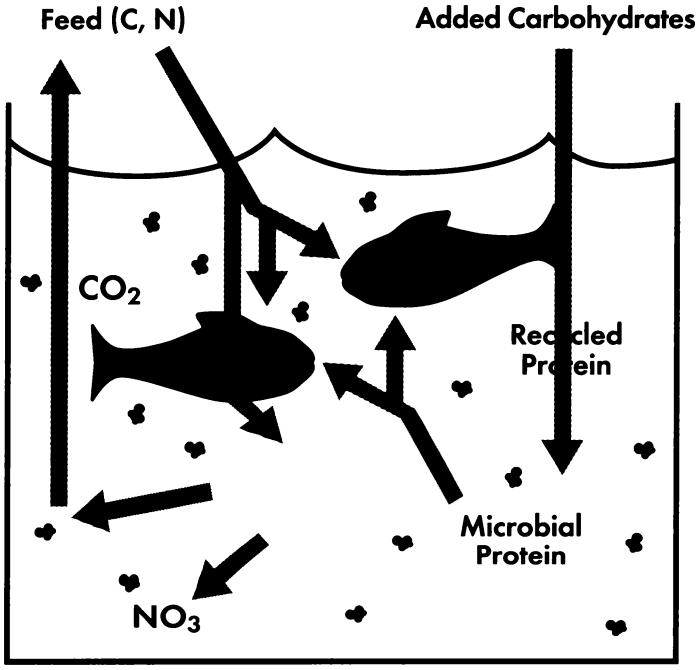
In addition to controlling inorganic nitrogen concentrations in the pond, the uptake of nitrogen by bacteria is in essence a process that enables the recycling of protein. The ammonium excreted as a waste material of the fed protein is reclaimed as microbial protein. The microbial biomass, when aggregated as microbial flocs, is a good source of protein for tilapia and

shrimp. Both McIntosh (2001) and Avnimelech *et al.* (1994) found that the utilization of protein, conventionally around 25% (Boyd and Tucker 1998), increases to about 45% in both shrimp and tilapia ASP ponds.

These findings were further elaborated by studying floc formation and characteristics in very detailed works published by Tacon and co-workers (Decamp *et al.* 2003, Tacon *et al.* 2002). It was found that there is more than 30% protein in the flocs, containing essential amino acids in sufficient quantities. In addition, it was demonstrated that the microbial flocs contain vitamins and trace metals, enabling emission from the feed, saving a significant fraction of the feed cost.

An important contribution to our understanding of ASP systems was made by the works of Burford and co-workers (2003) based on detailed studies of ponds in Belize. The uptake and utilization of microbial flocs by shrimp was evaluated using N^{15}-tagged flocs (Burford *et al.* 2004). The proportion of daily nitrogen uptake of the shrimp contributed by the natural biota was calculated to be 18-29%. Similar, though qualitative, results were found by Avnimelech *et al.* (1989), derived from the evaluation of the C^{13}/C^{12} ratios in feed and tilapia muscle samples.

Figure 2.



Activated Suspension Pond

The utilization of microbial flocs as a source of feed protein leads to a lower expenditure on feed. Avnimelech reported that feed cost for tilapia production was reduced from $0.84/kg of fish in conventional ponds to $0.58 in ASP. McIntosh (2001) reported that feed cost using the reduced protein diet in Belize ponds is about 50% as compared to conventional shrimp farming.

Protein is an expensive feed component. Generally, it is at least partially made of fish meal, a component that is becoming increasingly scarce as concerns increase over environmental damage and overharvesting in the oceans. The fact that protein utilization rises from 15-25% in conventional ponds to 45% in ASP is very important economically and environmentally.

The transition from algal-controlled conventional ponds to ponds with heterotrophic bacterial control has many implications. Algal activity is sensitive to environmental conditions, firstly to fluctuating light intensity. Heterotrophic bacteria are less dependent on environmental variability in ponds (Avnimelech 2003). The transition toward heterotrophic systems enables better control of the pond and is in essence a transition toward the change of aquaculture to a biotechnological industry. As an industry, it should follow a clear set of design parameters. Detailed ones have not been developed yet, but there are clear principles that should guide design of ASP ponds. Oxygen should not be a limiting factor. Aeration capacity on the order of 30 hp/ha is commonly used in shrimp ponds (ca 1 hp per 500 kg shrimp biomass), and higher aeration (more than 100 hp/ha) for more intensive tilapia ponds. In southern California, it was found that using pure oxygen may be more economical than using aerators (Dean Farrel, Seagreen Assoc., personal communication); however, this can be different in places where pure oxygen is more expensive. Ponds should be perfectly mixed, avoiding any stagnant zones where organic sludge might accumulate. Presently, the best aeration/mixing devices are paddle wheel aerators, placed radially in the pond, at a distance from the dikes of about one third the pond width. Aspirator-type aerators (or air lifts in small ponds) should augment the paddlewheels, in such a way that sludge settling near the center of the pond is resuspended. However, there is a need for aerators that are better designed and adjusted to ASP demands. Aerator placement and pond design should be made to prevent the formation of sites in the pond where sludge accumulates. However, it is difficult and not desired to resuspend the full amount of sludge generated. There is a need to concentrate the excessive sludge at a point in the pond and to drain it out. The common way to do it is by constructing

a sludge disposal pit in the center of the pond and periodically draining it. Sludge is drained daily (Avnimelech 1999), or even more frequently, in tilapia ponds and about weekly in shrimp ponds (Burford *et al.* 2003).

Size of intensive ponds varies from few dozen square meters to almost 2 ha. It is more difficult to control large ponds, yet, as demonstrated by Belize Aquaculture, it is possible to properly manage 1.6 ha ponds.

Anticipated Future Developments

How will ASP look in another 10 years? According to what we know of present plans to construct such ponds worldwide, it seems that in another 10 years, we will have many such ponds and vast practical information will be collected.

On initiating and developing ASP systems, the overall microbial activity has been considered, but very little is known as to the details of the relevant microbes and microbial ecology. Work done by Burford *et al.* (2004) and by Tacon *et al.* (2002) initiated efforts to better understand and control the microbial processes. McIntosh (2001) started with the selection of bacteria that form flocs. It is anticipated that with interest in ASP more studies will be made and more insight will be obtained. Specifically, it is anticipated that more control of floc formation will be obtained, in line with similar work done in water treatment technology.

Feeds and feeding of ASP systems are in their beginnings. We need specially formulated feeds with lower protein. Panjaitan (2004) recently demonstrated that the feed requirement in ASP shrimp systems is just about 70% of that needed in open systems where feed is not recycled and the non-eaten portion is wasted. Better and more accurate feeding schemes will be obtained. Adjusting the C/N ratios in feed has been done either empirically or based on approximated assumptions. Protein use efficiency was raised from 25% in conventional ponds to about 45% in ASP. Yet, obtaining more accurate data and modeling of pond dynamics will probably further raise protein utilization efficiency. The lower feed quantity required and lower cost of feed due to lower protein requirements and avoidance of vitamin and mineral inclusion in the feed will raise profitability when using ASP systems.

ASP systems are turbid. Turbidity can be controlled by mixing and through drainage of excess suspended matter. Presently, we do not know the optimal level of suspended matter in the water. This may well be

different for different species grown. It is rather easy to automatically control total suspended solids (TSS), probably using turbidity as a signal. Ponds can be drained so as to maintain roughly constant turbidity. Efficient resuspension, mixing, and draining of ponds call for use of efficient aerators – ones that will be better adapted as compared to ones we have presently – and to pond structures that assist efficient mixing and drainability.

A problem common to intensive and other ponds is the need to properly dispose or utilize the washed-out sludge. Until recently, many fish and shrimp farmers disposed of sludge in estuaries, the ocean, or in mangroves. However, this is no longer accepted, both due to environmental considerations and aquaculture disease prevention. We have learned to recycle the water from ponds. There is an urgent need to either recycle or properly dispose of the sludge. Among possible options is its reuse as an organic-rich amendment to ponds or agricultural soils, as a base material for composting or as a material for construction, either as such or following sanitation and stabilization processes (Eaton 2004, Evanylo *et al.* 2004, Marsh *et al.* 2004) .

With the rise in number of ASP systems, there is a need to develop means to commercially construct ponds. Presently, each farm has its special design, materials and operation protocol. Clearer methods will have to be developed in order to support a mass of such ponds. Possibly, companies that plan, produce components and construct such ponds will rise. Presently, operating ASP demands a thorough understanding of the system and a long learning process by the operators. Modeling efforts, building on what was presented initially by Bergeron *et al.* (2004), will enable a more user-friendly routine to operate such ponds.

REFERENCES

Avnimelech, Y. Minimal Discharge from Intensive Fish Ponds. *World Aquaculture*, 1998, 175:32-37.

Avnimelech, Y. Carbon/Nitrogen Ratio as a Control Element in Aquaculture Systems. *Aquaculture*, 1999, 176:227-235.

Avnimelech, Y. Control of Microbial Activity in Aquaculture Systems: Active Suspension Ponds. *World Aquaculture*, 2003, 34:19-21.

Avnimelech, Y., Mokady, S., and Schroeder, G.L. Circulated Ponds as Efficient Bioreactors for Single-cell Protein Production. *The Israeli Journal of Aquaculture-Bamidgeh*, 1989, 41(2):8-66.

Avnimelech, Y., Kochva, M., and Diab, S. Development of Controlled Intensive Aquaculture Systems with a Limited Water Exchange and Adjusted Carbon to Nitrogen Ratio. *Israel Journal of Aquaculture-Bamidgeh*, 1994, 46(3):119-131.

Avnimelech, Y., and Ritvo, G. Shrimp and Fish Pond Soils: Processes and Management. *Aquaculture*, 2003, 220:549-567.

Bergeron, J., Avnimelech, Y., and Malone, R. Modeling an Intensive Aquaculture System (IAS) for Growth of Tilapia. In *Proceedings of the Fifth International Conference on Recirculating Aquaculture*, July 22-25, 2004, Blacksburg, VA, USA.

Brune, D.E., Schwartz, G., Eversole, A.G., Collier, J.A., and Schwedler, T.E. Intensification of Pond Aquaculture and High Rate Photosynthetic Systems. *Aquaculture Engineering*, 2003, 28:65-86.

Boyd, C.E., and Tucker, C.S. 1998. *Pond Aquaculture Water Quality Management*. Kluwer Academic Publications, Boston, MA, USA.

Burford, M.A., Thompson, P.J., McIntosh, P.R., Bauman, R.H., and Pearson, D. Nutrient and Microbial Dynamics in High-intensity, Zero-exchange Shrimp Ponds in Belize. *Aquaculture*, 2003, 219:393-411.

Burford, M.A., Thompson, P.J., McIntosh, P.R., Bauman, R.H., and Pearson, D.C. The Contribution of Flocculated Material to Shrimp (*Litopenaeus vannamei*) Nutrition in a High-intensity, Zero-exchange System. *Aquaculture*, 2004, 232:525-537.

Decamp, O., Cody, J., Conquest, L., Delanoy, G., and Tacon, A.G.J. Effect of Salinity on Natural Community and Production of *Litopenaeus vannamei* (Boone) with Experimental Zero Water Exchange Culture Systems. *Aquaculture Research*, 2003, 34:345-355.

Eaton, G.K. The Utilization of Wastewater and Composted Recirculating Aquaculture Sludge in the Greenhouse Production of Ornamental Crops. In *Proceedings of the Fifth International Conference on Recirculating Aquaculture*, July 22-25, 2004, Blacksburg, VA, USA.

Evanylo, G., Gay, Z., Shernoy, C., Marsh, L., and Boardman, G. Process Considerations and Quality Use Standards for Composted Recirculating Aquaculture Sludge. In *Proceedings of the Fifth International Conference on Recirculating Aquaculture*, July 22-25, 2004, Blacksburg, VA, USA.

Hepher, B. 1988. *Nutrition of Pond Fish.* Cambridge University Press, UK.

Kochba M., Diab S., and Avnimelech Y. Modeling of Nitrogen Transformation in Intensively Aerated Fish Ponds. *Aquaculture*, 1994, 120:95-104.

Marsh, L.S., Evanylo, G., Gay, Z., Boardman, G., and Subler, S. Vermiculture of Aquaculture Sludge from an Indoor Intensive Recirculating Tilapia Production Facility. In *Proceedings of the Fifth International Conference on Recirculating Aquaculture*, July 22-25, 2004, Blacksburg, VA, USA.

McIntosh, P.R. Changing Paradigms in Shrimp Farming: V. Establishment of Heterotrophic Bacterial Communities. *Global Aquaculture Advocate,* 2001, 4:53-58.

Panjaitan, P. 2004. Field and Laboratory Study of *Penaeus monodon* Culture with Zero Water Exchange and Limited Water Exchange Model Using Molasses as a Carbon Source. Doctorate Thesis, Charles Darwin University, Australia.

Tacon, A.G.J., Cody, J., Conquest L., Divakaran, S., Forster, I.P., and Decamp, O. Effect of Culture System on the Nutrition and Growth Performance of Pacific White Shrimp *Litopenaeus vannamei* (Boone) Fed Different Diets. *Aquaculture Nutrition*, 2002, 8:121-137.

The Role of "Aquaponics" in Recirculating Aquaculture Systems

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ABSTRACT

Recirculating aquaculture systems (RAS) are designed to recondition "used" fish water so that it can be recycled back into the fish-rearing tank(s). These systems have become popular because of the ability to control water parameters, their high-density rearing capabilities, and their potential for water conservation. Because of the accumulation of nutrients in these systems, they offer an underutilized resource for persons willing to transform an existing RAS into one that integrates plants. A secondary crop of plants can add to the system's profit, with little overhead cost. The reduction of certain nutrients by the plants can also benefit the system by reducing or eliminating expensive filtration components. These integrated systems have gained recognition by researchers and commercial users alike, and have stimulated the interest of many because of their resource-efficient and "eco-friendly" status.

INTRODUCTION

Various types of intensive and extensive integrated fish/plant systems have been well documented and described. These include: utilization of wetlands for treatment of fish effluents (Schwartz and Boyd 1995),

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use of seaweeds for removal of nutrients from mariculture (Troell et al. 1999), irrigation of field crops (McIntosh and Fitzsimmons 2003), and aquaponics (Rakocy et al. 1992). Aquaponics is the integration of growing plants (hydroponically) and fish (aquaculture) in one system, usually in a recirculating system. In RAS that have a daily water exchange of less than 5%, nutrient concentrations approach levels found in commercial hydroponic solutions (Rakocy 2002); this makes an ideal situation for an integrated fish/plant system.

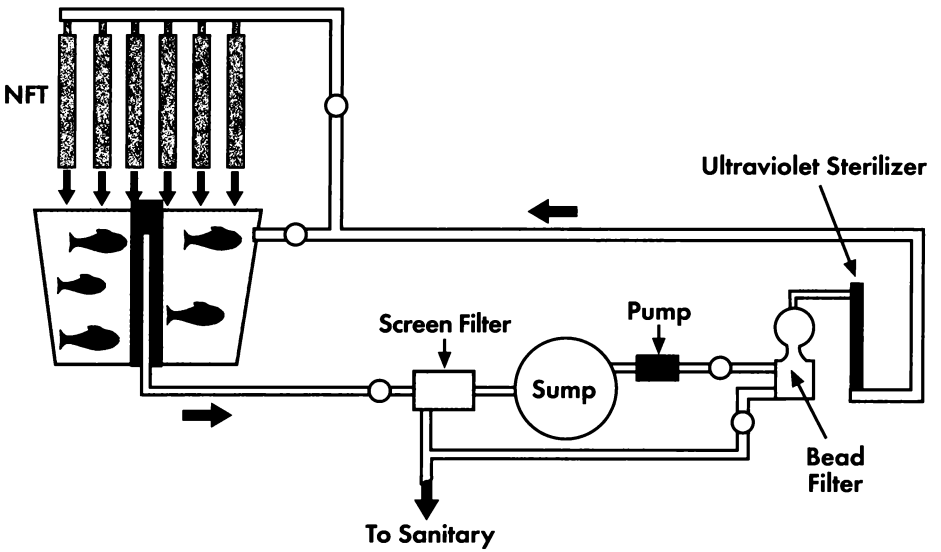
Recirculating aquaculture systems have many water treatment options available in their set-up. These may include: mechanical filtration, biological filtration, ultraviolet sterilization, ozonation, aeration, carbon filtration, or any combination of these steps. Various components of RAS, as well as numerous system configurations have been documented (Wheaton 1977, Piedrahita 1991, Lawson 1995, Summerfelt *et al.* 2001, Timmons *et al.* 2002). Total recirculating systems typically reuse 95-99% of the system water, while partial recycle systems reuse 50-85% of the water (Summerfelt *et al.* 2001). A daily water loss may be necessary due to filter maintenance and removal of nitrates (NO_3^-) (Lawson 1995).

Nutrients from fish wastes have the potential to become a nutrient source for the plants and thus are considered a valuable resource in an integrated system (Chamberlaine and Rosenthal 1995). This practice can be advantageous because, in addition to reconditioning the water, it has the potential to create a more cost-effective operation than a single-crop system. There are also many advantages of growing plants in an indoor recirculating system. These include: the elimination of soil-borne pathogens, controlled environment leading to increased harvests, and water conservation (Jones 1997).

System Requirements

Because of the various components available in aquaculture and hydroponics, the design of an integrated system is somewhat subjective to the grower. However, there are general recommendations for designing the filtration process for an aquaponic system. Rakocy (2002) points out that the design of an aquaponic system is based on the design of the RAS with the addition of a hydroponic component (Figure 1). The optimal arrangement for this would include: a fish rearing tank, a solids removal device, a biofilter, and a hydroponic system (Rakocy 1999).

Figure 1. Schematic of an aquaponic system using the nutrient film technique (NFT) for growing the plants.



Aquaponic systems vary in the techniques used for the removal of settleable solids (Table 1). The technique used for solids removal is probably the most subjective process in both research and commercial systems. These options include: immediate removal by screen filters; intermediate removal by settling tanks, sand filters, bead filters, and cartridge filters; and gradual removal by natural decomposition (gravel/ sand beds). There are many variables involved in choosing the optimal solids removal device. Daily feed input, plant species, and the size and type of plant growing area should all be considered in this decision process (Rakocy 2002).

The biofiltration of importance to aquaculture systems is nitrification. This process uses beneficial autotrophic bacteria to oxidize ammonium (NH_4^+) to nitrite (NO_2^-) and later to nitrate (NO_3^-) (Wheaton 1977). The primary biological filter (biofilter) may be located prior to the plant growing system, or the plant growing system itself may serve as the biofilter. The type of plant rearing system used will determine if additional biological filtration will be needed. Each plant growing technique provides a different amount of surface area needed for the colonization of beneficial nitrifying bacteria. Rakocy (1999) found that

Table 1. Various components and processes used in research and commercial aquaponic operations.

Reference	Research/ Commercial	Solids Removal	Bio-filtration	Plant System
Rakocy *et al.* 1997	R/C	Clarifier/ settling	Plant system	Floating raft
McMurtry *et al.* 1997	R	Sand beds[a]	Sand Beds	Sand beds
Adler *et al.* 2000	R	Settling tank	Fluidized bed	NFT
Seawright *et al.* 1993	R	Clarifier	Trickle filter	Floating raft
Harmon 2003	R	Bead filter	Bead filter	NFT
Sutton and Lewis 1982	R	Sedimentation	Three compartment	Gravel beds
Weaver and Shaw 2000[b]	C	Drum filter	Bead filter	Floating raft
Smith 1993[c]	C	Gravel beds[a]	Gravel Beds	Gravel Beds
Wilson 2002[d]	C	Screen filter	unknown	NFT
Nelson 2000 [e]	C	Gravel Beds	Gravel Beds	NFT

[a] *same unit serves as surface area for all three processes*

[b] *Integrated Aquatics, Welcome, Ontario*

[c] *S&S Aquafarms, West Plains, MO*

[d] *Tailor Made Fish Farms Pty Ltd., Australia*

[e] *Future Aqua Farms Ltd., Chezzetcook, NS*

when correct ratios of fish feed to plant growing area are used in raft hydroponic systems, sufficient nitrification is possible, whereas nutrient film technique (NFT) systems may require additional biofiltration.

A large ratio of plant growing area to fish growing area is needed to achieve a balanced system, but can vary from 2:1 to 10:1 depending on the system (Rakocy 1999). In a properly designed system, a small amount of fish can support a large number of plants. However, aquaculturists may

not necessarily want to maximize plant production, but only use them to supplement existing income and/or as tertiary filtration. Ultimately, it is the input of fish feed that determines the quantity of plants that can be successfully grown in the system. Rakocy (1992) found that lettuce production in a raft system was maximized with a daily feed input of 2.4 g/plant/day while Harmon (2003) found that 1.3 g/plant/day was sufficient for lettuce production in a NFT system.

In aquaponic systems, beneficial (nitrifying) bacteria, fish and plants all differ in optimal pH levels. Most literature shows that optimal pH for nitrifying bacteria (*Nitrosomonas* sp. and *Nitrobacter* sp.) is 7.8-9.0 (Hochheimer and Wheaton 1991). A typical hydroponic nutrient solution has a pH of 5.0-7.0, and it is known that plant growth may be affected if the pH is outside of this range (Jones 1997). Therefore, aquaponic systems should maintain a pH at or near 7.0 to meet the needs of the entire system.

Nutrient Removal

Most nutrients become available to plants after the decomposition of fish wastes. Many nutrients that accumulate in RAS do not have adverse effects on the fish, and in typical RAS are not utilized. However, a few of these can be of potential concern for fish culturists if they reach elevated levels. Un-ionized ammonia (NH_3) is toxic to fish at very low levels (Meade 1985) and is considered to be a limiting factor in high-density culture conditions. Phosphorus (P) and nitrate (NO_3^-) levels may also be a concern for some culturists as they may be monitored by regulatory agencies if the fish culture effluent is being discharged into surface waters. Plants have the ability to absorb ammonium (NH_4^+), phosphate ($H_2PO_4^-$), and nitrate (NO_3^-) ions, and therefore, are beneficial in the removal of these from the system. This situation makes for an ideal symbiotic relationship between the fish and plants.

Nutrient removal rates vary according to plant species, system design, and quantity of plants. Rakocy *et al.* (1997) recorded a 51% reduction in total ammonia nitrogen (TAN) and 38% nitrite (NO_2^-) reduction after flowing through a raft hydroponic system. Adler (1998) recorded a 99% reduction of dissolved phosphorus and a 60% reduction of nitrate concentration after flowing through a NFT conveyor system. Gloger *et al.* (1995) recorded a 24% reduction in total dissolved solids (TDS) in a lettuce raft culture system. Troell *et al.* (1999) found that in a mariculture system, *Gracilaria* sp. could remove 50-95% of the dissolved ammonium.

Plant System

Most hydroponic growing methods can be used in aquaponic systems (Table 1). Depending on the system, not all the fish culture water may be required to flow through the plant growing system. This will depend on the primary purpose for the plants in the integrated system as well as the size and type of the plant growing system. The plant growing system is usually the last component in an aquaponic system. Some systems utilize the hydroponic growing area (gravel/sand beds) as a means of mechanical filtration, while others do not rely on the use of the plant growing area as a solids filtration component (Table 1). In systems where the plant beds are also used to remove fish wastes, careful consideration must be given towards the accumulation of fecal matter vs. decomposition rate. These growing beds can easily become clogged and become anaerobic due to the rate of solids accumulation. However, it may be beneficial for some of the suspended solids to accumulate in the system and undergo decomposition to allow for mineralization of nutrients (Rakocy 1999).

Nutrient Supplementation

Typically, lettuce and herbs grow well with little or no nutrient supplementation to the aquaponic system. However, nutrient deficiencies do differ among systems, depending upon fish feed, feeding rates, plant species, filtration techniques, and the substrate in which the plants are grown. Nutrient concentrations must be monitored on a regular basis due to the possibility of nutrient deficiencies and salt accumulation (Seawright et al. 1998). Rakocy and Hargraves (1993) provide a good overview of nutrient supplementation for various crops in integrated systems.

The most common additions to a lettuce or herb aquaponic recirculating system include: chelated iron (Fe), potassium hydroxide (KOH), and calcium hydroxide ($Ca(OH)_2$). Because the required quantities of iron are not found in most aquaponic systems, a source must be added to the system on a regular basis. Iron is generally added to achieve a 2 mg/L concentration. Rakocy *et al.* (1997) added iron every three to four weeks for a lettuce crop, while Harmon (2003) added iron biweekly for a four-week crop of lettuce (2x per crop). The pH in RAS will decrease due to carbonic acid that is produced during the nitrification process (Wheaton *et al.* 1991). Therefore, it is necessary to make pH adjustments accordingly. Calcium hydroxide and potassium hydroxide provide a method of increasing pH as well as a source of calcium and potassium,

which are vital nutrients for plants and are often not found in the desired quantities for aquaponic systems (Rakocy *et al.* 1992).

CONCLUSION

Aquaculturists have the advantage over horticulturists in retrofitting an existing growing system into an integrated one. In an existing RAS, the requirements for an integrated system include: the plant growing system, minor modifications to the already existing filtration system, and the additional knowledge of hydroponic growing techniques. However, in an existing horticulture setting, all the RAS components, including fish, are required to set-up an aquaponic system. Generally, this is not feasible due to the large capital expense for filtration as well as extended knowledge of aquaculture and animal health.

The profit generated from plants would be determined by the plant species, growing system, and market prices. Adler *et al.* (2000) concluded that an integrated trout/basil operation could generate profit. Bailey *et al.* (1997) also determined that aquaponic farms in the U.S. Virgin Islands could be profitable. In an integrated system, the plant growing costs would be much less than in a commercial hydroponic operation because of the polyculture situation. If the operation is an existing aquaculture operation, the operating costs would be virtually unchanged or even reduced if it were converted into an aquaponic operation. This is providing that the additional space required for expansion is currently available. The second crop can also serve as an economic buffer if the market value of one crop declines or becomes a marketing issue. As with any type of farming enterprise, many variables should be considered when creating a business plan. Furthermore, in all aquaponic operations the grower must be well versed in all aspects of hydroponics and aquaculture. The knowledge of pests and disease in fish and plants and the toxicities associated with the supplementation of nutrients is critical to a successful aquaponics operation.

REFERENCES

Adler, P.R. Phytoremediation of Aquaculture Effluents. *Aquaponics Journal,* 1998, 4(4):10-15.

Adler, P.R., Harper, J.K., Wade, E.M., Takeda, F., and Summerfelt, S.T. Economic Analysis of an Aquaponic System for the Integrated Production of Rainbow Trout and Plants. *International Journal of Recirculating Aquaculture*, 2000, 1:15-34.

Bailey, D.S., Rakocy, J.E., Cole, W.M., and Shultz, K.A. Economic Analysis of a Commercial-Scale Aquaponic System for the Production of Tilapia and Lettuce. In *Proceedings from the Fourth International Symposium on Tilapia in Aquaculture*. Fitzsimmons, K. Ed., 1997. NRAES-106. Northeast Regional Agricultural Engineering Service, Ithaca, NY, USA.

Chamberlain, G., and Rosenthal, H. Aquaculture in the Next Century: Opportunities for Growth Challenges of Sustainability. *World Aquaculture*, 1995, 26(1):21-25.

Gloger, K.G., Rakocy, J.E., Cotner, J.B., Bailey, D.S., Cole, W.M., and Shultz, K.A. 1995. Contribution to Lettuce to Waste Water Treatment Capacity of Raft Hydroponics in a Closed Recirculating Fish Culture System. In *Book of Abstracts, World Aquaculture Society*, Baton Rouge, LA, USA. 1995.

Harmon, T. NFT Aquaponic Systems: A Closer Look. *Aquaponics Journal*, 2003, 8(4):8-11.

Hochheimer, J.N., and Wheaton, F.W. Understanding Biofilters, Practical Microbiology for Ammonia Removal in Aquaculture. In *Proceedings from Aquaculture Symposium.* 1991. NRAES-49. Northeast Regional Agricultural Engineering Service, Ithaca, NY, USA.

Jones, J.B. 1997. *Hydroponics: A practical guide for the soilless grower.* St. Lucie Press, Boca Raton, FL, USA.

Lawson, T.B. 1995. *Fundamentals of Aquacultural Engineering.* Chapman and Hall, New York, NY, USA.

McIntosh, D., and Fitzsimmons, K. Characterization of Effluent from an Inland, Low-Salinity Shrimp Farm: What Contribution Could This Make Water if Used for Irrigation. *Aquacultural Engineering*, 2003, 27:147-156.

McMurtry, M.R. Sanders, D.C., Cure, J.D., Hodson, R.G., Hanning, B.C., and St. Amad, P.C. Efficiency of Water Use of an Integrated Fish/Vegetable Co-Culture System. *Journal of the World Aquaculture Society*, 1997, 28:420-428.

Meade, J.W. Allowable Ammonia for Fish Culture. *The Progressive Fish-Culturist*, 1985, 47(2):135-145.

Nelson, R. Taking the Plunge into Aquaponics. *Aquaponics Journal*, 2000, 4(4):10-14.

Piedrahita, R.H. Engineering Aspects of Warmwater Hatchery Design. In *Proceedings Aquaculture Systems Engineering*, 1991, ASAE 02-91. American Society of Agricultural Engineers, St. Joseph, MI, USA.

Rakocy, J.E. Aquaculture Engineering: The Status of Aquaponics Part 1. *Aquaculture*, 1999, 25:83-87.

Rakocy, J.E. 2002. Aquaponics. In *Recirculating Aquaculture Systems*, Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T., Vinci, B.J., Eds. Cayuga Aqua Adventures, Ithaca, NY, USA.

Rakocy, J.E., Losordo, T.M., and Masser, M.P. 1992. *Recirculating Aquaculture Tank Production Systems, Integrating Fish and Plant Culture*. Southern Regional Aquaculture Center Publication No. 454.

Rakocy, J.E. and Hargraves, J.A. Integration of Vegetable Hydroponics. In *Proceedings of an Aquacultural Engineering Conference*. 1993. Wang, J.K., Ed., American Society of Agricultural Engineers, St. Joseph, MI, USA.

Rakocy, J.E., Bailey, D.S., Shultz, K., and Cole, W.M. Evaluation of a Commercial-Scale Aquaponic Unit for the Production of Tilapia and Lettuce. In *Proceedings from the Fourth International Symposium on Tilapia in Aquaculture*. Fitzsimmons, K., Ed., 1997. NRAES-106., Vol. 2. Northeast Regional Agricultural Engineering Service, Ithaca, NY, USA

Schwartz, M.F., and Boyd, C. Constructed Wetlands for Treatment of Channel Catfish Pond Effluent. *The Progressive Fish-Culturist*, 1995, 57:255-266.

Seawright, D.E., Stickney, R.R., and Walker, R.B. Nutrient Dynamics in Integrated Aquaculture-Hydroponic Systems. *Aquaculture*, 1998, 160:215-237.

Smith, W.S. The Genius of Simplicity. *The Growing Edge*, 1993, 5(2): 40-44.

Summerfelt, S. T., Bebak-Williams, J., and Tsukuda, S. 2001. Controlled Systems. In *Fish Hatchery Management*, Wedemeyer, G.A., Ed., second edition. American Fisheries Society, Bethesda, MD, USA.

Sutton, R.J., and Lewis, W.M. Further Observation on a Fish Production System that Incorporates Hydroponically Grown Plants. *The Progressive Fish-Culturist*, 1982, 44(1):55-59.

Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T., and Vinci, B.J. 2002. *Recirculating Aquaculture Systems*. Cayuga Aqua Adventures, Ithaca, NY, USA.

Troell, M., Ronnback, P., Halling, C., Kautsky, N., and Buschmann, A. Ecological Engineering in Aquaculture: Use of Seaweeds for Removing Nutrients from Intensive Mariculture. *Journal of Applied Phycology*, 1999, 11:89-97.

Weaver, C., and Shaw, T. Aquaponics in Welcome, Ontario. *Aquaponics Journal*, 2000, 4(2):10-15.

Wheaton, F.W. 1977. *Aquacultural Engineering*. John Wiley and Sons. New York, NY, USA.

Wheaton, F.W., Hochheimer, J.N., Kaiser, G.E., and Krones, M.J. Principles of Biological Filtration. In *Proceedings from Aquaculture Symposium*. 1991. NRAES-49. Northeast Regional Agricultural Engineering Service, Ithaca, NY, USA.

Wilson, G. Aquaponics Proves Profitable in Australia. *Aquaponics Journal*, 2002, 6(1):8-13.

Performance Characteristics of Rotating Biological Contactors Within Two Commercial Recirculating Aquaculture Systems

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ABSTRACT

Biological filtration is a critical determinant in the process train design of a recirculating aquaculture system. In addition to the mechanical and biological efficiency of the biofilter itself, this process must be co-developed with the various interrelated technologies involved in water-quality control. This study describes the performance of rotating biological contactors as an integral part of two commercial closed recirculating fish production systems. Data is presented from replicated systems employing paddlewheel-driven rotating biological contactors.

The RBC is a robust fixed-film bioreactor demonstrating excellent operational attributes in recirculating aquaculture systems. The efficiency of the RBC as biofilter is defined according to its mechanical and biological performance characteristics. In addition to highly efficient nitrification of ammonia under heavy feeding conditions (1.21 g/m^2/day), the RBC has significant influence on the control of secondary water-

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quality and hydraulic considerations affecting the overall design and performance of the system. RBCs off-gas carbon dioxide, providing a level of pH control, a significant benefit in closed recirculating systems. Additional data is presented for carbon dioxide sparging efficiency, and the capacity for versatile hydraulic loading and low-head operation.

This paper also provides a practical comparison of RBC design and performance considerations with other biofilter options, including the effects of design on the mechanical reliability, energy requirements, and spatial efficiency of this biofiltration system.

INTRODUCTION

Management of Nitrogenous Wastes – Biofilter Design Priorities

Ammonia, the principal nitrogenous waste of fish, results from the digestion of protein, and is therefore generated in proportion to the levels of feed administered. In recirculating aquaculture systems, without significant dilution, ammonia must be removed by a two-step process called nitrification. Nitrifying bacteria, concentrated on the biofilter media surfaces, convert ammonia to nitrite and then to relatively harmless nitrate. Nitrate is allowed to accumulate to levels determined by the amount of dilution (defining the % recirculation rate of the recycle system). Since both ammonia and nitrite are toxic to fish, their levels must be managed through the efficient design of biofiltration systems.

Biological filters must provide adequate surface area for the growth of nitrifying bacteria. *Nitrosomonas* and *Nitrosospira* convert ammonia to nitrite, and *Nitrobacter* and *Nitrospira* convert nitrite to nitrate. The water containing the dissolved waste must be brought into contact with the surface area supporting these populations of bacteria. The health of the bacterial film is affected by the availability of oxygen, the temperature, the organic loading, the pH, and the alkalinity of the water, all of which must be managed in tandem with the requirements of the fish. During operation, the filter cannot be permitted to clog with fish wastes or the sloughing bacterial biomass. The filter media must therefore be self-cleaning, or involve manual or automated management technologies to remain unclogged.

Ammonia

Ammonia dissolved in the water exists as two compounds in equilibrium: ionized ammonium (NH_4^+) and un-ionized ammonia (NH_3). While

un-ionized ammonia is extremely toxic to fish, the ionized portion is relatively harmless. The proportion of each is determined primarily by the pH of the water. The higher the pH, a measure of hydrogen ion (H^+) concentration, the higher the proportion of un-ionized ammonia. Therefore, pH control of the culture water is crucial to maintenance of acceptable levels of ammonia, and provides an opportunity for a wider range of water quality management parameters. Biofilters nitrify ammonia much more efficiently as the substrate concentration (level of total ammonia in the water) increases. Therefore, biofilter efficiency can be optimized by maintaining total ammonia at somewhat elevated levels, but at a pH which maintains the levels of un-ionized ammonia below that considered detrimental to the fish species being cultured. For example, with TAN (total ammonia nitrogen) levels at 3.0 mg/l and a pH of 7.2, the level of un-ionized ammonia (at 26°C) is only 0.029 mg/l, below the level of significant toxicity for many species. To maintain TAN levels at 1.0 mg/l would require a biofilter with three times the capacity, at a significant and unnecessary additional expense.

Nitrites

Nitrite (NO_2) is the intermediate product of nitrification and the biofiltration process. Under normal operating conditions, biofiltration should maintain a balance of nitrifying bacterial populations which will control both ammonia and nitrite levels. There are times when an imbalance in the nitrification efficiency of the biofilter may result in transient elevations in levels of nitrite in the culture water. This can usually be accommodated since the toxicity of nitrite is significantly reduced by the presence of chloride ions. By maintaining a minimal level of salt (NaCl) in the water (<1 ppt), it is possible to reduce the potential toxicity of nitrites. Rotating biological contactors have been used successfully in conditions of freshwater to full seawater concentrations of salt.

Rotating Biological Contactors (RBCs)

Biofilter design must take into account all of the stated water-quality management criteria, as well as considerations of space and cost efficiency. A rotating biological contactor or biodisc filter is a fixed film bioreactor composed of circular plates aligned on a central axle. The filter is usually staged within a flooded containment plumbed for a prescribed flow of water, with approximately half of the disc surfaces submerged,

and half exposed to the air. The discs are rotated slowly to alternately expose the biologically active media to the water carrying the nutrients (the nitrogenous wastes of the fish) and to the air, essentially providing an unlimited source of oxygen to the bacteria. The shear force on the surface of the discs as it passes through the water continuously sloughs senescent and thickening bacterial biomass, thereby maintaining a healthy biofilm.

Various mechanical designs of this biofilter configuration have been considered for recirculating aquaculture systems for decades (Lewis and Buynak 1976). The RBC has been shown to outperform many other fixed-film configurations applied to fish culture systems (Van Gorder and Fritch 1980; Miller and Libey 1984, 1985; Rogers and Klemetson 1985). Wheaton *et al.* (1994) number the inherent advantages of RBCs for aquaculture as:

1) the RBC is self-aerating, providing oxygen to the attached biofilm,

2) the RBC is a low-head device minimizing pumping energy needs,

3) the RBC is non-clogging due to shearing of loose biofilm caused by the rotation of the media through the water, with self-maintenance of an active biofilm, and

4) once established, RBC performance is reliable and resistant to sudden failures.

However, Wheaton also observes that almost all problems with RBCs "fall into the category of mechanical failures." Most reviews of RBCs disclose that failures with the drive motor, linkage, chain drive, bearings, breaking shafts, and the disassociation of the media from the shaft are problems with most RBCs designed for both municipal and aquacultural purposes.

Hochheimer and Wheaton (1998) state that RBCs are "generally quite stable in operation, have a high ammonia removal efficiency compared to some other biofilters, and operate with very little head loss." However, they indicate that "their primary disadvantage is that they require a power source to turn them, and mechanical breakdown can be a problem, particularly with a poorly designed unit." Timmons *et al.* (2001) affirm that RBCs "require little hydraulic head, have low operating costs, provide gas stripping, and can maintain a consistently aerobic treatment environment." They "also tend to be more self cleaning than static

trickling filters." But they state that "the main disadvantages of these systems are the mechanical nature of their operation and the substantial load on the shaft and bearings."

As noted, RBCs have various attributes, some positive and some negative, and can be compared with other biofilter designs in each of these categories. The following study of rotating biological contactors in commercial aquaculture applications illustrates these comparisons, and the consequences of the design of the biofilter on its integration with the other system components within an efficient recirculating aquaculture system. This study will consider the performance characteristics of RBCs within two commercial recirculating aquaculture systems in eastern Pennsylvania. All observations were made and data collected under fully operational, commercial production conditions during the culture of hybrid striped bass.

RBC Design – Mechanical Durability and Reliability

The RBC units evaluated in this study are manufactured by Fresh-Culture Systems, Inc. (Breinigsville, PA, USA). They are categorized as "floating/air-driven/rotating biological contactors. The units are comprised of flat and corrugated sheets mounted on a central PVC shaft. Appropriately positioned high-density styrofoam flotation provides the filters with neutral buoyancy, which allows for the near frictionless rotation of the central shaft within a guiding channel at each end of a fiberglass stage. Rotation is affected by the injection of air below, and/or water onto, a centrally placed paddlewheel. Using spokes and rigorous attachment methods, the media is secured tightly to the rotating shaft and central paddlewheel. The present design eliminates all requirements for a drive motor, chain, pillow blocks, or weight-supporting center shaft. The design of the RBC as a floating unit, with its weight supported by the water column rather than against the axle and pillow blocks, results in very little resistance to the rotation of the biofilter within the staging unit.

Traditionally designed RBCs must maintain the drive motor, and a direct-drive central axle, above the level of the water, thereby achieving only about 40% submergence of the active biofilter media. The present RBC design allows for a full 50% submergence (at full acclimation weight) through the integration of the appropriate level of buoyancy. This optimizes the alternate flooding of the media and exposure to the air.

Low-Energy Operational Characteristics

The energy required to maintain rotation of these RBCs is almost negligible. A low-pressure regenerative air blower provides the minimal volume of air (approximately 2.0 cfm directed below the paddlewheel) necessary to maintain rotation of the 186 m^2 and 557 m^2 RBCs. Considering this, a single 1HP blower (at 30 inches of water pressure) will supply enough air for the rotation of 32 RBCs. Considering the use of 18 kwh of energy per day to accomplish this, at $0.08/kwh, and a total daily expense of about $1.44, then each RBC would use about $0.05/day to provide rotation.

For redundancy, an additional torque was applied to the paddlewheel of the large 930 m^2 units being considered in this study, by the application of ~15 lpm of water flow over the paddlewheel. This minimal volume was diverted for biofilter rotation from the total 1,800 lpm (average) of flow through each of the biofilters. Under low-head pumping conditions, the application of a 2.0 HP pump to provide 900 lpm of flow will cost approximately $2.88/day. Diverting 1.7% of this flow for biofiltration rotation represents a cost of about $0.05/day. Therefore the total estimated cost for achieving rotation of the larger RBC, using both air and water, costs about $0.10/day. Either the air or water flow alone will maintain the rotation of these units, the weight of which, at full acclimation and loading, is estimated at over 700 kgs.

Unencumbered Hydraulic Loading

The hydraulic design of a biofilter will demonstrate an inherent capacity to allow a flow of water to pass through it, a feature that is usually dependent on the physical characteristics of the media. The blockage of flow over time varies with the quality of the clarification systems and the level of biomass loading, with the resulting resistance to flow adding to the system's additional energy requirements.

The RBC provides no restriction to the flow of water through the biofilter, even under conditions of heavy biomass loading and full acclimation, and can accommodate very high flow rates without requiring additional energy. When co-developed with associated unit processes, this provides for potential low-energy pumping options.

Low-Head Operation

Efficient system integration requires the determination of the proper

flow rate of water through the biofilter to provide for enough passes of the culture water daily to maintain the ammonia at desired levels, while minimizing the energy consumption requirements. The RBC, if properly plumbed using sufficiently sized influent and effluent pipes, provides unimpeded flow characteristics. The energy costs for pumping are minimized by operating with the biofilter water levels below tank water levels. Filters which must be elevated above the tank water level, including trickling and many fluidized media filters, must expend additional energy to elevate the pumped water.

Another measure of the energy costs involved in the operation of a biofilter is the head pressure under which it must be operated. Filters with fine media through which large volumes of water must be pumped, such as sand or bead filters, require correspondingly high water pressures, and subsequently increased electrical costs to operate. With fluidized sand filters, additional energy must be expended to fluidize the media and to elevate the water within the mixing chamber. The fluidized media must be elevated sufficiently to prevent the sand from exiting the chamber with the flow of water.

Within the biofilter, the flow characteristics must also allow for the contact of all of the available media surface area with the circulated water, with an appropriate retention period within the biofilter containment for optimal nitrification efficiency. The design of the rotating biological contactor does not involve passing a volume of water through a media bed, but instead allows for the unimpeded movement of the concentrated surface area of the biofilter *through* the moving volume of water. There is no requirement for high-pressure flow, or potential for the disruption of biological films due to these high-pressure flows, as in bead and sand bed filters.

Non-clogging Operation

Filter design must also eliminate the potential for clogging, since the inability to transport the culture water to the full area of media supporting the bacteria renders it less effective. Clogging can occur as a result of an accumulation of solid wastes due to inadequate clarification, or if the biofilter itself is not self-cleaning. The natural life cycle of the bacterial population results in significant quantities of senescent autotrophic and heterotrophic bacterial biomass, which must be sloughed from the filter media continuously and transported to the clarification system. This requires a biofilter with the proper balance of surface area and void space,

and a sufficient flow rate across the filter media to provide the necessary shearing force. RBCs provide an optimal surface and operational platform for this process, with the shearing force provided by sufficient rotational velocity (in the present design, 1.5 rpm).

Self-Aerating Capacity

Maintaining water quality within specific ranges of tolerance for the bacteria is critical to biofilter operation. A reduction in dissolved oxygen (DO) levels in the water passing through the biofilter will reduce the efficiency of nitrification. Levels must remain elevated above 2 mg/l (Wheaton *et al.* 1994) throughout the biofilter, or overall efficiency will suffer. The design of submerged biofilters must maintain adequate DO levels through filter aeration, optimal flow rate, and proper sizing of the filter, as well as by negating the possibility of clogging and the subsequent channeling of water through a reduced area within the biofilter.

As water moves through the media of submerged biofilters, dissolved oxygen levels are reduced by the Biological Oxygen Demand (BOD) of the bacterial populations to a point which subsequently reduces the nitrification efficiency of the biofilter. It is often necessary to aerate the water within the biofilter to maintain optimal nitrifying conditions. Timmons *et al.* (2001) provides a "rule of thumb" that for each gram of ammonia nitrified, 4.57 grams of oxygen are required to maintain the bacterial population. Unlike submerged biofilters, trickling filters and rotating biological contactors provide for an air/water interface at the surface of the bacterial film. These biofilters are thereby afforded an unlimited level of oxygen availability to the associated bacterial biomass. The RBC uses atmospheric oxygen, resulting in optimal conditions of nitrification, without additional costs for supplemental aeration or oxygenation, and without appropriating the dissolved oxygen being made available to the fish populations.

Carbon Dioxide Sparging Efficiency

Trickling filters and RBCs can also off-gas carbon dioxide under normal operating conditions. The significant air/water interface available to the respiring bacteria allows for the off-gassing of the carbon dioxide produced by the bacteria, as well as that within the water flow which is being sheeted over that surface. At all times, the RBCs in the present study present 50% of the total unit's surface area, or 465 m^2, to the air for gas exchange.

MATERIALS AND METHODS

Two separate aquaculture facilities, which used a total of 75 RBCs of the dimensions listed in Table 1, were employed in this study.

Data on the performance of RBCs was collected within two commercial indoor recirculating aquaculture facilities located in eastern Pennsylvania. Both facilities cultured hybrid striped bass over several years under intensive feeding regimens. RBCs were employed in nursery and grow-out aquaculture systems ranging in total volume from 10,000 liters to 115,000 liters. For this study, 12 separate grow-out systems were studied, each system employing the RBC model described above (RBC10000).

Table 1. Sizing of RBC systems used in this study.

RBC Model	Diameter	Surface Area
RBC10000	1.22m	930m^2

For each of the culture systems observed in this study, the flow rates through the system components permit the tank water volumes to be circulated through the biofilters in an average of 55 minutes. Each system was fed the same feed (40% protein, 16% fat) which was automatically administered several times daily over a 16-hour light cycle. Un-ionized ammonia concentration was maintained below 0.05 mg/l, with pH controlled (using automated NaOH injection) to maintain total ammonia concentration at approximately 3 mg/l.

RBC Nitrification Performance Characteristics

The efficiency of biofilter operation is usually reported as the nitrification of Total Ammonia Nitrogen (TAN)/m^2 of biofilter surface area/day. This study measures the comparative efficiency of the RBCs by two separate methods.

Feed Input-TAN Calculation Method

With Study #1, a theoretical level of TAN production is estimated as a function of the feeding levels. Biofilter efficiency is measured as a function of the removal of that estimated ammonia, thus establishing a steady state TAN concentration within the culture tanks. The daily replacement of 5% of the water as a function of the recirculation % of the system was also considered in the removal of ammonia.

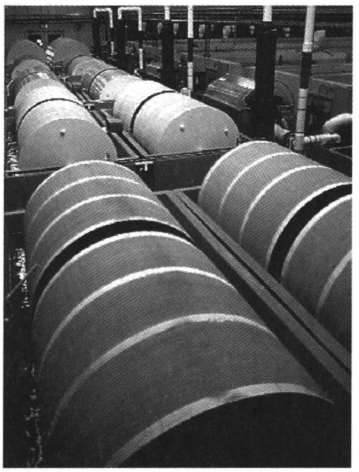
Study #1 involves eight systems, each with a volume of 150,000 liters, and each utilizing two RBCs. Each RBC has a surface area of 930 m^2 to handle the ammonia levels produced by populations of hybrid striped bass being cultured under intensive feeding conditions. Over a five-week period, the average level of feed per day was determined for each of eight production systems (System 1). This level of feeding was mathematically converted to levels of ammonia produced. Using Wheaton *et al.* (1994), an ammonia production rate of 0.03 kg TAN/kg feed is assigned, and represents the mass of ammonia that must be removed by biofiltration and dilution, in order to maintain equilibrium.

Direct Measurement Method

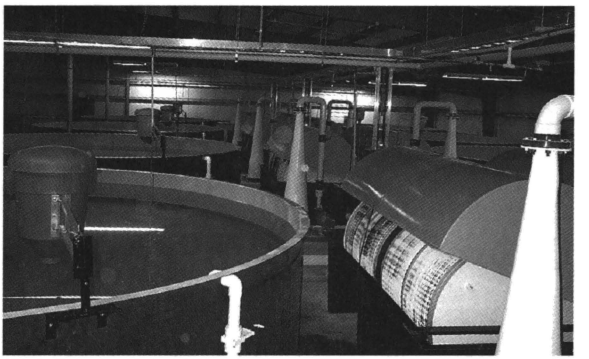
Study #2, carried out in four separate culture systems, each of 77,000 liters (System 2), involves the determination of ammonia levels within the flow of water before and after the individual biofilters, providing a direct measurement of the ammonia removed by filtration (ARF). Samples of water flowing through six RBCs, within four separate aquaculture systems were measured for TAN levels nephelometrically using the LaMotte Smart colorimeter (LaMotte Company, Chestertown, MD, USA), at the influent and effluent ports of the RBC stage. The level of TAN removed during the retention time within the filter is calculated as the difference between influent and effluent concentrations. Considering the measured

Table 2. Operating specifications for each of the two types of culture systems used in this study.

			Biofilter Specifications		
	Tank Design	**System Volume (liters)**	**Total Surface Area (m^2)**	**Total Specific Surface Area (m^2/m^3)**	**Total Flow Rate (liters/min)**
System 1	Cross-Flow Raceways (8 systems)	115,000 (2 tanks/ system)	1,860 (2 RBCs)	258 (2 RBCs)	1900
System 2	Round Tanks (4 systems)	77,000 (2 tanks/ system)	1,860 (2 RBCs)	258 (2 RBCs)	1,660



System #1 – RBCs servicing 115,000-liter cross-flow raceways.



System #2 – RBCs servicing 77,000-liter round-tank recirculating aquaculture systems.

flow rate through the biofilter, and the total surface area of the RBCs, the removal rate in g/m^2/day is calculated.

Carbon Dioxide Sparging Capacity

A replicated trial (*in situ*) was designed to quantify the potential for each RBC to off-gas carbon dioxide. The most direct measure of the levels of carbon dioxide being removed by the RBC is by the measurement of the pH of the water at the influent and the effluent ports of the biofilter. Over a two hour period, six separate pH measurements were made (using pH probes able to provide accuracy to 0.01 units) at the influent and effluent ports of two separate biofilters, each receiving 830 liters/minute. The pH and alkalinity (measured by Standard Methods) of each water sample were used to determine the ambient levels of carbon dioxide in each sample. The difference in the pH between influent and effluent concentrations provided the level of carbon dioxide sparged by the RBC.

RESULTS

Ammonia Nitrification Efficiency – Study #1

Table 3 lists the average feed levels administered to eight separate culture systems over a five-week period, and the average levels of TAN produced by the fish. With a steady-state situation, the levels of TAN produced, less 5% removed through water exchange, is assumed to indicate the levels of TAN removed by biofiltration.

Table 3. Average feed levels administered to eight separate culture systems over a 5-week period, and the average levels of TAN produced by the fish.

	Avg Feed/Tank/Day *Kg*					**TAN Removed/m²/Day** *Grams*				
Week #	1	2	3	4	5	1	2	3	4	5
Tank #										
1	38.4	37.1	31.7	28.1	33.0	1.43	1.09	1.17	1.03	1.22
2	33.3	18.6	24.1	36.4	43.4	1.22	0.70	0.88	1.44	1.60
3	20.4	34.9	40.9	38.0	41.0	0.75	1.23	1.50	1.39	1.51
4	35.0	41.0	42.5	38.2	37.3	1.28	1.50	1.56	1.40	1.37
5	36.5	40.6	12.7	-----	28.6	1.34	1.49	0.47	-----	1.05
6	32.8	26.8	19.4	37.6	38.0	1.21	0.99	0.71	1.37	1.40
7	27.5	35.7	35.2	30.7	48.0	1.00	1.31	1.29	1.13	1.74
8	33.3	38.1	20.9	23.3	37.5	1.23	1.39	0.77	0.85	1.38
Weekly Avg. TAN Removal Rate (g/m²/day)						1.18	1.21	1.04	1.23	1.41
Overall Average TAN Removal Rate						1.21 g/m²/day				

Ammonia Nitrification Efficiency – Study #2

Three samples of influent and effluent flow from each of six biofilters were tested for TAN. The average levels of TAN, and the removal rate through the RBCs, is provided in Table 4.

Table 4. Direct measured TAN removal rate.

Filter #	**Flow Rate liters/min**	**TAN (Influent) mg/l**	**TAN (Effluent) mg/l**	**TAN Removed gms/day**	**Removal Rate g/m²/day**
1	830	3.0	2.0	1195	1.3
2	828	3.4	2.6	954	1.0
3	812	2.9	1.8	1286	1.4
4	815	3.1	2.2	1056	1.1
5	821	2.9	1.9	1182	1.3
6	825	3.5	2.5	1236	1.3
				Avg. TAN removal rate (g/m²-day)	1.2

The direct measurement of ammonia influent and effluent levels through each of six separate biofilters demonstrates consistent removal rates with those obtained through feed metabolism calculations.

Comparative Performance Parameters by Surface Area

Table 5 demonstrates the comparative nitrification capacity for various types of biofilters:

Table 5. Comparative nitrification capacity for various types of biofilters.

Source	Ammonia Removal Rate
Submerged Filters (Wheaton *et al.* 1994)	0.3-0.6 gms/m^2-day
Bead Filters (Wheaton *et al.* 1994)	0.20-0.25 gms/m^2-day
Fluidized Sand Filters (Thomasson 1991)	0.25-0.35 gms/m^2-day
Rotating Biological Contactor (this study)	1.21 gms/m^2-day

For fine media biofilters such as fluidized sand or bead filters, volumetric comparisons of nitrification efficiency are often used. By volume, this RBC, with 258 m^2/m^3, demonstrates a nitrification rate of 312 gms/m^3-day. Tsukuda *et al.* (1997) estimate nitrification rates for cold-water fluidized sand filters at 150-410 gms/m^3-day. Malone *et al.* (1993), citing data from Thomasson (1991) and Monaghan *et al.* (1996), reported ammonia removal rates of 630-800 gms/m^3-day in water.

Carbon Dioxide Sparging Capacity

At six separate intervals, samples of influent and effluent flows in two separate 930 m^2 RBCs were tested, and the average levels for alkalinity, pH, and subsequent carbon dioxide levels were determined. The results are presented in Table 6.

Table 6. Carbon dioxide sparging capacity.

Avg. Alkalinity (mg/l)	Avg. Influent pH	Avg. CO_2 (mg/l)	Avg. Effluent pH	Avg. CO_2 (mg/l)	Avg. CO_2 removed
462	7.30	45	7.43	36	9 mg/l

On each pass through the RBC biofilter, the pH increased by an average of 0.13 units. At the recorded alkalinity (measured colorimetrically), this translates to the sparging of an average of 9 mg/l of carbon dioxide. Since

each biofilter is operating at an average flow rate of 830 liters/minute, the RBC off-gasses an average of 7.47 gms CO_2/minute. This translates to a carbon dioxide removal rate for one RBC of 10.8 kg of carbon dioxide each day. Timmons et al. (2001) calculates that for every gram of oxygen consumed, 1.38 grams of carbon dioxide is produced. For the systems in this trial, it is estimated (based on direct measurement over extended production cycles) that for every kg of feed provided, approximately 0.6 kg of oxygen is consumed.

Therefore, for these systems, receiving an average of 40 kg of feed daily and consuming an average of 24 kg/day of oxygen, carbon dioxide is being generated at a rate of approximately 33.1 kg/day. Each of these systems has two biofilters off-gassing a total of 21.6 kg/day of carbon dioxide, which is 65% of the estimated carbon dioxide generated. The systems require additional degassing capabilities to maintain carbon dioxide levels within an acceptable range, but this trial demonstrates that CO_2 sparging is a valuable function attributable to the rotating biological contactor.

DISCUSSION

RBCs have been demonstrated to be one of the most efficient and robust biofilters available for nitrification of aquaculture wastes. They demonstrate extremely high nitrification rates, while providing additional qualifications for self-aeration, off-gassing, and low-head operation. An ammonia removal rate of 1.2 g/m^2-day surpasses all other biofilter configurations cited. With a volumetric nitrification rate of 312 g/m^3-day, comparisons to fluidized sand filters demonstrate a nearly equal volumetric nitrification rate, and significant superiority in energy efficiency, ease of management, and reliability. Despite slightly increased spatial footprint requirements, the RBC minimizes facility height requisites, which lowers associated operational pumping costs. Staging of appropriately-sized RBCs with multiple and separate culture systems also provides a more versatile alternative than with the use of centralized biofiltration options, such as large fluidized sand filters. The separation of fish populations within independent systems provides valuable biosecurity and sequential rearing advantages.

The present RBC design has eliminated all previous concerns with mechanical durability and reliability of operation. Multiple replicates

of the latest full-scale iteration of this RBC design were observed in uninterrupted operation at full loading for over three years. No failures of shaft, disassociation of media, or interruption of rotation were observed throughout the three-year trial period. Considering this, in addition to the positive considerations that have always been attributed to this biofilter, the RBC provides a reliable and effective alternative for consideration in commercial recirculating aquaculture systems.

REFERENCES

Hochheimer, J.N., and Wheaton, F.W. Biological filters: trickling and RBC design. In *Proceedings of the 2nd International Conference on Recirculating Aquaculture* (Roanoke, VA, USA), Flick, G.J., and Rakestraw, T., (Eds.). 1998. Virginia Tech, Blacksburg, VA, USA.

Lewis, W.M., and Buynak, G.L. Evaluation of a Revolving Type Biofilter for use in Recirculating Fish Production and Holding Units. *Transactions of the American Fisheries Society*, 1976, 105:704-708.

Malone, R.F., Chitta, B.S., and Drennen, D.G. Optimizing Nitrification in Bead Filters for Warmwater Recirculating Systems. In *Techniques for Modern Aquaculture*. Wang, J.K. (Ed.) 1993. American Society of Agricultural Engineers, St. Joseph, MI, USA.

Miller, G.E., and Libey, G.S. Evaluation of a Trickling Filter in a Recirculating Aquaculture System Containing Channel Catfish. *Aquacultural Engineering*, 1984, 3:39-57.

Miller, G.E., and Libey, G.S. Evaluation of Three Biological Filters Suitable for Aquaculture Applications. *Journal World Aquaculture Society*, 1985, 16:158-168.

Monaghan, T.J., Delos Reyes, A.A., Jeansonne, T.M., and Malone, R.F. Effects of Media Size on Nitrification in Fluidized Sand Filters. In *World Aquaculture '97 Book of Abstracts* 1996. World Aquaculture Society, Baton Rouge, LA, USA.

Rogers, G.L., and Klemetson, S.L. Ammonia Removal in Selected Aquaculture Water Reuse Biofilters. *Aquacultural Engineering*, 1985, 4:135-154.

Thomasson, M.P. 1991. Nitrification in Fluidized Bed Sand Filters for use in Recirculating Aquaculture Systems. Unpublished Master's Thesis, LSU, Baton Rouge, LA, USA.

Timmons, M.B., Ebeling J.M., Wheaton F.W., Summerfelt, S.T., and Vinci B.J. 2001. *Recirculating Aquaculture Systems*. NRAC Publ. No. 01-002. Ithaca, NY, USA.

Tsukuda, S.M., Hankins, J.A., Marshall, C.P., Summerfelt, S.T., Bullock, G.L., and Sawyer, T.K. Effects of Sand Size on Fluidized-bed Biofilter Performance in Cold Water Systems. In *Recent Advances in Aquacultural Engineering*. Timmons, M.B., and Losordo, T.M. (Eds.). 1997. Northeast Regional Aquacultural Engineering Service, Ithaca, NY, USA.

Van Gorder, S.D., Fritch, J.D. Filtration Techniques for Small-scale Aquaculture in a Closed System. In *34th Annual Conference of the Southeast Association of Fish and Wildlife Agencies*. 1980. Nashville, TN, USA.

Wheaton, F.W., Hochheimer, J.N., Kaiser, G.E., Malone, R.F., Krones, M.J., Libey, G.S., and Easter, C.C. Nitrification Filter Design Methods. In *Aquaculture Water Reuse Systems: Engineering Design and Management*. Timmons, M.B., and Losordo, T.M. (Eds.). 1994. Volume 27, Development in Aquaculture and Fisheries Sciences. Elsevier, New York, NY, USA.

Total Gas Saturation Considerations for Recirculating Aquatic Systems

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Keywords: Zebrafish, *Danio rerio,* recirculating systems, water quality, total gas pressure, total gas supersaturation

ABSTRACT

Zebrafish (*Danio rerio*) are now widely used in aquatic research facilities for genetic and vertebrate development studies. Most of these facilities utilize recirculating systems for zebrafish production. Dependable production of high-quality fish is of vital concern in these recirculation systems as these fish are valuable and in many cases irreplaceable in terms of their significance to the research being conducted.

Water quality is of utmost concern in zebrafish systems. One critical parameter that has received attention in these facilities is that of total gas pressure. Under abnormal conditions, the partial pressures of dissolved gases in the water can be greater than saturation. When this is the case, there is a potential for problems with gas bubble trauma and an increasing chance for secondary microbial infections. This paper discusses total gas supersaturation theory, problems associated with supersaturation, methods of monitoring total gas pressure, and ways that gas bubble problems can be prevented in recirculating aquatic systems.

INTRODUCTION

Gas Transfer

Under steady-state conditions, the partial pressures of dissolved gases in water are in balance with the pressures of the same gases in the

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atmosphere above the water. Henry's law (Equation 1) is used to determine saturation concentrations of dissolved gases (Colt 1984).

$$C = 1000\ K\ ß\ X\ ((pATM - pH_2O)/760) \quad (1)$$

Where: C = Concentration of the gas (mg/L)

K = Ratio molecular wt of gas to volume (mg/mL)

ß = Bunsen coefficient for the gas

pH_2O = Vapor pressure of water (mm Hg)

pATM = Barometric pressure (mm Hg)

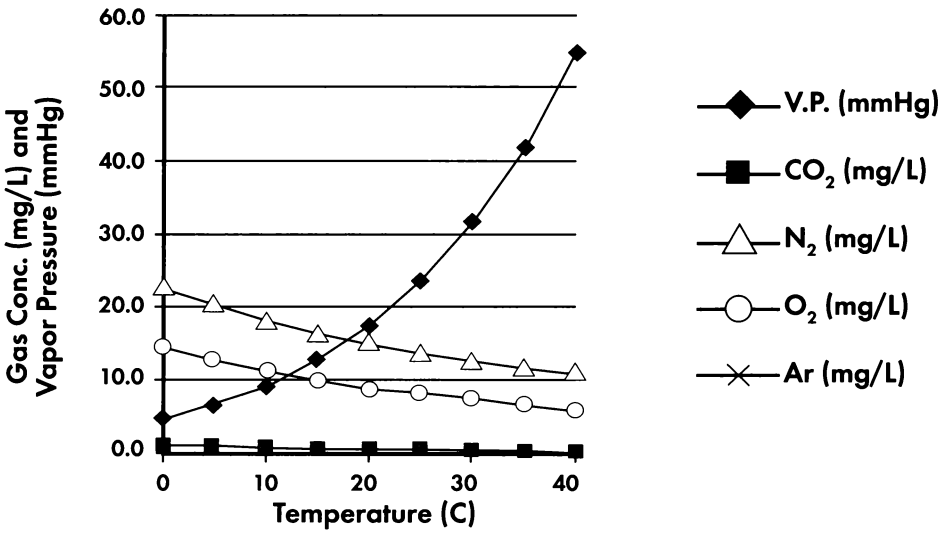
X = Mole fraction of the gas

According to Henry's Law, when the pressure of gas over the water is decreased, the amount of dissolved gas also decreases. In addition, the saturation concentrations of those gases will vary depending on temperature, salinity, and pressure. Higher pressure increases the amount of gas dissolved per unit volume, so the saturation concentration for a gas will be higher in deeper water. The inverse is the case for temperature and salinity. Water at higher temperature or salinity will have less gas per unit volume. Table 1 and Figure 1 present saturation concentrations for various gases in water at different temperatures.

Table 1. Sea level saturation concentrations of dissolved gases and water vapor pressure in freshwater at different temperatures (Colt 1984).

Temp (C)	N_2 (mg/L)	O_2 (mg/L)	Ar (mg/L)	CO_2 (mg/L)	pH_2O (mmHg)
0	23.0	14.6	0.89	1.09	4.6
5	20.3	12.8	0.78	0.89	6.5
10	18.1	11.3	0.69	0.75	9.2
15	16.4	10.1	0.62	0.63	12.8
20	14.9	9.1	0.56	0.54	17.5
25	13.6	8.2	0.51	0.46	23.7
30	12.6	7.5	0.46	0.40	31.8
35	11.7	6.9	0.42	0.35	42.2
40	10.9	6.4	0.39	0.31	55.3

Figure 1. Sea level saturation concentrations of dissolved gases and water vapor pressure in freshwater at different temperatures (Colt 1984).



The sum of the partial pressures of all dissolved gases plus the vapor pressure of water is referred to as the Total Gas Pressure (TGP). The difference between TGP and atmospheric pressure is defined as Delta P (ΔP). Both TGP and ΔP are usually reported in mm Hg (millimeters of mercury). TGP may also be reported as a percent of sea level or local atmospheric pressure (TGP%). The following equations (Colt 1984) present these relationships:

$$TGP = pN_2 + pO_2 + pCO_2 + pH_2O \quad (2)$$

$$\Delta P = pN_2 + pO_2 + pCO_2 + pH_2O - pATM \quad (3)$$

$$TGP = \Delta P + pATM \quad (4)$$

$$TGP\% = (\Delta P + pATM/\ pATM) \times 100 \quad (5)$$

$$\Delta P = (TGP\% \times pATM)\ 100 - pATM \quad (6)$$

Where: TGP = sum of the partial pressures of all dissolved gases and the vapor pressure of water (mm Hg)

ΔP = difference between TGP and atmospheric pressure (mm Hg)

TGP% = TGP as percent of atmospheric pressure (mm Hg)

pN_2 = partial pressure of dissolved nitrogen (mm Hg)

pO_2 = partial pressure of dissolved oxygen (mm Hg)

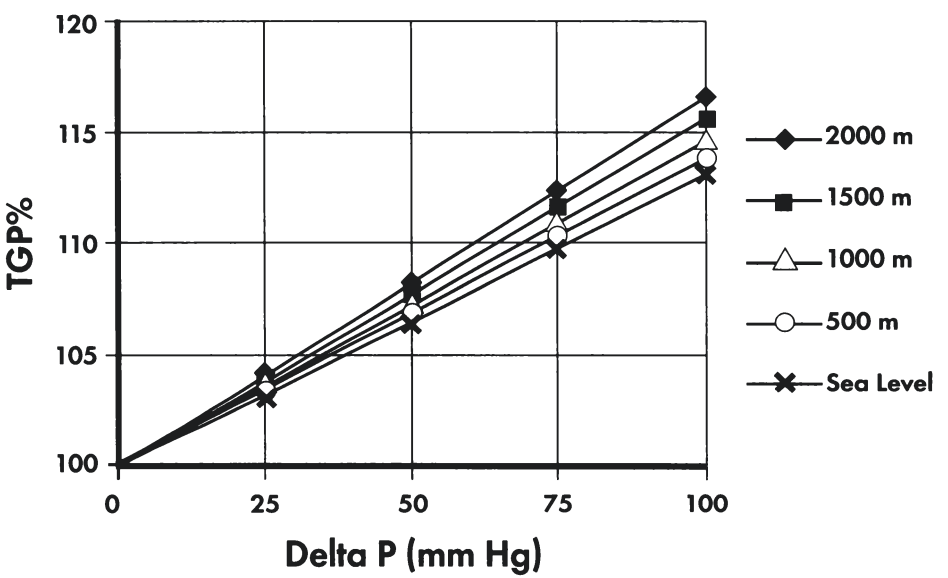
pCO_2 = partial pressure of carbon dioxide (mm Hg)

pH_2O = vapor pressure of water (mm Hg)

pAtm = atmospheric pressure (mm Hg)

Standard Methods for the Examination of Water and Wastewater (APHA/AWWA/WEF 1992) recommends reporting values of ΔP rather than TGP% (percent saturation). However, percent of saturation has been widely used in the past and is probably the most familiar method of reporting total gas pressure data. The problem with the old data is that it was reported in terms of TGP% without the corresponding barometric pressure data. As a result it cannot be accurately converted to ΔP values. Figure 2 presents the relationship between ΔP and TGP% for different elevations.

Figure 2. Relationship between ΔP and TGP% at different elevations.



Measurement of Dissolved Gases

Dissolved gases have been measured using manometry, volumetric tests, mass spectrometry, gas chromatography, chemical titration, direct sensing of pressure, and by headspace partial pressures (Tanner *et al.* 2003; Watten *et al.* 2004). The latter method is the most common means of total dissolved gas measurement and is completed using a saturometer or tensionometer. These devices use a gas permeable membrane (silicone rubber tube) that isolates the dissolved gases and water vapor from the surrounding water. The membrane is connected to a manometer or pressure transducer for pressure measurement.

These instruments measure either ΔP or TGP. When a manometer is used, ΔP (difference between TGP and barometric pressure) is recorded. If a pressure transducer calibrated to absolute pressure is used, then TGP is reported. In this case, ΔP may be calculated using the local barometric pressure. Other instruments utilize a pressure transducer set to zero at the local barometric pressure. The ΔP is reported at that barometric pressure. These devices must be corrected for changes in local barometric pressure.

The actual ΔP experienced by aquatic animals is the difference between TGP and local pressure (barometric pressure plus the hydrostatic pressure). This would be the pressure inside and outside the fish. Gas bubbles will form only when the TGP is greater than the sum of the compensating pressures (APHA/AWWA/WEF 1992; Colt 1984). The compensating pressure is the hydrostatic water pressure, the barometric pressure, and the pressure in the blood or tissues. Gas bubble trauma or gas bubble disease can result if the TDG_{uncomp} is greater than 100% or if the ΔP_{uncomp} is greater than zero (see Equations 7 and 8). The depth of water where the ΔP_{uncomp} is equal to zero is referred to as the hydrostatic compensation depth. Below this depth, it is not possible for dissolved gases to form bubbles or for bubbles to come out of solution. Above this depth, bubbles may form both in the water column and in the blood and tissues of aquatic organisms (Colt 1984).

$$\Delta P_{uncomp} = \Delta P - \rho g Z \quad (7)$$

$$TGP_{uncomp} = [(pAtm + \Delta P)/(pAtm + \rho g Z)] \times 100 \quad (8)$$

Where: ΔP_{uncomp} = uncompensated ΔP (mm Hg)

TGP_{uncomp} = uncompensated total gas pressure (mm Hg)

ρ = the density of water (kg/m^3)

g = acceleration of gravity ($9.8066\ m/s^2$)

Z = depth (m)

Before bubble growth can begin, a threshold ΔP must be exceeded. Thus, the ΔP is a direct indicator of the potential for aquatic and marine organisms to develop signs of gas bubble disease.

Causes of Gas Supersaturation

Numerous sources of gas supersaturation have been reported in the literature. Some of these include; 1) spill from dams, 2) power generation cooling water effluent, 3) solar heating, 4) geothermal heating, 5) photosynthesis, 6) groundwater, 7) airlift aeration and gas injection systems, 8) waterfalls, 9) pumping systems, 10) ice formation, 11) barometric pressure changes, 12) aircraft transport, and 13) ocean waves. For additional information on each of these processes, see the publication by Fidler and Miller (1994).

Gas supersaturation seldom develops in recirculating zebrafish growout systems. However, when it has occurred, there have been two primary causes. The first cause is the rapid heating of water that is under pressure. This can occur in systems utilizing temperature-mixing valves. Because gas solubility decreases as the temperature rises, cold supersaturated water can release bubbles as it is warmed (due to the reduced capacity of the warm water to hold dissolved gas). This may be the case when cold tap water (pressurized to about 50 psi) in piping is depressurized to the local atmospheric pressure. A good practice would be to always degas source water prior to use, especially if it has been heated by more than 5° C (10° F).

The other cause of supersaturation in recirculating aquatic systems can be the result of air drawn into a water pump. Within the pump, the air is forced into solution under high pressure resulting in supersaturation. This may be the case if there is an air leak in the piping on the suction side of the pump, if air is introduced in a sump near the pump inlet, or if a vortex forms in the sump or tank near the pump inlet. Air bubbles introduced into the inlet of the pump by one of these methods may result in total dissolved gas supersaturation levels as high as 110% in less than 5 minutes (unpublished data). Thus, it is very important to routinely ensure that none of the following conditions are occurring: air leaks in suction piping, low

water levels causing a vortex in sumps, and the introduction of air bubbles near the suction inlet of the pump.

Problems Associated with Gas Supersaturation

Dissolved gas supersaturation has been shown to result in several problems for aquatic animals. Some of these conditions include; 1) bubble formation in the cardiovascular system, 2) over-inflation and/or rupture of the swim bladder, 3) bubble formation in the gills, 4) blistering in the skin particularly around the eyes, 5) bubbles in internal organs, 6) loss of swimming ability, 7) susceptibility to secondary infections, and 8) altered blood chemistry (Weitkamp and Katz 1980, Colt 1986, Fidler and Miller 1994, Speare 1998).

Figure 3. Macroscopic gas bubbles in the tissues around eyes of zebrafish exposed to gas supersaturated water (signs of exophthalmia or "pop-eyed" appearance).

(Photo courtesy of Jennifer L. Matthews, D.V.M., Ph.D., Zebrafish International Resource Center, University of Oregon.)

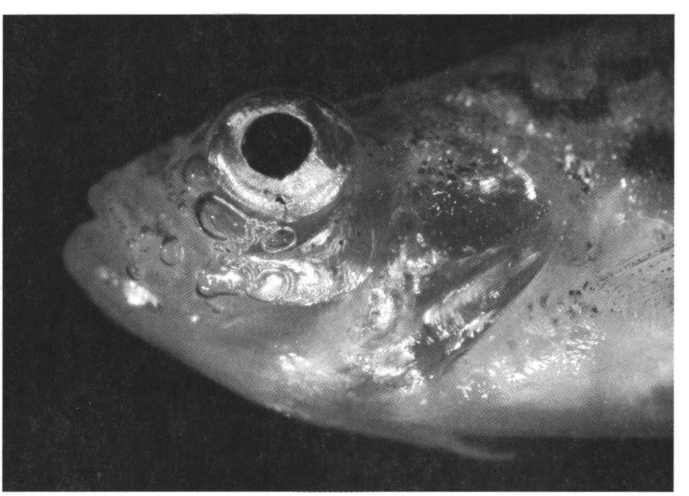


Figure 4. Formation of gas bubbles in tissues near the eyes of zebrafish exposed to gas supersaturated water.

(Photo courtesy of Jennifer L. Matthews, D.V.M., Ph.D., Zebrafish International Resource Center, University of Oregon.)

When zebrafish are exposed to supersaturated water they can show signs of gas bubble disease. This is a non-infectious condition in which gases from supersaturated water come out of solution forming gas bubbles in the circulatory system and tissues of the fish. The major signs of gas bubble disease in zebrafish are exophthalmia (pop-eyed appearance), abdominal distension and hyper-buoyancy, gas bubbles in the skin, or general malaise (Jennifer L. Matthews, DVM, PhD, Zebrafish International Resource Center, University of Oregon, personal communication). The bubbles under the skin can be visible to the naked eye (Figures 3 and 4). This condition can further develop into areas of necrosis, secondary bacterial infections, and eventually death. Diagnosis is based on the observation of gas emboli in capillaries of the gills or internal organs on wet mount exam or by observation of macroscopic gas bubbles in the eyes or skin.

Dissolved Gas Levels of Concern

The U.S. EPA has published a water-quality guideline that recommends a maximum TGP% of 110% of the local atmospheric pressure (U.S. EPA, 1986). This guideline has been accepted by most states. However, there have been numerous studies completed on dissolved gas supersaturation and gas bubble disease since the EPA guideline was developed. These studies suggest that in some cases, the EPA guideline of 110% is too high.

This is definitely the case in shallow applications and/or for certain life stages of aquatic animals. Gas supersaturation values as low as 103% can be dangerous for zebrafish, other aquatic species, or during particular life stages. Adult fish have been shown to be more tolerant of higher total gas pressure than fry or juvenile fishes.

In general, fish will move to deeper water to compress the gases. This prevents bubble formation in their circulatory system and body tissues. However, at a pressure of 103% gas supersaturation, the compensation depth, or depth at which bubbles will not form in the blood of the fish, is only about 12 inches. For every 1% increase in gas tension, the fish must descend 4 inches to compensate for the elevated gas pressure. If the fish tank is only 8-10 inches deep (as is the case with many tanks and aquaria used in research-scale-aquatic systems), the fish would not be able to compensate for a gas supersaturation level of 103%.

CONCLUSIONS

Total dissolved gas pressure is the sum of the partial pressures of all gases dissolved in water plus the water vapor pressure. When the total gas pressure in water is greater than the barometric pressure, the water is supersaturated. These conditions may cause gas bubble disease in fish and other aquatic animals. In lakes and rivers, the aquatic animals can usually move to deeper water to compress the gases. However, for tanks, aquaria, and other shallow settings their vertical movement is limited. Prevention of supersaturation can be extremely important in these cases.

Though instances are rare, it is still possible for supersaturated conditions to occur in laboratory recirculating systems. Supersaturation can result if source water is prepared by mixing cold and warm water, or if air bubbles are drawn into the water pump. Under these conditions, there is an increased chance of gas bubble disease and/or a fish kill. Problems may be minimized or eliminated if source water that has been heated more than 5°C (10° F) is degassed prior to use. In addition, routine care must be taken to ensure that air bubbles do not enter the water pump. This means not allowing the water level of the sump to drop so low that a vortex forms and also ensuring that air bubbles are not drawn into the pump.

REFERENCES

APHA/AWWA/WEF. *Standard Methods for the Examination of Water and Wastewater*, 18th Edition. 1992.

Colt, J. Computation of Dissolved Gas Concentrations in Water as Functions of Temperature, Salinity, and Pressure. *American Fisheries Society Special Publication 14*, 1984.

Colt, J. Gas Supersaturation – *Impact on the Design and Operation of Aquatic Systems. Aquaculture Engineering*, 1986, 5:49-85.

Fidler, L.E., and Miller, S.B. British Columbia Water Quality Guidelines for Dissolved Gas Supersaturation, 1994.

Speare, D.J. Disorders Associated With Exposure to Excess Dissolved Gases. *In Fish Diseases and Disorders*. Wallington, Oxon, UK. 1998. Leatherland, J.F., and Woo, P.T.K., (Eds.). CAB International Publications: Wallington, Oxon, UK.

Tanner, D.Q., Bragg, H.M., and Johnston, H.M. Total Dissolved Gas and Water Temperature in the Lower Columbia River, Oregon and Washington, 2003: Quality-Assurance Data and Comparison to Water-Quality Standards. *Water Resources Investigations Report,* 2003, 03-4306.

U.S. Environmental Protection Agency. *Quality Criteria for Water*. EPA-440-5-86-001. 1986.

Watten, B.J., Boyd, C.E., Schwartz, M.F., Summerfelt, S.T., and Brazil, B.L. Feasibility of Measuring Dissolved Carbon Dioxide Based on Headspace Partial Pressures. *Aquacultural Engineering*, 2004, 30 (3-4):83-101.

Weitkamp, D.E., and Katz, M. A Review of Dissolved Gas Supersaturation Literature. *Transactions of the American Fisheries Society*, 1980, 109:659-702.

Intensive Zero-Exchange Shrimp Production Systems – Incorporation of Filtration Technologies to Improve Survival and Growth

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Keywords: Shrimp, production, aquaculture, filtration, waste products, biofilters, clarification, nitrification

ABSTRACT

Cost effective application of superintensive, biosecure marine production systems in the U.S. will depend upon proactive management of culture-water quality. More efficient production practices and effective management of waste materials from the shrimp aquaculture industry can allow for higher productivity, improved growth and survival, and pave the way for eventual application away from coastal areas. These improved production strategies are key factors contributing to profitability and environmental sustainability. Development of cost-effective management strategies includes application of mechanical and biological filtration devices to remove solids and nitrogenous products from culture systems. Accumulation of these waste products can limit system productivity and negatively impact cultured animals, increasing the potential for stress,

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disease, and mortality. Technologies developed to remove solids and maintain concentrations of nitrogenous waste products within acceptable limits include different types of filters used alone or in combination with a variety of media types. All of these technologies have achieved varying degrees of success. While use of expandable granular biofilters is not new, improvements have been made in the design and composition of the filtration media. This, in conjunction with an appropriate backwash regimen, encourages attachment and growth of nitrifying bacteria to accomplish clarification and nitrification in a single unit. The purpose of this study was to evaluate the effects of biological and mechanical filtration on production and selected water-quality criteria in zero-exchange, biosecure, superintensive shrimp production systems.

MATERIALS AND METHODS

The efficacy of two different filtration medias, alone and mixed 1:1 was evaluated using airlift-driven marine recirculating bubble-washed bead filters (MRBF). A foam fractionator (FF) using bubbled air was used to evaluate mechanical filtration. Both treatments were fitted to green-water tank systems stocked at high density (287 animals/m^2) with Pacific white shrimp (*Litopenaeus vannamei*). The two types of media used were Enhanced Nitrification (EN), a floating modified polyethylene bead (Beecher *et al.* 1997); Kaldnes Miljøteknologi moving bed filter media (KMT, Tonsberg, Norway), a neutrally buoyant polyethylene wheel (Lekang and Kleppe 2000); and a 1:1 mix of EN and KMT. Both EN and KMT media have a density <1 and a specific surface area of 500-1050 m^2/m^3 so that biofilm formation can occur while allowing the media to remain positively buoyant. Media used in this experiment were either new or bleached, reused beads which had no organic material associated with them.

Twenty 3.35 m diameter (8.8 m^2) polyethylene tanks were used to evaluate five treatments: no filtration (control); mechanical filtration (FF); biological filtration (EN Media), biological filtration (KMT Media); and biological filtration (mixed media EN/KMT). There were four replicate tanks (Figure 1) for each treatment. Tanks (each holding 6,279 L) were filled with filtered (25 μm) sea water from South Carolina's Colleton River (~28 g/L) and were maintained without water exchange. A commercial liquid fertilizer (Tri-Chek liquid polyphosphate pond fertilizer 10-34-0, Tri-Chek Seeds, Inc., Augusta, GA, USA) was applied on Days 1 and 3 (post fill) at 100 ml/tank and on Day 7 at 50 ml/tank to promote algal

bloom development. Continuous aeration and circulation were supplied to the tanks and filters by two 5-hp regenerative blowers (Metek Model DR3D89, Rotron Industrial Products, Saugerties, NY, USA) delivering air to six fine pore airstones and one central 12-inch porous pipe diffuser per tank. Bead filters (Aquatic Systems Technologies LLC, New Orleans, LA, USA) were filled with 0.84 m^3 of media and automatically backwashed using air from a 1.6-hp compressor regulated at 40 psi (Ironforce 6.25 hp, Campbell Hausfeld, Harrison, OH, USA). Filter air flow was adjusted over a period of three weeks to establish a backwash periodicity of 2.5-3.0 h with a duration of roughly 60 s. The FF units were airlift-driven, prototype units (model # PS8 8.5", Aquaneering, Inc., San Diego, CA, USA) 20.3 cm x 169 cm with a rated flow of 45-94 L/m. Tanks were covered with white netting to prevent escape and juvenile (mean weight = 2.0 g) Pacific white shrimp were stocked at a density of 287/m^2 on Day 11 post-fill (June 13, 2003 - study Day 0). To better control tank temperatures the entire tank complex was covered with a roof of 63% shade cloth. Shrimp were fed a 35% protein, 8% lipid, 2.5% squid meal diet (Rangen, Inc., Buhl, ID, USA) applied twice daily (at 0800 and 1600 h) to single feed trays in each tank. Feed rate was adjusted based upon shrimp growth and feed consumption. Feed quantity applied and consumed was recorded at each feeding. Shrimp growth was measured weekly (treatments divided in half and each half measured every other week) by obtaining individual weights of 50 randomly collected shrimp from each tank.

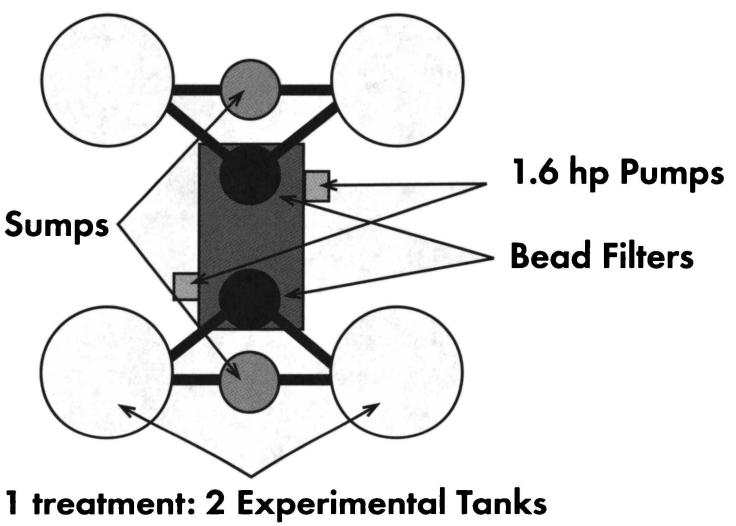


Figure 1. Design of experimental system.

Dissolved oxygen (mg/L), temperature (C), salinity (g/L), and pH (YSI Model 556 Multiprobe System, Yellow Springs Instrument, Yellow Springs, OH, USA) were recorded every morning (~0800 h). Temperature and pH were again recorded every afternoon (~1600 h). To maintain pH levels >7.0 and alkalinity >120 mg/L (as $CaCO_3$), sodium bicarbonate was added to each system. Alkalinity, total ammonia-N, nitrite-N, nitrate-N, and chlorophyll *a* were measured weekly according to standard water quality methods (APHA 1989). Total suspended solids (TSS) was measured weekly and turbidity (ntu) was measured daily using a turbidometer (Micro 100 Turbidometer, HF Scientific, Fort Myers, FL, USA) and by Secchi disc depth. Light and dark BOD bottles were used to measure water column gross oxygen production (change in light bottle minus change in dark bottle) and demand (change in dark bottle) and calculate net primary productivity (gross oxygen production divided by oxygen demand) once a week (Bratvold and Browdy 1998). Bead-filter maintenance included monitoring flow rates, inlet/outlet dissolved oxygen levels, sludge volume, percent solids, and filter backwash regularity. Flow rates and dissolved oxygen levels associated with bead-filter intake/outflow and foam fractionator return flow rates were measured twice during the week. Flow rate through the filter was adjusted for a turn over rate of roughly 10 water exchanges daily. Sludge was removed and total volume measured twice a week. To ensure maximum removal, sludge was purged from the filters until the discharge was clear (tank volume lost to sludge removal was <1%). The sludge was then mixed to remove bead-filter media and create a homogenous sample, and total volume was recorded before aliquots were removed and allowed to settle to determine percent solids. Discharge from the foam fractionators was also collected (collection buckets were removed and replaced every morning and afternoon), quantified and allowed to settle for percent solids determination. Sludge samples were collected weekly for total and volatile suspended solids (TSS, VSS), Kjeldahl nitrogen (TKN), and total organic carbon (TOC), and either frozen or the pH reduced to <2 by addition of H_2SO_4 for later analysis. Filter backwash periodicity and duration were monitored, recorded, and adjusted once a week. Filter air flow was checked daily. For all measured parameters, the treatments were compared with ANOVA tests when data exhibited normal distribution. Student's t-test was used to compare treatment means ($\rho = 0.05$). An ANOVA on ranks (Wilcoxon test) was performed for data that were not normally distributed.

RESULTS

Average shrimp weight at harvest, mean growth/week, survival, production, and food conversion ratio (FCR) are listed in Table 1. KMT treatments showed significant increases in growth rate relative to mixed media, foam fractionation, and control treatments. A similar trend was observed for the EN treatment. Survival and production, on the other hand, were not significantly different than control and FF treatments. Mixed-media filtered tanks performed least effectively, with production and FCR significantly different from other treatments.

Table 1. Mean values for harvest weight, growth/week, survival, food conversion ratio (FCR) and production. Values in a column with different superscripts are significantly different.

	Weight (g)	Growth/week (g)	Survival (%)	FCR	Production (kg/m^3)
Control	7.9 ± 0.7^{b}	0.8 ± 0.1^{b}	77.0 ± 3.1^{a}	1.9 ± 0.1^{ab}	2.4 ± 0.1^{b}
FF	8.5 ± 1.0^{ab}	0.8 ± 0.1^{ab}	63.9 ± 5.4^{ab}	2.2 ± 0.1^{ab}	2.2 ± 0.2^{b}
EN	9.4 ± 0.5^{ab}	0.9 ± 0.1^{ab}	65.8 ± 24.3^{ab}	2.3 ± 1.2^{ab}	2.5 ± 1.0^{b}
KMT	9.9 ± 1.0^{a}	0.9 ± 0.1^{a}	69.3 ± 3.9^{ab}	1.8 ± 0.2^{a}	2.8 ± 0.4^{b}
EN/KMT	8.4 ± 1.6^{b}	0.8 ± 0.2^{b}	43.8 ± 35.0^{b}	4.7 ± 2.9^{b}	1.6 ± 1.4^{a}

There were significant water-quality differences between treatments (Table 2). Dissolved oxygen levels and daily pH values were significantly higher in bead-filter treatments than in unfiltered treatments. The DO range reflects two power outages that interrupted tank aeration. Salinity in FF tanks was significantly different from other treatments, including controls.

Total ammonia-nitrogen (TA-N) and nitrite-nitrogen (NO_2-N) were significantly different in filtered and unfiltered treatments (Table 3). Mixed media tanks had significantly higher TA-N and NO_2-N concentrations than all other treatments and all filtered treatments had higher NO_2-N concentration than unfiltered treatments. Unfiltered treatments had significantly higher NO_3-N concentrations. Unfiltered tank TA-N dropped to <1.0 mg/L by Day 14 while filtered tanks, especially those with KMT media, never appeared to stabilize and decrease. By day 45 the NO_2-N in unfiltered tanks dropped to <0.5 mg/L while filtered tanks continued to have higher, fluctuating nitrite levels. In all tanks NO_3-N concentration

Table 2. Mean values for salinity, temperature, pH, and dissolved oxygen. Values in a column with different superscripts are significantly different.

	Salinity (g/L)	AM Temp. (C)	PM Temp. (C)	AM pH	PM pH	D.O. (mg/L)
Control	21.9 ± 2.1^{b}	27.0 ± 0.9^{a}	28.2 ± 1.0^{a}	7.3 ± 0.3^{d}	7.4 ± 0.2^{e}	5.7 ± 0.8^{c}
FF	22.8 ± 2.0^{a}	27.0 ± 0.9^{a}	28.1 ± 1.0^{a}	7.4 ± 0.3^{c}	7.5 ± 0.2^{d}	6.0 ± 0.7^{b}
EN	21.9 ± 2.0^{b}	26.8 ± 0.9^{a}	28.2 ± 1.1^{a}	7.5 ± 0.2^{b}	7.7 ± 0.2^{c}	6.3 ± 0.6^{a}
KMT	22.0 ± 2.5^{b}	26.9 ± 0.9a	28.2 ± 1.1^{a}	7.6 ± 0.2^{a}	7.8 ± 0.2^{a}	6.2 ± 0.7^{a}
EN/KMT	21.9 ± 2.2^{b}	26.9 ± 0.9^{a}	28.2 ± 1.1^{a}	7.6 ± 0.2^{a}	7.7 ± 0.2^{b}	6.2 ± 0.7^{a}
Mean	22.1 ± 2.1	26.9 ± 0.9	28.2 ± 1.0	7.5 ± 0.3	7.6 ± 0.3	6.1 ± 0.8
Range	18.0 - 22.1	24.5 - 30.9		7.5 - 8.5		2.1 - 8.2

showed a slight decrease around Day 30 but then increased again and continued to increase for the duration of the production trial. Survival was similar to that of previous production trials with the exception of three filtered tanks which experienced elevated NO_2-N levels.

Table 3. Mean values for dissolved inorganic nitrogen: A. TA-N; B. NO_2-N; C. NO_3-N. Values in a column with different superscripts are significantly different.

	TA-N (mg/L)	NO_2- N (mg/L)	NO_3-N (mg/L)
Control	1.1 ± 2.0^{b}	3.1 ± 5.0^{d}	25.5 ± 13.9^{a}
FF	1.3 ± 1.9^{b}	3.2 ± 4.4^{d}	24.3 ± 14.2^{ab}
EN	1.2 ± 1.8^{b}	9.1 ± 9.5^{abc}	17.5 ± 11.1^{cd}
KMT	1.6 ± 1.5^{b}	7.4 ± 5.3^{c}	14.5 ± 11.6^{cd}
EN/KMT	3.9 ± 9.6^{a}	11.9 ± 8.9^{a}	19.4 ± 13.1^{bc}
Mean	2.1 ± 5.2	7.8 ± 7.9	18.6 ± 13.0
Range	0 -57.0	0 - 34.1	0 - 64.0

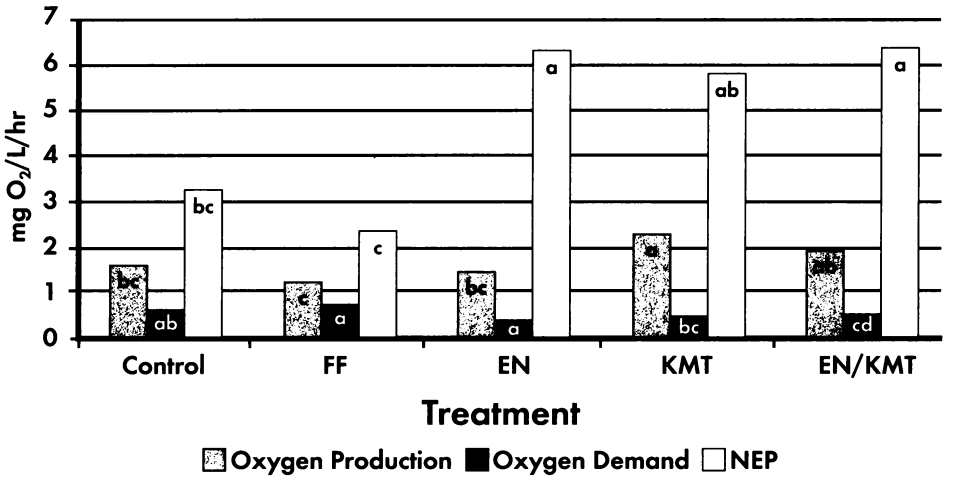


Figure 2. Overall mean treatment values for oxygen production, oxygen demand, and net ecosystem production (NEP). Values across columns not sharing the same letter are significantly different.

Figure 2 illustrates the relationship between oxygen production, demand, and net ecosystem productivity (NEP). An NEP >1 indicates that there is more oxygen production than oxygen consumption while an NEP <1 indicates that consumption exceeds production. Filtered tanks containing KMT media (alone or with EN media) had the highest oxygen production while tanks containing EN media (alone or with KMT media) had the lowest oxygen consumption compared to unfiltered treatments. Filtered tanks also had the highest NEP.

Chlorophyll *a* levels were significantly lower in filtered treatments (Table 4) even though oxygen production was significantly higher than unfiltered tanks. In addition to reduced chlorophyll *a*, filtered treatments had less VSS and TSS. An exception to this trend was observed in two EN tanks where TSS and VSS increased at about Day 30. Despite demonstrated solids removal, filtered treatments turbidity fluctuated during the production trial and the turbidity actually increased in EN media tanks (Table 4) even though sludge output remained high. This increase in solids load was accompanied by an increase in dissolved oxygen consumption within the two affected EN filters (Figure 3). Unfiltered tank turbidity increased and Secchi depth decreased throughout the production trial.

Table 4. Overall mean values for suspended solids and water clarity. All levels were significantly different for filtered tanks.

	Chlorophyll *a* (mg/m^3)	VSS (mg/L)	TSS (mg/L)	Turbidity (ntu)	Secchi (cm)
Control	226.7 ± 95.9^{a}	263.3 ± 96.9^{a}	383.6 ± 134.2^{a}	119.4 ± 37.4^{a}	17.6 ±5.4^{b}
FF	195.7 ± 97.9^{ab}	225.6 ± 100.6^{b}	318.6 ± 125.3^{b}	100.8 ± 24.5^{b}	18.2 ± 5.5^{b}
EN	150.8 ± 100.4^{b}	74.7 ± 89.9^{c}	113.4 ± 132.8^{c}	30.1 ± 13.8^{c}	42.4 ± 8.2^{a}
KMT	179.5 ± 132.9^{b}	40.0 ± 43.4^{de}	58.4 ± 61.8^{d}	20.8 ± 7.3^{c}	44.4 ± 9.9^{a}
ENKMT	153.3 ± 109.8^{b}	34.0 ± 20.6^{e}	50.5 ± 29.0^{d}	20.2 ± 7.2^{c}	41.7 ± 8.0^{a}
Mean	176.5 ± 114.8	97.0 ± 112.3	141.1 ± 159.0	58.3 ± 47.9	33.1 ± 14.3
Range	2.0 - 533.2	0 - 486.0	5.0 - 636.0	9.4 - 210.0	10.3 - 63.0

Feed rates were adjusted as shrimp growth and water quality parameters changed. Throughout the production trial feed loading never exceeded 600 g/day or 0.7 kg/m^3 of bead media. Cumulative sludge removal and % settleable solids were highest for EN media filters and lowest for FF tanks with no significant difference in either sludge removed or settleable solids between KMT and EN/KMT filters. In sampled filters there was no appreciable change in TKN across the EN filter. TKN increased in water returning from the KMT filter and was only reduced after passing through the EN/KMT filter. TKN increased in sampled sludge from all three filters with the greatest increase in organic nitrogen loading occurring in the EN filter (six times higher than the initial sample) which also had the highest initial concentration.

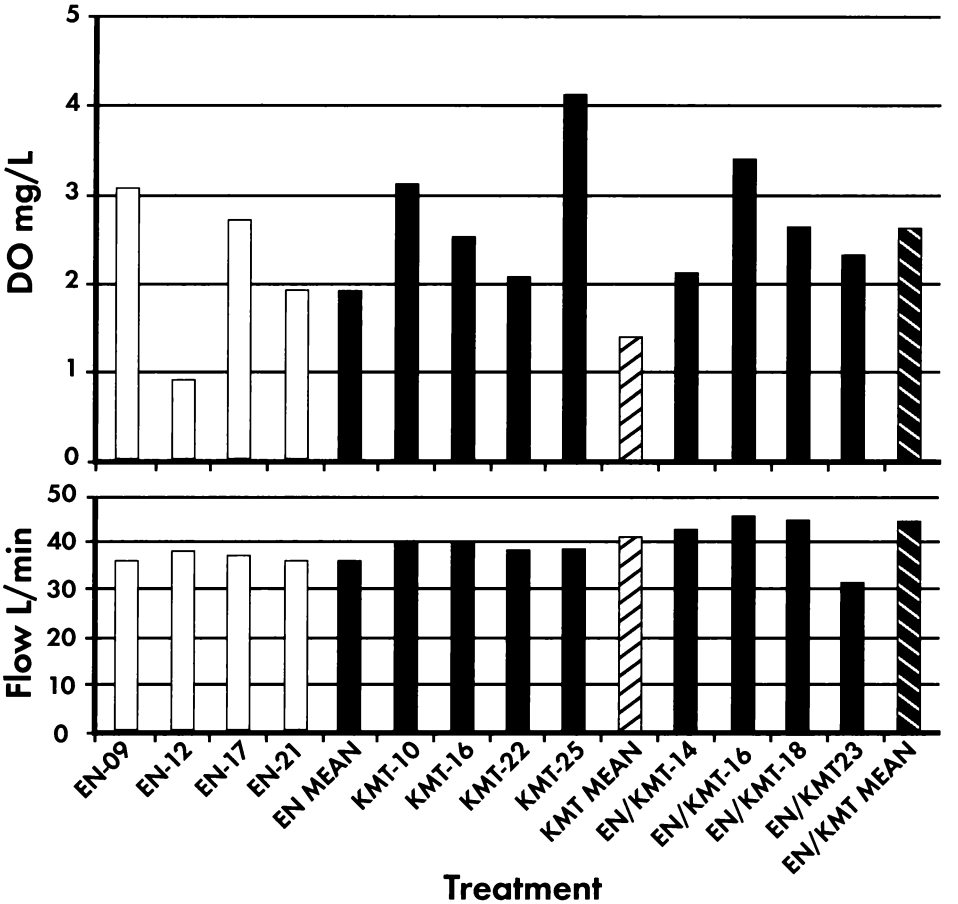
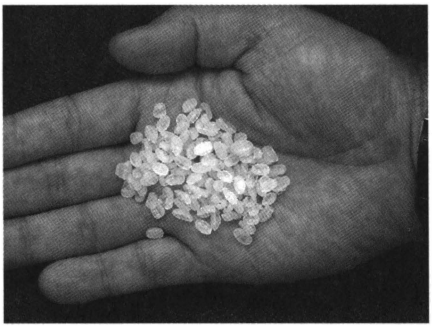


Figure 3. Filter respiration and tank return flow for all filtered tanks. Although mean filter flow appears consistent, there was a significant decrease in flow in both FF and filtered treatments over the course of the production trial.

DISCUSSION

The purpose of this study was to evaluate air lift-driven bead filters and foam fractionation units for their potential as management strategies for suspended solids and nitrogenous waste removal in superintensive, zero exchange shrimp production systems. The type of bead filter used was particularly attractive because it had the capacity to function as both a biological and mechanical filtration unit while requiring no electric pump for operation or removal of accumulated solids. Filtration was to be accomplished through the use of two dissimilar polyethylene media (Figure 4). EN media as a small modified bead has a much greater composite surface area and smaller packed volume pore space than the

larger wheel shaped KMT media. The larger surface area for adhesion coupled with the smaller pore space was expected to remove more small particulate material. The modified shape provided a concave surface to protect nitrifying bacteria under backwash conditions. The KMT media, with its protected interior surface for colonization, was expected to remove and accumulate larger solids while retaining a greater population of nitrifying bacteria under backwash conditions. Because solids capture efficiency varies when media size is fixed, it was expected that the combination of these two dissimilar media would achieve the solids capture efficiency of the individual media types. Especially under the green-water conditions of this study, determining the appropriate backwash frequency to maximize solids removal while enhancing biofiltration is critical if the detrimental effects of retained solids decay and subsequent ammonia loading is to be avoided. Under normal organic loads the small, tightly packed EN media should be backwashed more frequently than the larger, less densely packed KMT media for optimal function (Moore *et al.* 2001). Foam fractionation as a mechanical filtration unit was expected to efficiently remove fine suspended solids and dissolved solids using bubbled air moving upwards against the downward flow of water from the tank (Cripps and Bergheim 2000). As with the bead filter used, the design of the foam fractionator was attractive because it required no electric pump for operation.



EN Media

KMT Media

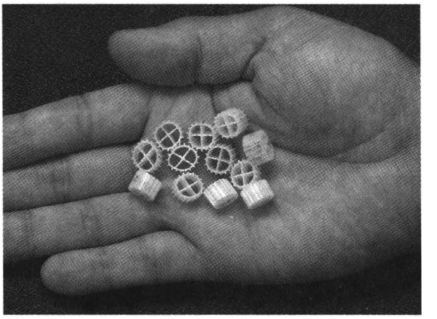


Figure 4. Comparison of EN media to KMT media.

High-density production trials conducted in zero-exchange superintensive raceway systems at the Waddell Mariculture Center have achieved production rates approaching 3 kg/m^2 (McAbee *et al.* 2001, Weirich *et al.* 2002). At these biomass levels, problems with unexplained reductions in survival rates, cuticular lesions and chronic mortality were thought to be indicative of a system at or near its carrying capacity (Weirich *et al.* 2002). During this experiment, weight at harvest and mean growth rate (g/week) were less than that seen in previous production trials under similar conditions while survival, FCR, and production numbers, however, were consistent with those achieved during previous production trials. Although bead-filtered treatments were expected to improve survival and growth, results were quite variable.

Previous studies in these systems demonstrated that cropping of organic material increased system carrying capacity while enhancing growth of the target crop (Weirich *et al.* 2002, McAbee *et al.* 2001). The results of this study confirm that incorporating filtration into a eutrophic, zero-water-exchange intensive production system can offer clear benefits as a mechanical means of reducing solids, managing the algal community and improving general water quality. This improvement is reflected by higher pH and oxygen levels in the presence of reduced chlorophyll *a* and increased water clarity in filtered treatments. Significant reduction in TSS and VSS were also as expected for bead filtered systems which function well at removing particles larger than 50 μm (Chen *et al.* 1993). The mean percentage of TSS that was VSS ranged from 62-74%, consistent with observed values for aquacultural (Ning 1996) and domestic sludge (Metcalf and Eddy Inc. 1991). The percentage of nitrogen (as TKN) in sludge was much greater than in aquacultural (Ning 1986) and domestic sludge (Metcalf and Eddy Inc. 1991) with mean values ranging as high as 74%.

Although this may at first appear to be counterintuitive, cropping of senescent algal cells and suspended organic material by filter media may have been conducive to the production of a younger, more active phototrophic community. This type of natural productivity has been suggested to contribute to the improved growth of Pacific white shrimp associated with pond water based systems (Moss *et al.* 1992, Moss 1995). In the present study this may have been reflected in the significant improvement in growth rate in the KMT filter treatment.

The bead filters, however, did not effectively reduce nitrogenous wastes. Although all tanks were filled and fertilized prior to being stocked, it is

likely that there was inadequate time for nitrifying bacteria to become well established and to maintain a density sufficient for effective nitrification while backwash frequency and periodicity were being adjusted. Additionally, while backwash frequency and periodicity were adequate for removing solids from the filtered systems, sludge removal needed to be more frequent to mitigate the effects of organic solids accumulation and decomposition. Conditions within the filters tended to favor proliferation of faster growing heterotrophic bacteria rather than the slower growing nitrifying bacteria (Sastry *et al.* 1999). It was apparent that even with a relatively low feed loading, media type affected filter performance under the gentle backwash conditions present in the airlift driven MRBF system. The configuration of the KMT media did not function effectively under the relatively low feed loading at the established backwash frequency. Solids trapped within the interior of the bead clogged the media, reduced the available surface area for nitrification and reduced bead buoyancy causing the media to sink and become mired in the bottom of the filter. The EN media was also problematic. Because it removed smaller particles more efficiently than the larger, less densely packed KMT or mixed media, it removed too much of the suspended material associated with attached nitrifying bacteria and beneficial flocculent material which can contribute to shrimp growth (Moss *et al.* 1992, Burford *et al.* 2004). Although EN tanks clearly removed more solids throughout the production trial, at about Day 30, two of the EN filters had significant increases in suspended solids and decreases in water clarity. The increase in TSS and VSS, indicative of reduced filter function possibly due to excessive solids retention and to clogging, was accompanied by a significant increase in filter oxygen consumption. This increase in consumption is to be expected as every 1 mg increase in VSS requires roughly 1.42 mg O_2 for oxidation (McCarty 1965). Mixed-media tanks operated with efficiency intermediate to the filters filled with a single media type. Despite constant cleaning both bead filters and foam fractionators were affected by fouling within the pipes by filamentous algae and other organisms which attached themselves to the air stones driving the system. Because the filters were gravity-fed, they had to be buried below ground to circulate water with airlifts. As a result, sludge removal above ground was incomplete in all filters. Effective removal was complicated by media, especially the larger KMT media, which was subject to fouling as discussed previously, becoming trapped in the sludge drain. Repetitive backwashes, forcing air back through the filter and "bumping" the filter did not always break up bead/biofloc clumps and

when the systems were taken down at the end of the study a substantial amount of solids with embedded media were stuck in the bottoms of the filters.

It was expected that the FF units would function better in conjunction with a marine system than a freshwater system and would have been capable of removing both fine and suspended solids (Chen *et al.* 1992; Cripps and Bergheim 2000). Although turbidity and suspended solids were significantly less in FF tanks as compared to control tanks, solids removal was inconsistent and directly related to the intermittent generation of bubbles with a consistency capable of entrapping and removing solids from the water column. When foam was produced it was *en masse* and despite trying several strategies to cause the bubbles to break up and collect as sludge, much of the material remained stuck to the inside of the clear plastic sleeve at the top of the unit.

Aquaculture systems with or without filtration have some nitrification capacity. Nitrifying bacteria actively colonize available tank surfaces when optimal water conditions are met. Indeed, in the bacterial floc dominated, zero-exchange shrimp production systems, nitrification *in situ* has been shown to effectively control ammonia and nitrite levels within the system (Browdy *et al.* 2001, Bratvold and Browdy 2001, Weirich *et al.* 2002). With environmental parameters within tolerance in both filtered and unfiltered tanks, *in situ* nitrification should account for 30-60% of total nitrification (Malone *et al.* 1993). Control and FF treatments had successful populations of nitrifying bacteria and had no problems with ammonia or nitrite. Filtered treatments having the same available surface area in addition to filter media were not as successful in managing these compounds. It seems likely that while not optimized for nitrification, the efficiency of solids removal may have depleted this inherent nitrifying capacity. As a result, high nitrite levels may account for the negative results on survival and production found in the mixed media treatment.

CONCLUSION

Both the bead filters and foam fractionators were effective at removing solids from the high-density zero exchange shrimp production tanks. Although the cropping of organic material stimulated algal primary productivity and did result in improved growth in one treatment, overall results were variable. In many tanks the removal of too much of the

bacterial floc from the system and the breakdown of organic material within the filter may have contributed to increased ammonia and nitrite levels and reductions in growth and survival. Operated as they were in this production trial, filtration did not make a positive contribution to the management of nitrogenous waste. To optimize filtration and nitrification and to negate these problems in subsequent production trials, refinements to the filter systems and operating protocols have been designed. The goal of these refinements is to improve the operation of the filters by reducing organic loading and improving control of the level of cropping of organic material from production tanks. Refinements include using EN media exclusively, reducing the backwash rate to once every eight hours, raising the filters above ground level using 1/3-hp pumps to circulate the water, and removing settled solids more frequently. To preclude over-cropping of algae and beneficial flocculent material in the tank water from cycling through the filter, filtration will be more precisely controlled through the use of a sump coupled with a recirculation loop. The ultimate goal of these studies is to maximize growth and carrying capacity while improving production efficiency, as measured in terms of nitrogen transfer from feeds to the target crop and waste recycling within the system.

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REFERENCES

APHA (American Public Health Association) American Water Works Association and Water Pollution Control Federation. 1989. *Standard methods for the examination of water and wastewater*. 17th edition. APHA, Washington, DC, USA.

Beecher, L., DeLosReyes, A., Drennan, D., and Malone, R. Alternative Media for Enhanced Nitrification Rates in Propeller-washed Bead Filters. In *Advances in Aquacultural Engineering, Aquacultural Engineering Society Proceedings III*, Orlando, FL, November 9-12, 1997. Timmons, M., and Losordo, T. (Eds.). ISTA IV: New York, NY, USA.

Bratvold, D., and Browdy, C. Simple Electrometric Methods for Estimating Microbial Activity in Aquaculture Ponds. *Aquacultural Engineering*, 1998, 19:29-39.

Bratvold, D., and Browdy, C. Effects of Sand Sediment and Vertical Surfaces (Aquamats) on Production, Water Quality, and Microbial Ecology in an Intensive *Litopenaeus vannamei* Culture System. *Aquaculture*, 2001, 195:81-94.

Browdy, C., Bratvold, D., Stokes, A., and Mcintosh, R.P. Perspectives on the Application of Closed Shrimp Culture Systems. In: *The New Wave, Proceedings of the Special Session on Sustainable Shrimp Farming Aquaculture*, Baton Rouge, 2001. Browdy, C., and Jory, E. (Eds.). World Aquaculture Society, Baton Rouge, LA, USA.

Burford, M., Thompson, P., McIntosh, R., Bauman, R., and Pearson, D. The Contribution of Flocculated Material to Shrimp (*Litopenaeus vannamei*) Nutrition in a High-intensity, Zero-exchange System. *Aquaculture*, 2004, 232:525-537.

Chen, S., Timmons, M., Biogni Jr., J., and Aneshansley, D. Suspended Solids Removed by Foam Fractionation. *Progressive Fish Culturist*, 1992, 55:69-75.

Chen, S., Timmons, M., Aneshansley, D., and Biogni Jr., J. Suspended Solids Characteristics from Recirculating Aquacultural Systems and Design Implications. *Aquaculture*, 1993, 112:143-155.

Cripps, S., and Bergheim, A. Solids Management and Removal for Intensive Land-based Aquaculture Production Systems. *Aquacultural Engineering*, 2000, 22:33-56.

Lekang, O-I., and Kleppe, H. Efficiency of Nitrification in Trickling Filters Using Different Filter Media. *Aquacultural Engineering*, 2000, 21:181-199.

Malone, R., Chitta, B., and Drennan, D. Optimizing Nitrification in Bead Filters for Warmwater Recirculating Aquaculture. In *Techniques for Modern Aquaculture*, 1993. Wang, J. (Ed.). American Society of Agricultural Engineers.

McAbee, B., Browdy, C., Rhodes, R., and Stokes, A. The Use of Greenhouse-enclosed Raceway Systems for the Super-intensive Production of Pacific White Shrimp, Book Review 6:40-43.

McCarty, P. Thermodynamics of Biological Synthesis and Growth. *Journal of Air and Water Pollution* 1965, 9:621-639.

Metcalf and Eddy, Inc. 1991. *Wastewater Engineering: Treatment, Disposal, Reuse*. McGraw-Hill, New York, NY, USA.

Moore, R., Quarmby, J., and Stephenson, T. The Effects of Media Size on the Performance of Biological Aerated Filters. *Water Research* 2001, 10:2514- 2522.

Moss, S. Production of Growth-enhancing Particles in a Plastic Lined Shrimp Pond. *Aquaculture* 1995, 132:253-260.

Moss, S., Pruder, G., Leber, K., and Wyban, J. The Relative Enhancement of *Penaeus vannamei* Growth by Selected Fractions of Shrimp Pond Water. *Aquaculture* 1992, 101:229-239.

Ning, Z. 1996. Characteristics and Digestibility of Aquacultural Sludge. Masters Thesis, Louisiana State University, Baton Rouge, LA, USA.

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Weirich, C., Browdy, C., Bratvold, D., McAbee, B., and Stokes, A. Preliminary Characterization of a Prototype Minimal Exchange Super-Intensive Shrimp Production System. In Proceedings of the 4th International Conference on Recirculating Aquaculture, Roanoke, Virginia, 2002; Virginia Tech, Blacksburg, VA, USA.

Book Review

Aquaculture and Fisheries Biotechnology: Genetic Approaches

R.A. Dunham

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With the increased global demand for aquaculture products comes the need for developing more efficient production systems. The emergence of aquatic biotechnology over the past 25 years poses the possibility of manipulating a wide variety of traits valued by aquaculturists and fisheries managers. In addition to benefits for aquaculture and fisheries, however, aquaculture biotechnology also presents a number of controversies. Against this background, Rex Dunham presents *Aquaculture and Fisheries Biotechnology: Genetic Approaches*. His aim, stated in the preface, is to explain to students, farmers, fisheries biologists, and scientists how theory relates to reality and to provide a strong review of the current status of key biotechnology topics, illustrating concepts with key research results. He aims to be objective regarding controversial topics, presenting various viewpoints and then discussing differing perspectives in the context of the available data. The book has 19 chapters, with a supporting glossary, extensive references to the technical literature, and an index.

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Chapter 1 presents a short, concise history of genetics and biotechnology, placing aquaculture biotechnology within its larger context. Genetics is traced from Robert Hooke (cell theory) through Charles Darwin (natural selection), R.A. Fisher and Sewall Wright (quantitative and population genetics), and the rise of molecular genetics and biotechnology. Developments in the breeding of aquatic organisms are traced from non-scientific carp breeding by the Romans and Chinese to the emergence of science-based selective breeding, to the rise of aquaculture biotechnology, including ploidy manipulation, sex reversal, gene transfer, and genomics. The chapter effectively provides the historic context for aquatic biotechnology, and the few glitches (e.g., Frank Ruddle's group produced the first transgenic mouse – see Gordon *et al.* 1980, Dolly the sheep was cloned in 1996) will not distract most readers.

To effectively measure and exploit the genetic components of variation for purposes of selective breeding, environmental sources of phenotypic variation must be recognized and then controlled for experimentally or corrected. Chapter 2 proceeds from a brief explanation of partitioning of phenotypic variance to a discussion of the numerous types of environmental effects, including stocking density, age, and maternal effects. Making the point that many published papers in molecular genetics and biotechnology ignore details of how the fish were cultured, Dunham provides important background for evaluating the validity and contribution of aquaculture biotechnology studies.

Manipulation of entire chromosome sets has proven a key area of aquatic biotechnology, producing triploid stocks that are sterile or rapidly growing for particular species. In Chapter 3, Dunham reviews the literature on polyploidy induction in fish and shellfish, considering methods for ploidy manipulation, ploidy assessment, and performance of triploid stocks. He then considers the implications of using existing triploids stocks for a range of potential applications in aquaculture and fishery management. This is generally a strong chapter; Dunham explains why triploidy is or is not a viable option for various species in aquaculture or fisheries management contexts, and concludes that polyploidy has good potential for genetic conservation.

Chapter 4 begins by considering gynogenesis and androgenesis, respectively, chromosome set manipulation-based methods for propagating individuals with all-maternal or all-paternal inheritance. Methods for induction of gynogens and androgens are explained, and their use for

producing monosex stocks and clonal lines is considered from the viewpoints of phenotypic variability, regeneration of genetic variation, growth, disease resistance, and production of clonal hybrids. I would have valued a presentation of the author's evaluation of the utility or limited utility of gynogens and androgens in aquaculture and fisheries. Material on chromosome set manipulations is followed by material on nuclear transplantation, because both sets of methods relate to cloning. Dunham begins by explaining the cloning of individuals by transplantation of nuclei from donor cells to enucleated eggs, citing key studies outside of aquatics and then focusing on fishes. While the piscine literature is covered rather fully, some key issues are overlooked. The different implications of transplanting nuclei from embryonic cells or from fully differentiated cells on subsequent gene expression and performance of the cloned individual (see, for example, NRC 2002) are not discussed. A section on possible applications of nuclear transplantation, e.g., for rapid generation of true-breeding transgenic lines would have added to the practical utility of the chapter.

Utilization of monosex or sterile populations of fish is a solution or partial solution to problems associated with sex-related differences in performance, early sexual maturation, and unwanted reproduction. Techniques available for producing monosex or sterile populations – including chemical and mechanical sterilization and hormonal sex reversal – are discussed in Chapter 5. There is a strong section on genetics of sex determination in Nile tilapia, and a strong concluding section shedding light on practical issues constraining commercialization of monosex aquaculture stocks.

Development and screening of genetic markers has had a huge impact on aquaculture and fisheries management, an impact that will grow in the future. Chapter 6 discusses the development of biochemical and genetic markers and selected applications, mostly on management of aquaculture species. Methods are discussed in their historical order of development, and include isozymes, DNA restriction fragment length polymorphisms, mitochondrial DNA, randomly amplified polymorphic DNA, amplified fragment length polymorphism, microsatellite DNA, expressed sequence tags, and single nucleotide polymorhisms. The relative costs and effectiveness of the respective marker methods are compared. While the chapter covers methodological and aquaculture-related topics well, the huge impact of genetic marker methods on fishery management

is not well addressed. Landmark publications are not mentioned in the context of discussions of the respective marker methods, e.g., Ryman and Utter (1987) in the context of isozymes, and the work of John Avise and many other researchers regarding mitochondrial DNA. Microsatellite DNA markers are now the most widely used markers for population genetics and have had a huge impact on fisheries management, which is not discussed or referenced. The last section of the chapter begins to address issues posed by management of natural populations, but does not draw on numerous case studies that could illustrate applications of molecular markers in fisheries management, for example, in management of anadromous salmonids.

Interactions of hatchery and wild fish – a focus of continuing controversy among aquaculturists, fishery managers, ecologists, and population geneticists – are discussed in Chapter 7. The first part of the chapter is intended to provide a foundation for understanding population genetics. The reader would be served by mention and definition of all population genetic processes, but the section is very uneven, covering some processes in detail, while covering others only in cursory fashion. For example, discussion of coadaptation and outbreeding depression does not address the multilocus nature of coadaptation, as modeled in Sewall Wright's (1932) adaptive landscape. There is a growing literature on coadaptation in fishes (Hallerman 2003) to which interested readers should refer. Mention of many landmark studies or reviews of population genetics of fish is omitted, e.g., regarding inbreeding, Allendorf and Leary (1986) on fluctuating asymmetry, or Kincaid (1976a, 1976b, 1983) on inbreeding depression. Reviews of the huge literature on how population genetics affects fisheries management (e.g., Ryman and Utter 1987, Utter and Ryman, 1993) might have been cited to give depth to what is necessarily limited coverage in a single chapter. The issue of how population genetics affects conservation was not given sufficient coverage; critical concepts such as evolutionarily significant units (ESUs - Waples 1991, Moritz 1994, Nielsen 1995) should have been defined in depth, if only to refer the reader to the technical literature. Instead, the issue of what unit to conserve (page 109) was approached from the viewpoint of "performance" as opposed to adaptive characters. Mention that negative impacts of cultured fish upon wild gene pools might be ameliorated by natural selection was not appropriately qualified by discussion of the long time required, of the impact of intermittent introductions, and of loss of

between-population genetic diversity. These are perhaps the best examples of how, while thought-provoking and citing many interesting studies, the chapter is uneven and written from an aquaculture geneticist's view. Discussion of geographic distribution of genetic variation did not mention such key driving factors as the recolonization of North America by fishes after deglaciation. Discussion of the effects of stocking hatchery brook trout did not mention the landmark work of McCracken's group (e.g., McCracken *et al.* 1993), and of interspecific hybridization did not mention introgression of rainbow trout into cutthroat trout gene pools (Leary *et al.* 1995). The concluding discussion of an integrated management strategy, describing designation of areas where different degrees of preservation or manipulation of aquatic gene pools would be practiced, omitted discussion of such key operational issues as how areas would be designated and subtleties of management.

Development of large numbers of genetic markers gave rise to genetic mapping of entire genomes, detection of loci affecting expression of quantitative traits (QTLs), and application of such knowledge for genetic marker-assisted selection (MAS) (Chapter 8). This is a very thorough chapter in terms of reviewing research to date with aquatic organisms. However, the chapter has its quirks and minimizes treatment of certain key issues. For example, in a very thorough section on isozyme-based genetic maps is a long passage on DNA marker-based mapping of puffer fish. The section on QTL mapping does not mention that segregation is followed *within families*. The section on MAS might have discussed how marker and phenotypic performance information are combined in a selection index. There is a huge literature on these topics, and these should have been major sections with appropriate citations to the technical literature. Certain omissions will make the chapter more difficult for most readers to understand. For example, there is no figure showing a genetic map, nor a figure comparing genetic maps to show conservation of linkage groups.

Elucidation of gene function is a rapidly-developing area of genomics, and Chapter 9 discusses gene expression, isolation, and cloning. These procedures are discussed in the context of what we have learned of such key processes as development and growth, reproduction, disease resistance, brain function, cold tolerance, and osmoregulation. Genome-level issues such as genetic imprinting, transposable elements, and ribosome function are described, and the chapter ends with a very brief presentation on

proteomics. This is generally a well-done chapter, but has its quirks and omissions. Microarrays are not described or shown, leaving the reader with only a description of their applications. A DNA dot-blot is shown, but neither the genes spotted on it, nor the probe used to screen it are specified. Much more progress has been achieved on understanding of MHC (major histocompatibility complex) function and on proteomics than is alluded to. The notion that differential performance of reciprocal crosses of many species may be attributable to genetic imprinting was not considered.

Applied with highly visible success in fishes, gene transfer technology is discussed in Chapter 10. Given the author's notable involvement in gene transfer in common carp and channel catfish, it is not surprising that this is one of the stronger, more thorough chapters in the book. Dunham covers gene transfer techniques, expression vectors, integration and transmission of gene constructs, and pleiotropic effects of transgenes. He considers the performance of transgenic fishes for traits relating to growth, cold tolerance, and disease resistance. In a more prospective analysis, he discusses transgenic production of pharmaceuticals, gene knock-out technology, and potential role of mitochondrial DNA in gene transfer. The chapter is well illustrated. However, there are a few questionable assertions and glitches. The suggestion that use of active transposons for gene transfer is a good idea should be questioned (NRC 2002). Neither the Anderson *et al.* nor Liang *et al.* citations seem related to cold tolerance. Hybrid breakdown in F_2 and F_x generations is widely regarded as caused by disruption of coadapted gene complexes (Hallerman 2003), not by nuclear-mitochondrial DNA interactions.

Maximum genetic progress in development of genetic lines of aquatic organisms will come from combining different sorts of genetic enhancement programs. A brief Chapter 11 considers combinations of, for example, sex reversal and triploidy and gene transfer and crossbreeding. The author is correct in his assertion that combinations of approaches may be especially powerful when combined, and cites many interesting examples. However, some biotechnological approaches were not combined for the purposes suggested by their inclusion in this chapter. Notably, Devlin et al. (2001) did not crossbreed a transgenic line with a wild population in order to increase the effect of transgene expression, but rather to assess the risks posed by introgression of a transgene into a wild stock. In the context of a passage on gynogenesis and selection to improve immune response, it is never asked whether a high antibody response

is necessarily a desirable trait. These aspects of the chapter can leave readers unfamiliar with the material without a critical perspective.

Aquaculture stocks genetically improved in a research environment are not necessarily high-performance stocks in a commercial production environment, and such genotype-environment (GE) interactions are considered in a brief Chapter 12. The material is straightforward, leading to the appropriate conclusion that GE interactions on performance should be evaluated before manipulated stocks are commercialized. More detail on how to set up, analyze, or evaluate studies of GE interaction would have been helpful to many readers

Commercialization of transgenic aquatic organisms on a large scale poses environmental hazards (Chapter 13). Consideration of these hazards from the theoretical and empirical viewpoints comprises a large literature. Dunham draws from the literature but sometimes adds inferences that ought to be questioned, resulting in a mix of well-reasoned and less well-reasoned arguments. The reader must consider these critically for him/herself. For example, empirical estimations of fitness components generally show that fitness-related characters are decreased in transgenics. Recent work on the net fitness of transgenics indicates, however, that it is not enough to identify *a priori* a key fitness-determining factor (e.g., vulnerability to predators), show lessened fitness for this trait, and then conclude that selection will remove the transgene from a receiving population. The *net* effect of the transgene on *overall* fitness will determine the genetic impacts of transgenics on a receiving population, impacts that may take many generations to reach equilibrium. To argue that selection would remove maladaptive genotypes from a population, posing only temporary harm (pp. 203, 205) does not consider either the (generally slow) rate at which selection operates in natural populations or the effect of recurring introductions of such genotypes into a receiving population. The assertions that transgenic fish may not have greater genetic impact on natural populations than domestic conspecifics (p. 204) or that "all available data indicate that transgenic fish are less fit than non-transgenics fish and would probably have little, if any, environmental impact" (p. 206) are bold speculations. Dunham goes on to suggest that escaped transgenic fish, by adding genetic diversity to populations, could increase fitness and render such populations more viable, an assertion made by no one else in the sector. This part of the chapter does not come to the key bottom-line conclusion, that risk assessment

and risk management must be done on a case-by-case basis, considering species, transgene construct, integration event, and receiving ecosystem. Conspicuously missing from the chapter was discussion of studies of non-transgenic fish injected with the growth hormone (GH) protein, a ready model for GH transgenic fish which can be stocked in the wild for realistic ecological risk assessment. For example, brown trout injected with GH were more willing to risk exposure to a predator than non-injected fish (Johnsson *et al.* 1996). Similarly, among non-transgenic rainbow trout, aggression was lowest in the control pairs, intermediate in the control/GH-injected pairs, and highest in the GH-injected pairs (Jonsson *et al.* (1998a), supporting the hypothesis that GH increases aggression levels. Reproductive confinement would go a long way toward addressing genetic and ecological hazard posed by transgenic fish and Dunham presents a detailed consideration of progress towards achieving transgenesis-mediated sterility. This is generally a strong passage, although hazard posed by progressive reversion of triploid oysters towards diploidy is not mentioned.

Foremost in the minds of many consumers is the issue of safety of food products from transgenic organisms. The safety of GM foods has been examined thoroughly and is reviewed in Chapters 14 and 15. Chapter 14 provides a brief general review and correctly identifies allergenicity as posing the greatest potential for harm to consumers. U.S. oversight by the Food and Drug Administration and international guidelines promulgated by the Codex Alimentarius Commission are briefly described. Interested readers might note that subsequent to the book going to press, FAO and WHO (2004) published the recommendations to Codex Alimentarius of a workshop on food safety of products of animal biotechnology including fish. Issues posed by labeling of GM foods are briefly discussed. Against this background, Chapter 15 presents a very detailed case study on safety of consumption of transgenic salmon expressing elevated levels of growth hormone and insulin-like growth factor. Dunham leads the reader through the technical literature to show lack of bioavailability to the human consumer and lack of bioactivity of these piscine hormones in humans. Appropriately, he briefly reviews results of Cuban studies of food safety of growth hormone transgenic tilapia. In the best review of the subject that I have read, he concludes that GH and IGF levels in transgenic fish products pose no hazards to human health.

Policies adopted by nations and international bodies will have major bearing on the pace and extent of adoption of aquatic biotechnology. Chapter 16 considers regulation of transgenic fish, reviewing development of biotechnology policy in the United States, the European Union, Canada, and the United Kingdom. Developments in Cuba and China, countries with transgenic lines in advanced stages of development, are not discussed. Development of biotechnology policy under the U.N. Convention on Environment and Development is discussed in terms of its impact on adoption of GM organisms in developing countries. The effects of World Trade Organization decisions and intellectual property rights (IPR) will have a large impact on international trade in GM products. While generally a sound treatment, there is no consideration of how IPR came to be applied to living materials and to animals in particular, a surprising omission given that the landmark case, *Ex parte Allen*, involved triploid oysters and that a key review (Hallerman and Kapuscinski 1990) appears in the list of references. For interested readers, a detailed review of 10 states' regulatory policies on GM aquatic organisms is presented by Stenquist (1998).

Certain products of biotechnology are reaching commercial application (Chapter 17). Triploid salmon, rainbow trout, grass carp, and Pacific oysters are now produced, variously for reasons of reproductive confinement and maintenance of product quality after age of maturation. Sex reversal is used to produce all-female salmonid and silver barb and all-male Nile tilapia stocks. Dunham also mentions progress towards possible commercialization of transgenic fishes, notably the AquaBounty Atlantic salmon (which was not approved by FDA in 2004 as the author had expected). Subsequent to the book going to press, GloFish fluorescent zebrafish were commercialized as ornamentals (Hallerman 2004).

Chapter 18 – discussing strategies for genetic conservation, gene banking, and maintaining genetic quality – in many respects picks up where Chapter 7 leaves off. The first section, discussing population size, inbreeding, and maintenance of genetic quality – while a review of interesting studies – does not convey a straightforward picture of principles and procedures that a breeder should apply when establishing and maintaining a captive population. For straightforward guidelines, interested readers might consult Tave (1993) for aquaculture broodstocks or Frankham *et al.* (2002) for stocks of imperiled species. Discussion of how to avoid inbreeding notably lacked mention of rotational line crossing

(Kincaid 1977). A particular level of inbreeding where inbreeding depression becomes likely was put forward without qualification; the reader would have been better served by mentioning that the critical level varies among species, stocks, and environments and supporting the statement with reference to case studies. The possibility of purging deleterious alleles from small captive populations omits mention of the classical work with Speke's gazelle (Templeton and Read 1984), and of studies questioning the approach (e.g., Hedrick 1994). Discussion of genetic drift does not acknowledge the relevant case where a transgenic line is established by one founder with one integration event. Pathbreaking isozyme-based studies of sperm competition in mixed milt of salmonids by Gharrett and Shirley (1985) and Withler (1988) are not cited. The chapter ends with a section on genetic conservation emphasizing biotechnological approaches such as cryopreservation and cloning. Considerations regarding living gene banks are not discussed. The flagship genetic conservation program – the Norwegian living and cryopreserved gene banks for Atlantic salmon (Gausen 1993) – is not mentioned. For a complete assessment of the range of issues, interested readers might consult Cloud and Thorgaard (1993).

Various environmental, research infrastructure, economic, and political issues will have to be resolved for aquaculture genetics to make its maximum contribution. Chapter 19 presents recommendations for resolution of these issues made by a working group chaired by Dunham in anticipation of the 2001 International Conference on Aquaculture in the Third Millennium. These recommendations provide a succinct and appropriate way to put the development of aquatic biotechnology into perspective at the end of the book.

While providing a useful resource, the glossary and references sections have some omissions. Key undefined terms pertaining, for example, to genome mapping, include ortholog, paralog, Kosambi cM, paint probe, and centirays. The references section lacks the supporting citation for certain key studies cited.

While I have my criticisms, *Aquaculture and Fisheries Biotechnology: Genetic Approaches* is the best review to date and will prove useful to a variety of readers. The general strength of this book is the thorough review of applications of aquatic biotechnology to aquaculture, and it will serve as a useful reference to a range of professionals. Its general weakness regards applications to population genetics and fishery

management. For teachers, combining a book focusing on selective breeding with this volume focusing on biotechnology would support a state-of-knowledge course on genetics in aquaculture.

REFERENCES

Allendorf, F.W., and Leary, R.F. 1986. Heterozygosity and Fitness in Natural Populations of Animals. Pages 57-76 in M.E. Soule, ed. *Conservation Biology: The Science of Scarcity and Diversity.* Sinauer Associates, Sunderland, MA, USA.

Cloud, J.G., and Thorgaard, G.H. (Eds.). 1993. *Genetic Conservation of Salmonid Fishes.* Plenum Press, New York, NY, USA.

Devlin, R.H., Biagi, C.A., Yesaki, T.Y., Smailus, D.E., and Byatt, J.C. A Growth-hormone Transgene Boosts the Size of Wild but not Domesticated Trout. *Nature*, 2001, 409:781-782.

FAO-WHO (Food and Agriculture Organization of the United Nations and World Health Organization). 2004. Safety Assessment of Foods Derived from Genetically Modified Animals, Including Fish. FAO Food and Nutrition Paper 79. FAO, Rome, Italy.

Frankham, R., Ballou, J.D., and Briscoe, D.A. 2002. *Introduction to Conservation Genetics.* Cambridge University Press, Cambridge, UK.

Gausen, D. 1993. The Norwegian Gene Bank Programme for Atlantic Salmon (*Salmo salar*). Pages 181-188 in J.G. Cloud and G.H. Thorgaard (Eds.) *Genetic Conservation of Salmonid Fishes.* Plenum Press, New York, NY, USA.

Gharrett, A.J., and S.M. Shirley. A Genetic Examination of Spawning Methodology in a Salmon Hatchery. *Aquaculture* 47:245-256.

Gordon, G.W., G.A. Scangos, D.J. Plotkin, J.A. Barbosa, and F.H. Ruddle. 1980. Genetic Transformation of Mouse Embryos by Injection of Purified DNA. *Proceedings of the National Academy of Sciences USA*, 1985, 77:7380-7384.

Hallerman, E.M. 2003. Coadaptation and Outbreeding Depression. Pages 238-259 in E.M. Hallerman (Ed.). *Population Genetics: Principles and Applications for Fisheries Scientists*. American Fisheries Society, Bethesda, MD, USA.

Hallerman, E.M. 2004. GloFish, the First GM Animal Commercialized, Profits Amid Controversy. ISB (Information Systems for Biotechnology) News Report, June 2004. http://www.isb.vt.edu.

Hallerman, E.M., and Kapuscinski, A.R. Transgenic Fish and Public Policy: Patenting of Transgenic Fish. *Fisheries*, 1990, 15(1):21-24.

Hedrick, P.W. 1994. Purging Inbreeding Depression and the Probability of Extinction: Full-sib Mating. *Heredity* 73:363-372.

Johnsson, J.I., E. Petersson, E. Jonsson, B.T. Bjornsson, and T. Jarvi. 1996. Domestication and Growth Hormone Alter Antipredator Behavior and Growth Patterns in Juvenile Brown Trout, *Salmo trutta*. *Canadian Journal of Fisheries and Aquatic Sciences*, 53:1546-1554.

Jonsson, E. J.I. Johnsson, and B.T. Bjornsson. 1998a. Growth Hormone Increases Aggressive Behavior in Juvenile Rainbow Trout. *Hormones and Behavior*, 33:9-15.

Kincaid, H.L. 1976a. Effects of Inbreeding on Rainbow Trout Populations. *Transactions of the American Fisheries Society*, 105:273-280.

Kincaid, H.L. 1976b. Inbreeding in Rainbow Trout (*Salmo giardneri*). *Journal of the Fisheries Research Board of Canada*, 33:2420-2426.

Kincaid, H.L. 1977. Rotational Line Crossing: An Approach to the Reduction of Inbreeding Accumulation in Rainbow Trout Stocks. *Progressive Fish-Culturist*, 39:179-181.

Kincaid, H.L 1983. Inbreeding in Fish Populations Used in Aquaculture. *Aquaculture*, 33:215-227.

Leary, R.F., F.W. Allendorf, and G.K. Sage. 1995. Hybridization and Introgression between Introduced and Native Fish. *American Fisheries Society Symposium*, 15:91-101.

Lynch, M., and B. Walsh. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Sunderland, MA, USA.

McCracken, G.F., C.R. Parker, and S.Z. Guffey. 1993. Genetic Differentiation and Hybridization between Stocked Hatchery and Native Brook Trout in Great Smoky Mountains National Park. *Transactions of the American Fisheries Society*, 122:533-542.

Moritz, C. 1994. Defining 'Evolutionary Significant Units' for Conservation. *Trends in Ecology and Evolution*, 9:373-375.

Nielsen, J.L. 1995. Evolution and the Aquatic Ecosystem: Defining Unique Units in Population Conservation. American Fisheries Society Symposium 17. American Fisheries Society, Bethesda, MD, USA.

NRC (National Research Council). 2002. *Animal Biotechnology: Scientific Concerns*. National Academy Press, Washington, DC, USA.

Ryman, N. and F.M. Utter, eds. 1987. *Population Genetics and Fishery Management*. University of Washington Press, Seattle, WA., USA.

Stenquist, S. 1998. Federal and State Regulations Relevant to Uncontained Applications of Genetically Engineered Marine Organisms. Pages 139-180 in R.A. Zilinskas and P.J. Balint, eds. *Genetically Engineered Marine Organisms: Environmental and Economic Risks and Benefits*. Kluwer Academic Publishers, Boston, MA, USA.

Tave, D. 1993. *Genetics for Fish Hatchery Managers, 2nd ed.* Van Nostrand Reinhold, New York, NY, USA.

Templeton, A. R., and B. Read. 1984. Factors Eliminating Inbreeding Depression in a Captive Herd of Speke's Gazelle (*Gazella spekei*). *Zoo Biology*, 3:177199.

Utter, F., and N. Ryman. 1993. Genetic Markers and Mixed Stock Fisheries. *Fisheries*, 18(8):11-21.

Waples, R.S. Pacific Salmon, *Oncorhynchus* spp., and the Definition of "Species" under the Endangered Species Act. *U.S. National Marine Fisheries Service Marine Fisheries Review*, 1991, 53:11-22.

Withler, R.E. Genetic Consequences of Fertilizing Chinook Salmon (*Oncorhynchus tshawhytscha*) Eggs with Pooled Milt. *Aquaculture*, 1988, 68:15-25.

Wright, S. The Roles of Mutation, Inbreeding, Crossbreeding, and Selection in Evolution. *Proceedings of the Sixth International Congress on Genetics*, 1932, 1:356-366.

McCarty, P. Thermodynamics of Biological Synthesis and Growth. *Journal of Air and Water Pollution*, 1965, 9:621-639.

Metcalf and Eddy, Inc. 1991. *Wastewater Engineering: Treatment, Disposal, Reuse*. McGraw-Hill, New York, NY, USA.

Moore, R., Quarmby, J., and Stephenson, T. The Effects of Media Size on the Performance of Biological Aerated Filters. *Water Research*, 2001, 10:2514- 2522.

Moss, S. Production of Growth-enhancing Particles in a Plastic Lined Shrimp Pond. *Aquaculture*, 1995, 132:253-260.

Moss, S., Pruder, G., Leber, K., and Wyban, J. The Relative Enhancement of *Penaeus vannamei* Growth by Selected Fractions of Shrimp Pond Water. *Aquaculture*, 1992, 101:229-239.

Ning, Z. 1996. Characteristics and Digestibility of Aquacultural Sludge. Masters Thesis, Louisiana State University, Baton Rouge, LA, USA.

Sastry, B., DeLosReyes, A., Rusch, K., and Malone, R. Nitrification Performance of a Bubble-washed Bead Filter for Combined Solids Removal and Biological Function in a Recirculating Aquaculture System. *Aquacultural Engineering*, 1999, 19:105-117.

Weirich, C., Browdy, C., Bratvold, D., McAbee, B., and Stokes, A. Preliminary Characterization of a Prototype Minimal Exchange Super-Intensive Shrimp Production System. In *Proceedings of the 4th International Conference on Recirculating Aquaculture*, Roanoke, VA, 2002. Virginia Tech, Blacksburg, VA, USA.

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