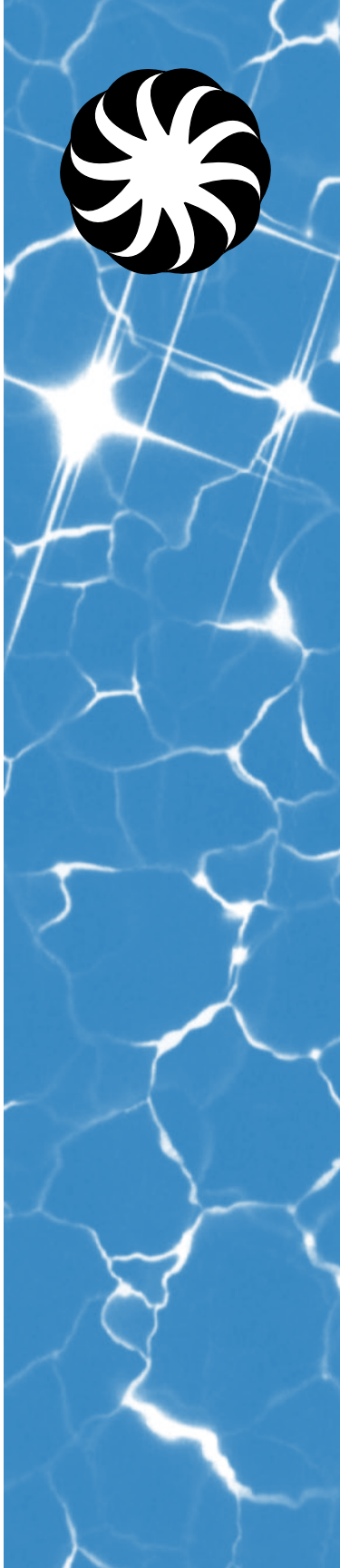


International Journal of Recirculating Aquaculture

June 2010
Volume 11

Published by

Virginia Cooperative Extension

VirginiaTech
Invent the Future

VIRGINIA STATE

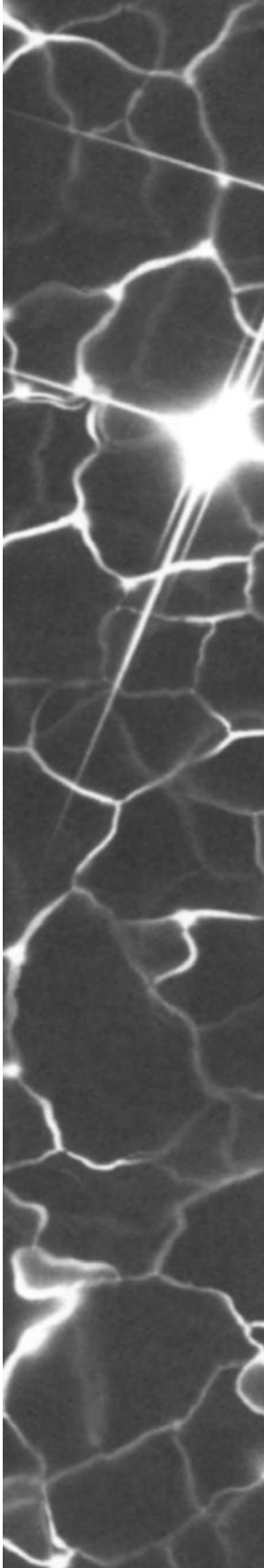
International Journal of Recirculating Aquaculture

International Journal of Recirculating Aquaculture

June 2010
Volume 11

Printed in the United States of America

ISSN 1547-917X



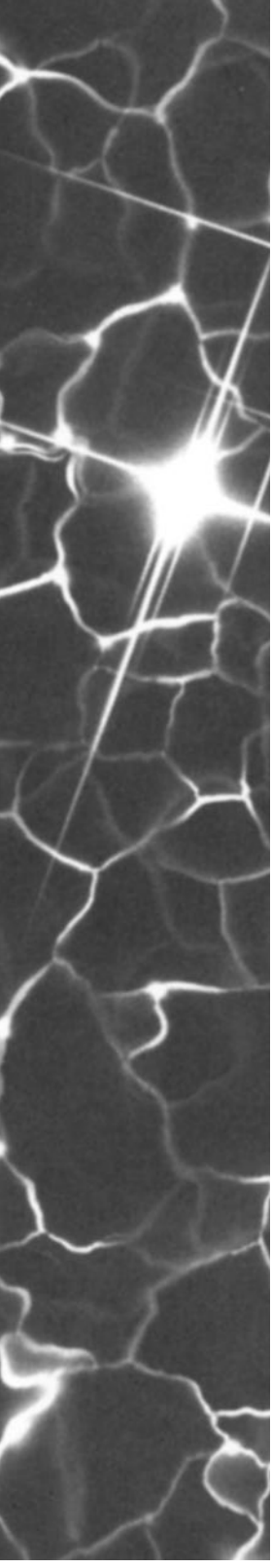
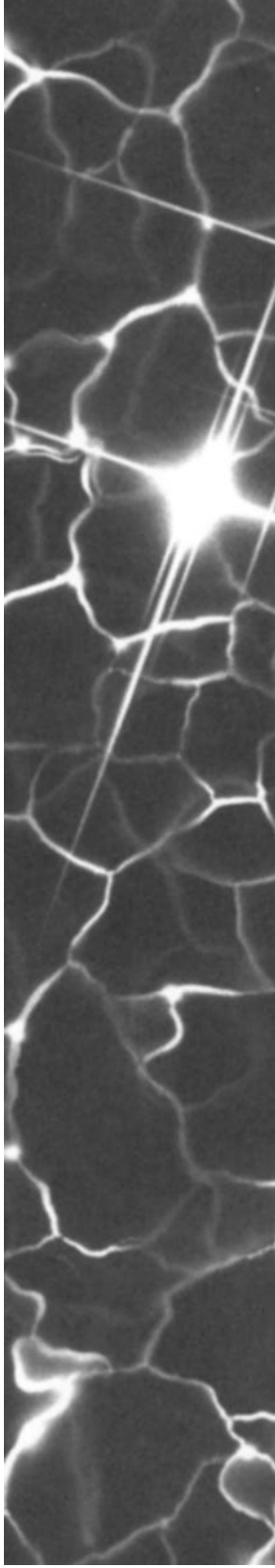


Table of Contents (Volume 11)



Dear Reader:

We welcome the submission of high quality manuscripts and reports on all topics relating to recirculating aquaculture. We encourage our readers to contact our production office with any comments, suggestions or ideas they may have concerning the journal. Articles published in IJRA are listed by several citation and abstracting services, which means that the journal is fully searchable by author, title and/or subject. Numerous university and aquaculture/agriculture libraries worldwide continue to receive issues of IJRA for inclusion in their collections. Visit the journal's website (www.ijra.com) for archives of past and current abstracts, book reviews, and author contact information, as well as a downloadable file providing detailed instructions for prospective authors. Our goal is to make IJRA widely available for use by aquaculturists, academicians and researchers around the world.

As always, we would like to thank our readers, authors, and reviewers for their continued efforts in support of IJRA.

The Passive Vacuum Degasser; Research Test Setup and Preliminary Observations

R.N. Patterson*[1] and K.C. Watts[1]

[1]Department of Process Engineering and Applied Science
Dalhousie University
Halifax, Nova Scotia, Canada

*Corresponding author: Dick.Patterson@dal.ca

Keywords: Passive vacuum degasser, degassing, vacuum-assisted, recirculating aquaculture systems

ABSTRACT

Some form of vacuum-assisted degassing is often required in both production and research facilities to bring the total pressure of dissolved gasses in the culture water below the saturation value. One form of vacuum degasser is the passive vacuum degasser, a device that consists of a column, packed or unpacked, that has its tailpipe exiting below the surface of the water in the receiving vessel. Such an arrangement causes a vacuum to self-form in the column. The strength of this vacuum appears to correlate to both geometric and operational parameters in relationships that have not yet been clearly defined.

An elaborate recirculating apparatus, with degassing columns equivalent in size to a commercial system, has been set up to explore the various physical parameters of passive degassers. Initially, to observe the degassing process, the column being used is a 10 ft (3 m) long, 1 ft (0.3 m) diameter clear plastic pipe into which water, supersaturated with air, is introduced at its upper end. The pump has been selected to operate at rates adjustable up to 210 USGPM (800 Lpm). A chiller is used to maintain a constant temperature. The re-saturation of the water is accomplished by means of a separate pressurized packed column. The geometric parameters that will be investigated are: column diameter to length ratio, distribution plate design, tailpiece diameter and length,

International Journal of Recirculating Aquaculture 11 (2010) 1-18. All Rights Reserved

and packing/no packing. The operational parameters include water flow rate, air saturation rate, water temperature, and column water height. Instrumentation includes a paddle wheel flowmeter, ultrasonic flowmeter, total gas pressure, oxygen level, temperature sensors before and after the column, column vacuum probe, column height differential pressure transducer, cross-over pipe pressure and pump pressure.

The entire setup is linked to a computer for data logging. The aim of this paper is to describe the apparatus and its instrumentation, and report some preliminary findings.

INTRODUCTION

It has been well established that gas-supersaturated supply water causes gas bubble disease in aquatic animals, as the gas comes out of solution in conditions of reduced pressure or solubility. (e.g. Colt and Bouck 1984, Bouck et al. 1984, Westers et al. 1991) Ideally the unsaturated level should be -5 to -10% to ensure that further conditioning, such as warming, will not cause the water to again become saturated or even supersaturated.

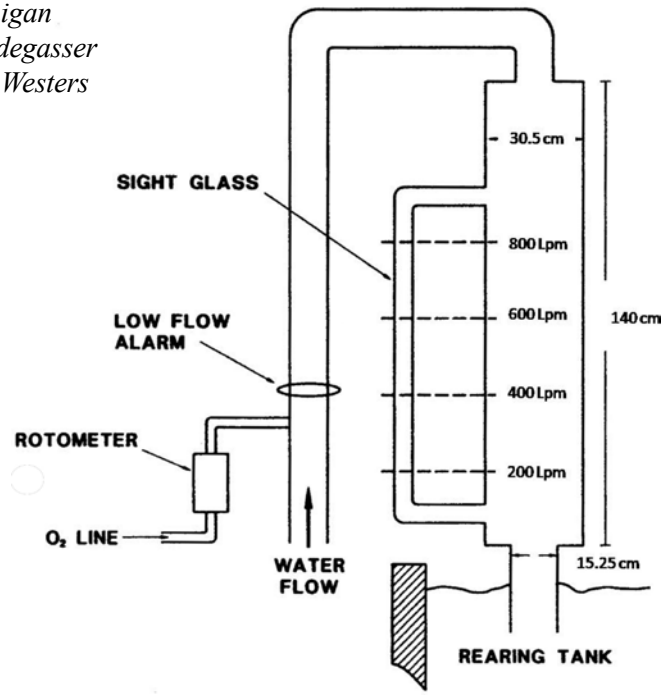
One device in common use to achieve desaturation of supply water is the passive vacuum degasser (PVD). This device, essentially a vertical column, packed or unpacked, with a restricting tailpipe exiting below the surface of the receiving tank, creates a vacuum in the column merely from the characteristics of the water flow through the column and tailpipe. Exactly how that vacuum is created and what range of system characteristics can optimize the transfer is largely unreported.

Background

A paper by Westers et al. (1991) based on the sealed columns at a Michigan state hatchery (Figure 1) initiated this investigation. Their primary focus was gas transfer, but flow rate, column height and vacuum data were also recorded. Regretfully, the length of the tailpipe to the receiving tank water surface was not noted.

When one plots Westers et al. (1991) column water level data against the vacuum created in the same terms, a straight line relationship emerges (Figure 2, solid line).

Figure 1: Michigan state hatchery degasser diagram (from Westers et al. (1991).



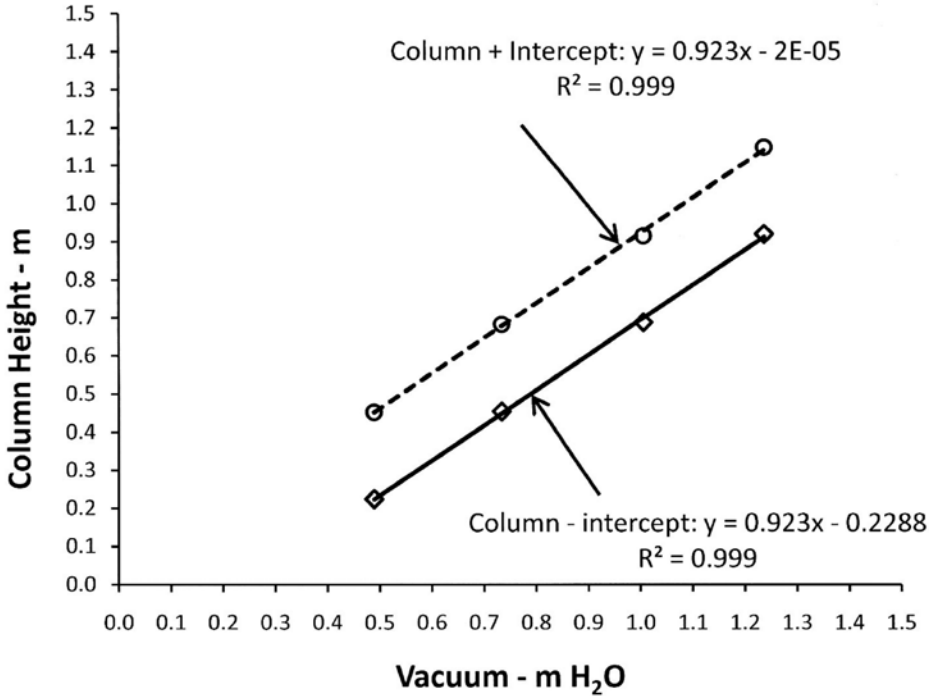


Figure 2: Water column height vs. vacuum measured, with column bottom as datum (solid line) and the data with the intercept removed (dotted line). From Westers et al. (1991)

As noted above, the datum for the water column height measurements in Figure 1 is the bottom of the column. If it is assumed that the intercept reflects the height of this datum above the surface of the receiving tank, and if that value is added to the column values to reflect the total height of the column above the tank water surface, Figure 2 (dashed line) emerges which suggests a very close relationship between the height of the water column (with the tank surface as datum) and the vacuum produced.

The data also show a relationship between the vacuum and column height and the flow rate (Figure 3), applying the tailpipe length/intercept assumption suggested above.

Purpose of the Study

This study was initiated to examine the physical parameters that cause the passive degasser to function. The aim is to develop guidelines and a model to assist the engineer in designing a device given the water parameters (temperature and salinity), flow rate, and degree of desaturation required. Thus a test bed was required to examine a whole range of factors that might influence gas transfer; e.g. degree of input

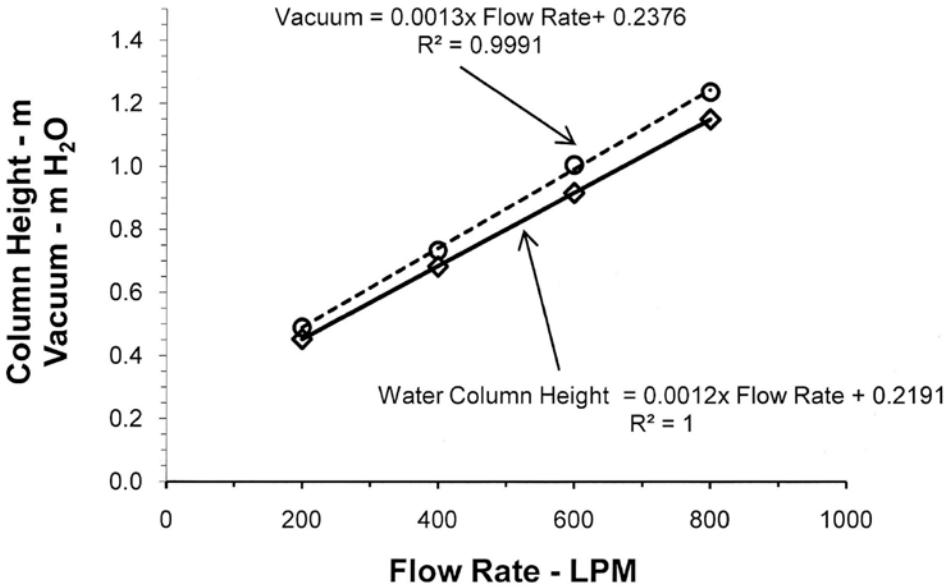


Figure 3: Water column height, assuming that the intercept in Figure 2 is the distance from the bottom of the column to the tank surface, and the vacuum recorded versus the flow rate. From Westers et al. (1991)

supersaturation, flow rate, residency time, the vacuum created, and dimensional effects of the column and tailpipe.

Initially, the study examines the questions: what causes the vacuum and can a design model based on flow rate and desired vacuum be developed? The experimental set-up at Dalhousie University is still being commissioned and so this is a report on the design and construction of the system with some preliminary results.

MATERIALS AND METHODS

The Dalhousie Engineering Test Setup *(Figure 4)*

The Main Flow Route

The water flow route from the holding tank, 1.9 m (6.23 ft) diameter by 1.4 m (4.5 ft) deep, begins with a 2 hp sump pump (Hydromatic SB3S, 5.69 inch impeller, Hydromatic, Kitchener, ON, Canada) pumping up to a tee fitting. Using baffles, the holding tank is divided into three parts: tailpipe section, underflow to a probe section and overflow to the pump section. This arrangement is to present the probes with the deepest (least amount of entrained air) water.

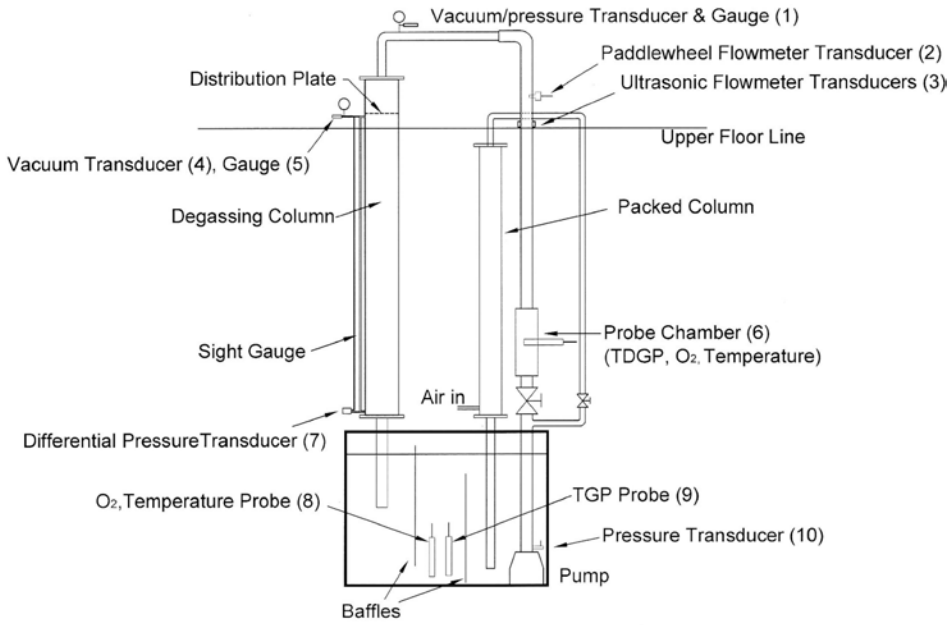


Figure 4: Dalhousie Engineering Research test setup for passive vacuum degasser studies. The numbered sensors are referred to in the text.

The main flow rises past a nominal 4 inch gate valve through a Probe Chamber (6) [basically a section of 0.2 m (nominal 8 in Schedule 40) PVC with ports], followed by a section of clear nominal 0.102 m (4 in) Schedule 40 PVC pipe. The crossover to the top of the degassing column is nominal 0.075 m (3 in) PVC pipe which also has a section of clear pipe.

The present column is a 3.05 m (10 ft) long, 0.305 m (nominal 12 inch Schedule 40) clear PVC tube. There is a distribution plate at the top and the flow exits through a 0.075 m (nominal 3 inch Schedule 40) clear PVC tailpipe with tattle tails (clear pipe was used in four locations in order to visually inspect the flow for any entrained air.) It is intended that the dimensions of these last two items will be changed to vary the test parameters as noted previously.

In order to re-saturate the water, a side stream is diverted from below the main gate valve to the top of a pressurized packed column of 0.203 m (nominal 8 inch) Schedule 40 PVC, 2.44 m (8 ft) tall. The packing is 1.27 cm diameter by 0.95 cm polypropylene wheels from Coffin World Water Systems (Irvine, CA, USA). The laboratory air supply is not detailed, but pressurized air is fed to the bottom of this column through a manifold.

Instrumentation

Flow rate

As this parameter is thought to be very important, flow is measured by three methods: an Omega PX482A-030 pressure transducer (Omega, Laval, QC, Canada) on the pump outlet (10) (to be compared with the pump operating curve), an FLS F3.3 paddlewheel flow sensor with K330 4-20 mA transmitter (2) (Northeast Equipment Co., Dartmouth, NS, Canada) and the transducers from the Omega F7000 ultrasonic flowmeter (Omega, Laval, QC, Canada) (3).

Vacuum/pressure

The main degasser vacuum is sensed by a vacuum transducer [Winters PT30HGV (4) CTH Instruments, Dartmouth, NS, Canada] and a vacuum gauge [Winters P304 V 100 inches of water (5) CTH Instruments, Dartmouth, NS, Canada] tapped in just below the distribution plate. There is a second Omega PX1 82B-01 5CI (-14.7 to +15 psi) (Omega, Laval, QC, Canada) transducer with a Winters P861 (30” Hg - 15 psi,

CTH Instruments, Dartmouth, NS, Canada) tapped into the end of the crossover nearest the degassing column.

Column height

Running up the degassing column is a clear plastic sight gauge (Figure 5). Tape measures run along the column by the sight gauge and down into the tank for measuring column height with respect to the water surface. The clear column showed that the degassing action in the column created considerable foaming. The sight gauge gives a clearer indication of the height of the water alone. However, there is an undulation in the rate of flow from the pump (detailed later). Thus while the flow rate was being averaged electronically, the column height reading was essentially a snapshot. Consequently, an Omega PX2300-10 DI (0-10 psid) differential pressure transducer (Omega, Laval, QC, Canada) was paralleled to the sight gauge.

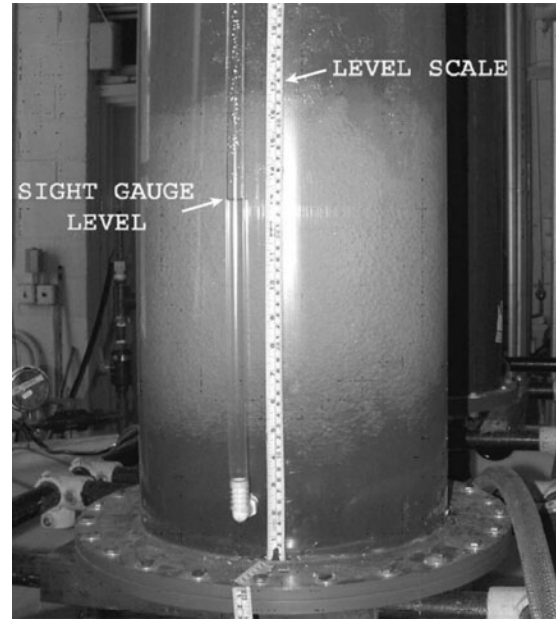


Figure 5: Lower end of the degassing column showing the sight gauge and height tape.

Total dissolved gas pressure (TDGP), dissolved oxygen (DO), and water temperature

For the in-flow to the degassing column, these parameters are sensed in the Probe Chamber (6) by the probe of a TBO-DL6F (6) (Common Sensing, Inc. Clark Fork, ID, USA). This probe senses total dissolved gas pressure, dissolved oxygen and temperature. The TBO box itself reads barometric pressure and water vapor pressure. After the column, these parameters are sensed in the mid division of the tank by a dissolved gas probe [Alpha 300c (8) Alpha Designs, Ltd., Victoria, BC, Canada] and a dissolved oxygen/temperature probe [Royce 900 (7) Royce Instrument Corp., New Orleans, LA, USA].

Re-saturation air

Laboratory air is supplied to the bottom of the packed column through a manifold with variable area flow meters. A pressure gauge and a humidity/temperature probe (Omegaette HH3 14, Omega, Laval, QC, Canada), are included in the air supply line.

Ancillaries

There is an external 1/2 hp recirculating chiller (Aquanetics Systems, San Diego, CA, USA) plumbed into the system should temperature become a factor. If testing uses other than freshwater, salinity will be measured with a Hach Sension5 conductivity meter (Northeast Equipment Co., Dartmouth, NS, Canada).

Data recording

While the TBO-6DLF and the Omegaette HH3 14 have on-board logging, the remainder of the electronic inputs are logged on two LabJack U12 data loggers in parallel (LabJack Corp., Lakewood, CO, USA) with the data being sent to a notebook computer using the program DAQFactory Express (Azeotech, Inc., Ashland, OR, USA).

Flowrate determination

As some of the expected parameters of the flow are directly affected by the velocity of flow, primarily to the second power, considerable effort has been expended in assuring that an accurate flow rate could be obtained. Three methods were examined and reported in Table 1: the paddle wheel flowmeter (PWFM), the pump head pressure transducer (PTD) against a digitized version of the published pump curve and the ultrasonic flowmeter (USFM).

Initially, while the paddlewheel flow meter (PWFM) and the ultrasonic flow meter (USFM) were in 0.5 to 7% accordance, the pump pressure transducer (PTD) differed considerably, up to 37%. The original pump order was for a 5.69 inch impeller. Back plotting the pressure head vs. PWFM flow rate data on the manufacturer's set of curves (Figure 6), the plot came out along the 5.88 inch impeller line. Either there is about a 1.5 m (5 ft) of water head difference error or the impeller is really 5.88 inch.

Manual Data - Ultrasonic				Labjack-DAQ Averaged VDC Data					Derived Data	
Run	Time	Avg GPM	LPM	Run	Time	PWFM	Ultrasonic	PTD	PWFM LPM	PTD LPM
1	9:23:15	31.83	120.48	1	9:22:16	2.449265	2.608109	6.442795	56.1	125.10
2	9:26:40	47.05	178.10	2	9:26:41	3.092936	2.912435	6.319824	139.7	165.86
3	9:30:10	77.22	292.32	3	9:30:12	3.954791	3.502844	5.887667	251.6	315.82
4	9:33:56	125.34	474.45	4	9:33:51	5.138672	4.44401	5.719076	405.2	377.17
5	9:36:30	134.80	510.29	5	9:36:32	5.503267	4.617289	5.740504	452.5	369.29
6	9:39:50	144.06	545.33	6	9:39:51	5.736202	4.818864	5.478673	482.8	467.42
7	9:42:20	156.95	594.13	7	9:42:31	6.162705	5.066573	5.435523	538.1	483.97
8	9:44:50	169.28	640.80	8	9:44:31	6.460612	5.303385	5.379557	576.8	505.58
9	0:46:50	181.72	687.87	9	9:46:51	6.786784	5.546712	5.261393	619.1	551.79
10	9:51:40	196.21	742.73	19	9:51:42	7.302857	5.832214	5.059509	686.1	632.56
11	9:54:00	208.96	790.99	11	9:54:01	7.516113	6.076823	4.977376	713.8	666.07
12	9:56:30	221.68	839.16	12	9:56:31	8.001302	6.32959	4.831543	776.8	726.51
13	9:58:50	237.37	898.55	13	9:58:51	8.385522	6.63968	4.626544	826.7	813.50
14	10:01:00	243.21	920.65	14	10:01:01	8.583287	6.740897	4.638672	852.3	808.29

Table 1: Consolidated Flow Rate Test Data

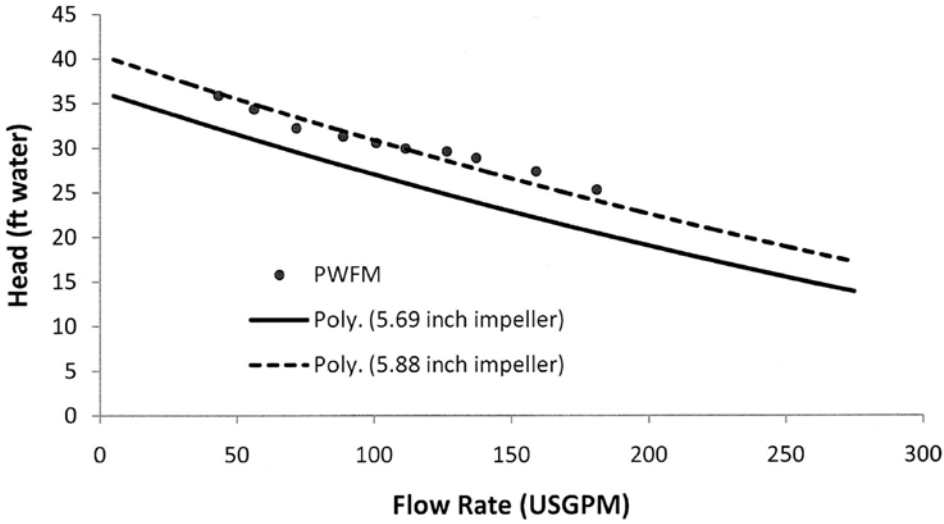


Figure 6: Reproduced Hydrostatic S3SD pump curves for 5.69 inch and 5.88 inch impellers with the paddlewheel flowmeter (PWFM) data vs. pump head pressure data (PTD)

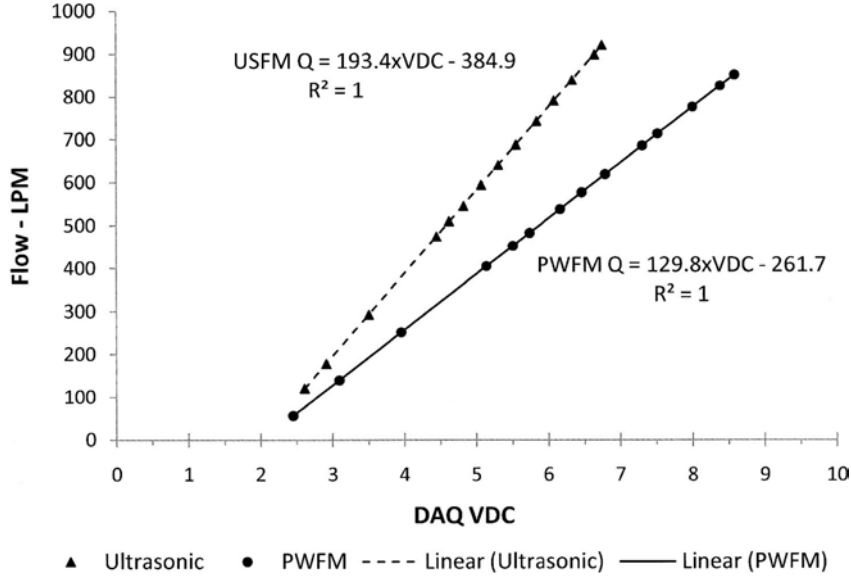


Figure 7: Plots from calibration data of the paddlewheel flowmeter (PWFM) and the ultrasonic flowmeter (USFM).

Note in Figure 7 that the ultrasonic flowmeter calibration (F7000 panel reading vs. voltage on the LabJack/DAQFactory Express) was linear but with a different slope and intercept from the paddlewheel flowmeter (paddlewheel flow values were derived from the LabJack recorded voltages according to the manufacturer's setup directions).

Figure 8 compares methods with each other. The pump head PTD supports neither the USFM nor the PWFM and its scatter is still evident despite the averaging. This is further discussed later in the paper.

The ultimate question is: which of the flowmeter outputs, the paddlewheel flowmeter or the ultrasonic flowmeter is most accurate? The probe chamber represents a reducer in the line and the recommended distance is 15 diameters, or about 1.5 m (5 ft). As the present distance is over 1.8 m (6 ft), this sensor is in a valid location.

For the ultrasonic flowmeter transducers, there is less certainty about the location and the fluid echo quality of the ultrasonic signal. There are three conditional cases:

1. Liquid with suspended solids or aeration bubbles 25 to 10,000 PPM of 30 µm in size, or larger.

2. Liquid with suspended solids or aeration bubbles greater than 10,000 PPM 30 µm size, or larger.

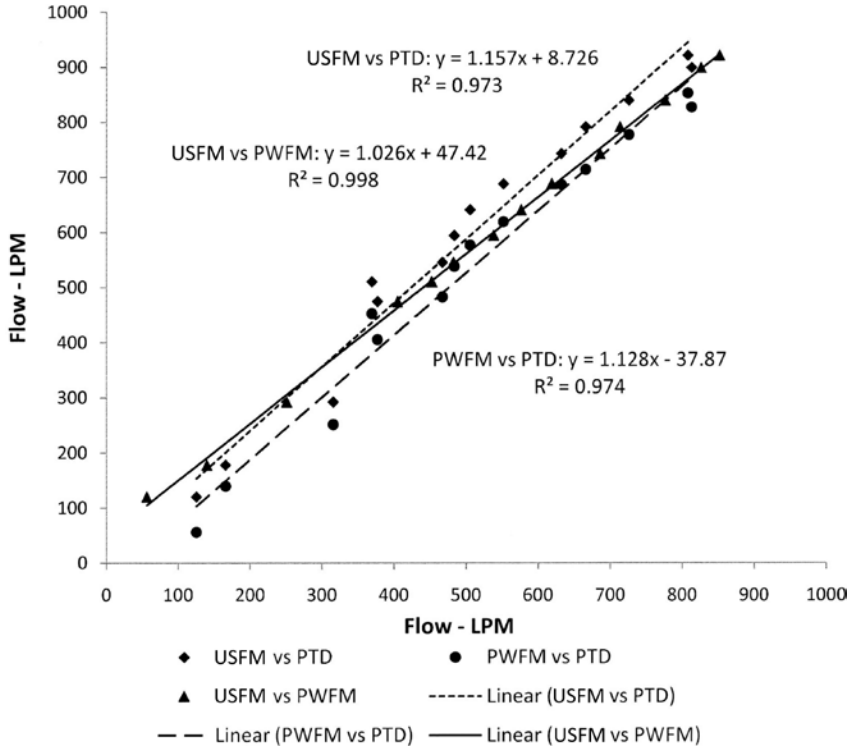


Figure 8: Cross-comparison of the three methods, paddlewheel flowmeter (PWFM), ultrasonic flow meter (USFM), and pump outlet pressure (PTD).

3. Liquid with less than 25 PPM suspended solids or aeration of 30 µm or larger and suspended solids or aeration content smaller than 30 µm (clean water).

All three cases require different mounting arrangements. Being unsure of the liquid condition and hence the mounting of the ultrasonic transducers in this case, it was decided to use the paddlewheel as the standard. However, the ultrasonic voltage output is a valid calibration line if compared to the paddlewheel output, and a good check on the paddlewheel.

The paddlewheel flowmeter indicated that there is a slight undulating character to the flow with a period of about 45 seconds as shown in an expanded form in Figure 9. This undulation can also be seen in Figures 6 and 8.

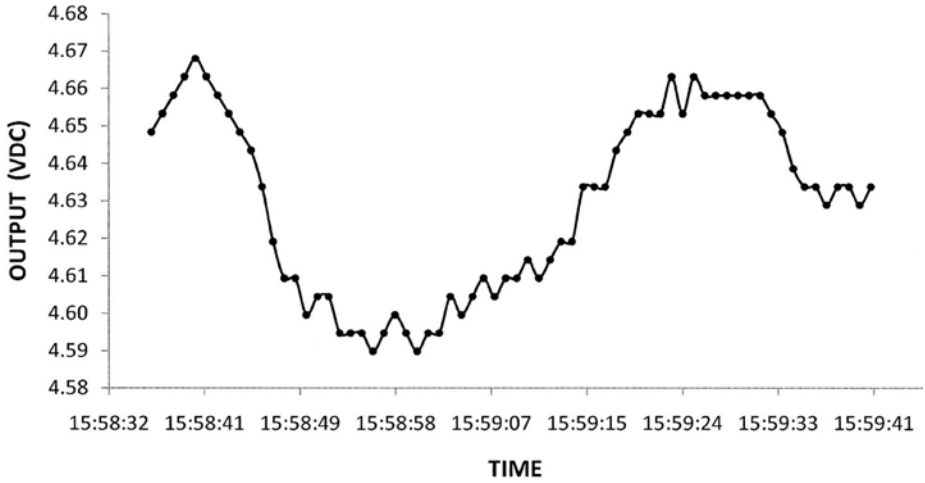


Figure 9: Paddlewheel flowmeter (PWFM) voltage readings over approximately one minute.

The pulse of the two-bladed impeller is clear in the upper plot of Figure 10. The averaging of the results tends to smooth out the data as shown in the derived flows for the paddlewheel and ultrasonic flowmeters (Figure 10, lower plots).

For the pressure transducer on the pump outlet, while the flow rate data is of the same order as that for the paddlewheel flowmeter, it does not line up with the paddlewheel data (Figure 10), despite the close adherence to the 5.88 inch impeller curve published by the manufacturer referred to

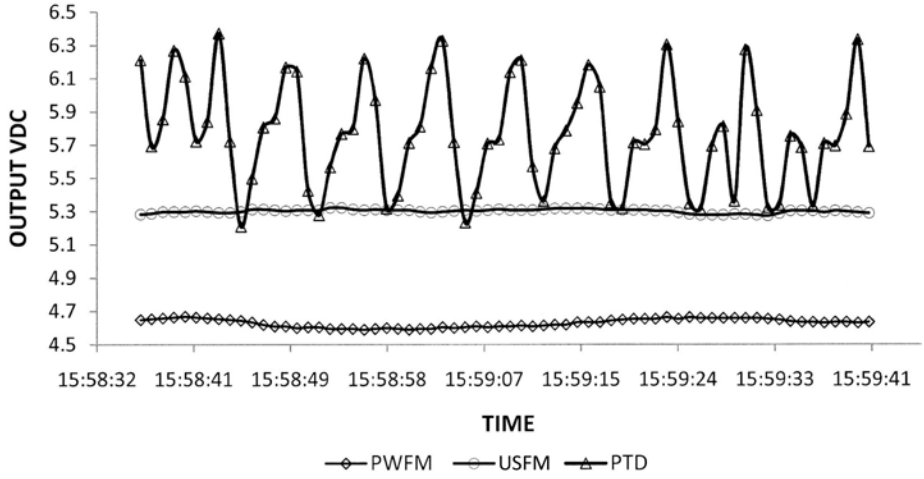


Figure 10: Comparison of recorded voltages of the three flow rate transducers with time.

earlier. The pressure transducer must be considered the third best method of determining flow rate in part because it depends so much on having an accurate characteristic curve for this particular pump.

Re-supersaturating the flow

These columns, normally used on flow-through water supplies, are very efficient at removing the dissolved gasses. The challenge in this recirculating system is to re-supersaturate the flow for the next cycle. Both a side-stream venturi and air stones were tried without sufficient success. The packed column is much more efficient and supersaturates the water, as would be expected. Trial and error is used to determine the correct mix of air/water flows. Even at maximum re-aeration flow rates, the degasser eventually removes more gasses than can be replaced in the cycle. It is expected that, instead of a continuous series of runs in a trial, the method will be to supersaturate the water, do a run, re-supersaturate the water, do a run, etc. This methodology is expected to be valid, as depending on conditions (degree of supersaturation, flow rate), the desaturation rate is about 0.1% per minute.

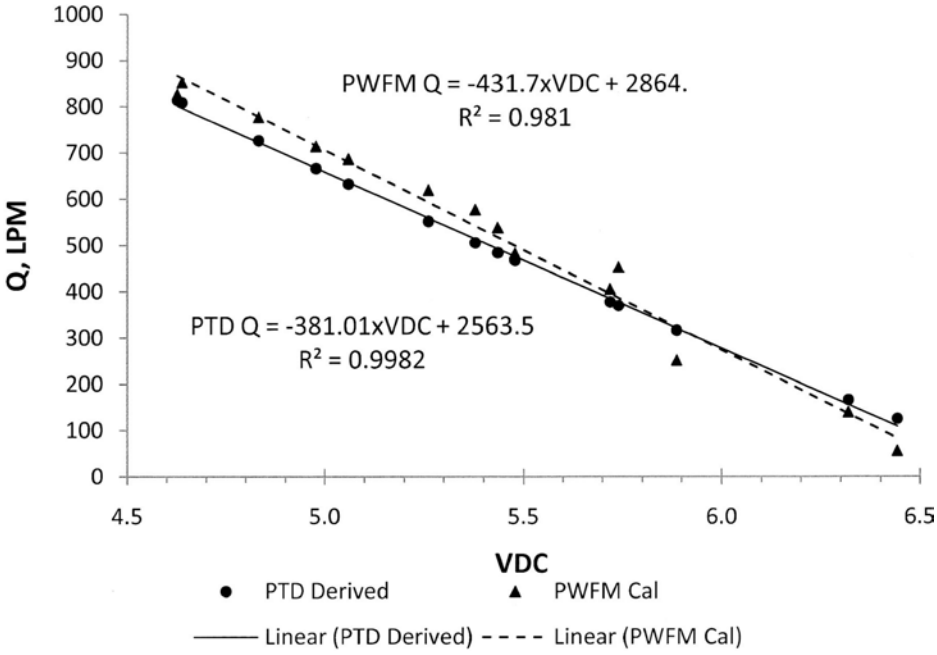


Figure 11: Derived flow rates (Q) for the pump pressure transducer (PTD) vs. voltage compared to the paddlewheel data (PWFM).

RESULTS AND DISCUSSION

Static tests

The column can be locked at any column height by closing the main valve. A static test to prove vacuum tightness was performed by running the column up to about 2/3rds full, closing the main valve (and shutting off the pump) waiting for settling, taking readings, venting in a little air to allow the height to drop a little, taking readings, etc. A typical result is shown in Figure 12. The strong correlation between the column height (top of tank water datum) and the vacuum created is evident.

Nature of the column flow

The clear column and tailpipe allow a view of the activity in these sections not observable in commercial systems. The water head in the column is a two-phase mixture of water and extracted gases in the form of bubbles (Figure 5). Whereas the foam head is churning, the sight gauge is virtually bubble free. Its water level is lower than the foam head height, depending on flow and the amount of air being extracted. This gauge gives a truer indication of the height of water.

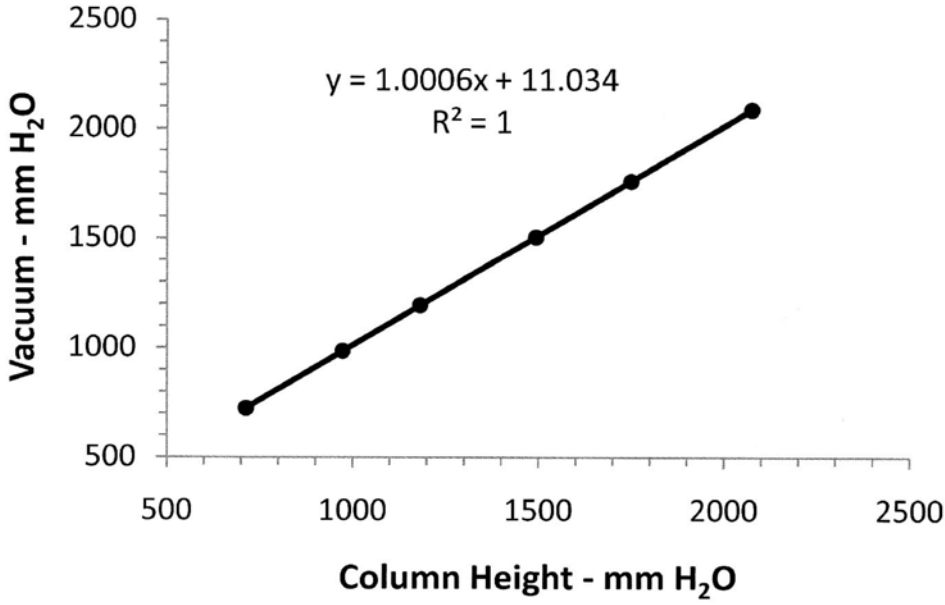


Figure 12: Static test data.

While the sight gauge level varies about 1 to 5 cm (½ to 2 in) with the flow, this is nearly stable compared to the foam head. Even so, as mentioned in the system description, a differential pressure transducer was installed across this sight gauge so that a reading can be recorded electronically and multi-sampling and averaging used as for the other data streams.

System gas removal is by bubble transport in the flow down through the tail piece. The entrained gas bubbles then float up to the tank surface. Cotton thread tattle tails were mounted in the clear PVC tailpipe to observe if vorticity existed through this section. None was observed.

Dynamic tests

A dynamic test is performed by adjusting the flow rate and allowing the column to stabilize before readings are taken. Early results of such tests gave a very different correlation between vacuum and column height. An example is shown in Figure 13.

The column vacuum is much less than the column height would indicate. The variation increases with greater column height, from 11% at the lowest point to 28% at the highest value for this data set. This result differs widely from that reported by Westers et al. (1991). A parabolic correlation fits the data better, as the R^2 value is greater.

Several more test runs will be needed to confirm these observations. The theoretical basis for the phenomenon shown in Figure 14 has not yet been established.

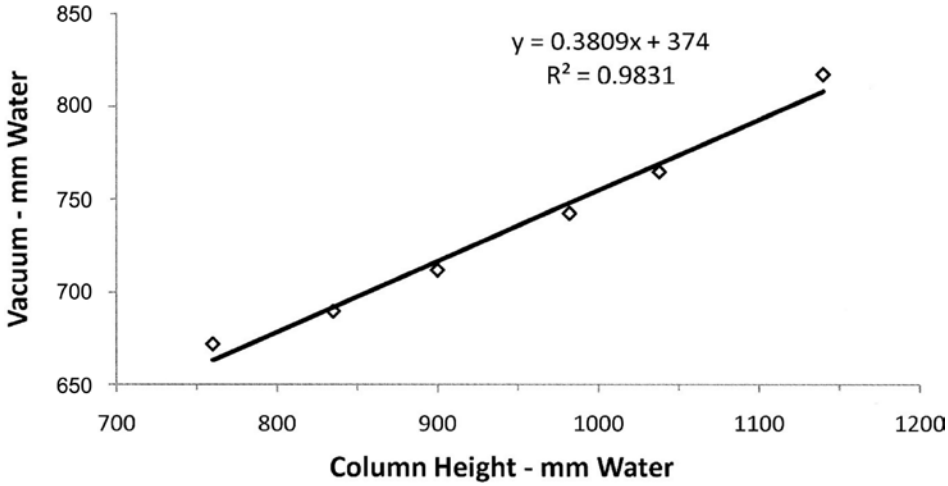


Figure 13: Vacuum produced vs. column height (tank surface datum) for one dynamic test.

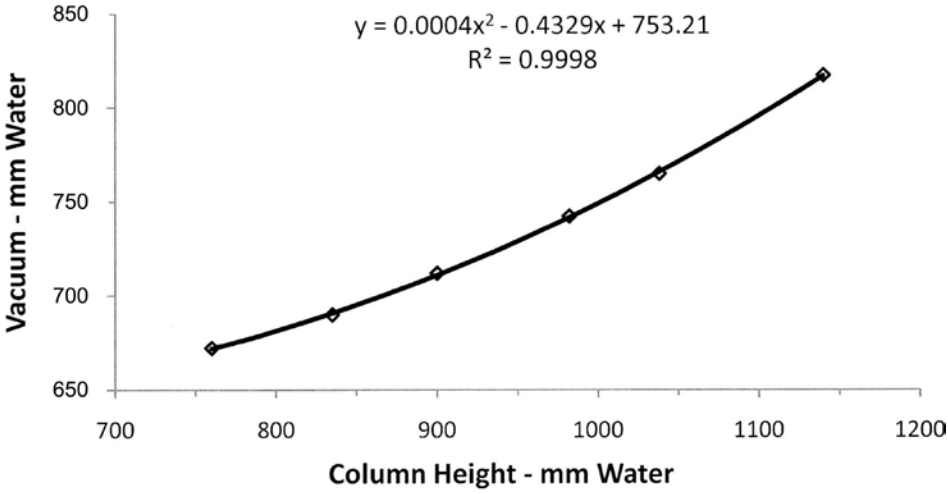


Figure 14: Figure 13 with parabolic curve fitting

CONCLUSION

The ultimate aim of this study is the production of a model or models to assist the engineer in designing a column to specifications of flow rate and degassing capability. The set-up has the capability of testing, at flow rates from 100 to 800 LPM, various combinations of column length, column diameter, tailpiece diameter, length and depth submerged, and water supersaturation versus desaturation. The purpose is to look for optimal combinations. That work is ongoing, but the simple premises that spawned it are being rethought.

REFERENCES

Bouck, G.R., King, R.E., and Bouck-Schmidt, G. Comparative Removal of Gas Supersaturation by Plunges, Screens and Packed Columns. *Aquacultural Engineering* **1984**, 3:159-176.

Colt, J. and Bouck, G. Design of Packed Columns for Degassing. *Aquacultural Engineering* **1984**, 3:251-237.

Westers, H., Boersen, G., and Bennett, V. Design and Operation of Sealed Columns to Remove Nitrogen and Add Oxygen. *American Fisheries Society Symposium* **1991**, 10:445-449.

Passive Vacuum Degasser Test Setup

Use of Coral Rubble, Aquamat™ and Aquaponic Biofiltration in the Recirculating System of a Marine Fish Hatchery

A. Estim[1]* and S. Mustafa[1]

[1]Borneo Marine Research Institute
Universiti Malaysia Sabah
Jalan UMS, 88400 Kota Kinabalu
Sabah, Malaysia

*Corresponding Author: bentin@ums.edu.my

Keywords: Aquaponic, biofilter, coral rubble, marine fish hatchery, water quality, *Eucheuma spp.*

ABSTRACT

A preliminary study on the effect of combination biofilters, including coral rubble, geotextile Aquamat™ (Meridian Aquatic Technology, Silver Spring, MD, USA), and algal aquaponics in a marine fish recirculating system was investigated. Aquamat™ is an innovative product fabricated from highly specialized synthetic polymer substrates. Aquamat™ forms a complex three-dimensional structure that resembles seagrass in appearance, and has been used to support high stocking densities in fish culture ponds and enhance biological processes. In addition, coral rubble was used, and two seaweed species, *Eucheuma spinosum* and *E. cottonii*, were evaluated for their usefulness as aquaponic biofilters in a recirculating system. Results showed that the four different biofilters operating within the recirculating system were significantly different ($P<0.05$) in NH_3-N and NO_3-N concentrations. The lowest mean NH_3-N concentration was recorded in the recirculating tank using Aquamat™ + seaweed + coral rubble, while the highest mean NO_3-N concentration was recorded in the recirculating tank using Aquamat™ + coral rubble. Fish weight gain and survival rates were not significantly different ($p<0.05$) in the four recirculating systems. In the second experiment, three varieties

International Journal of Recirculating Aquaculture 11 (2010) 19-36.

of *Eucheuma spp.* grew poorly, and produced no noticeable effects on NH_3-N, NO_2-N and NO_3-N concentrations. *Eucheuma cottonii* decayed in the early days, while the two varieties of *E. spinosum* decayed after 35 days. Once decayed, water quality impairment followed. This study concluded that *Eucheuma* species were not suitable as a method of biofiltration in a recirculating culture system. While these seaweeds do remediate water quality, they themselves require a good environment to perform this role. When conditions are not optimal for the stocked organisms, the co-culture system can produce negative results. Follow-up investigation is needed to determine the suitability of such integrated aquatic systems for a large-scale fish production in recirculation systems.

INTRODUCTION

In recent years, there has been growing concern over the impact of aquaculture, especially the nutrient-rich wastewaters discharged from fish holding facilities into the environment. Scientific interest in nutrient pollution from aquaculture facilities has increased markedly since the 1980s (Camargo and Alonso 2006). It is estimated that 52-95% of the nitrogen, 85% of the phosphorus, 80-88% of the carbon and 60% of the total feed input in aquaculture ends up as particulate matter, dissolved chemicals or gasses (Wu 1995). Aquaculture has increasingly been viewed as environmentally detrimental (Naylor et al. 2000). Gutierrez-Wing and Malone (2006) explained that recirculating systems have been identified as one of the two main research areas in aquaculture that address this problem. These kinds of systems are gaining wider acceptance because of their ability to reduce waste discharge, improve water quality control and reduce cost of production.

The processes crucial to the treatment of water in recirculating systems are solids capture, biofiltration, aeration, degassification, and ion balance. There are many alternative technologies available for each of these processes. There is a great potential to realize significant cost reductions depending on the development of designs that integrate two or more of these processes (Losordo et al. 1999). The selection of a particular technology depends upon the species being reared, production site infrastructure, production management expertise, and other factors. In a recirculating system, the three most common types of water purification treatments include earthen ponds (sedimentation), a combination of

solids removal and nitrification, and a combination of solids removal and macrophyte-based nutrient removal (Van Rijn 1996). The combined culture of marine algae and animals has been tested in China and Taiwan (Qian et al. 1996), as well as Israel (Shpigel and Neori 2007). These systems are based on the concept that algae actively uptake CO_2, release O_2 to the surrounding environment, and utilize the nutrients in metabolic waste originating from the stocked fish.

In this study, a combination of biofilters, including geotextile (Aquamat™), aquaponic algae, and coral rubble were incorporated into a marine fish recirculating system, and evaluated for their effectiveness (Estim et al. 2009, Estim and Mustafa 2010). Aquamat™ is a new and innovative product fabricated from highly-specialized synthetic polymer substrates. It forms a complex three-dimensional structure that resembles seagrass in appearance. This product has been principally used to support high stocking densities in fish culture ponds (Scott and McNeil 2001) and enhance biological processes that reduce ammonia concentrations (Bratvold and Browdy 2001, Estim et al. 2009). Additionally, two seaweed species, *Eucheuma spinosum* and *E. cottonii* (also known as *Kappaphycus alvarezii*) were tested as aquaponic biofilters in a recirculating system. These seaweed species are already cultured in the coastal areas of Sabah, Indonesia and the Philippines for their carrageenan contents, and were therefore easily available for integration with the fish aquaculture system. The objectives of this study were a) to compare dissolved inorganic nitrogen concentrations, fish weight gain, growth rates and survival rates in the four different recirculating systems and b) to measure the growth rate and biomass yield of three different seaweed varieties in a fish recirculating system.

Several studies have reported enhanced growth rates of seaweed and animals in integrated culture (Qian et al. 1996, Troell et al. 1999, Shpigel and Neori 2007). Schuenhoff et al. (2006) further elaborated that enhanced growth rates are achievable by integrated recirculating mariculture systems, which capture excess nutrients, making it possible to diversify the final products, provide a more efficient use of resources, and increase the income from the system while reducing operating costs.

MATERIALS AND METHODS

Aquamat™, Aquaponic Algae and Coral Rubble in Recirculating Systems

Twelve rectangular fiberglass tanks (0.5 x 0.55 x 0.5 m) were selected for the experiment. Each tank was equipped with a rectangular polyethylene bucket (0.2 x 0.15 x 0.1 m), which contained coral rubble (CR) in sizes ranging from 1.0 – 2.5 cm in diameter (Figure 1). Four combinations of recirculating biofilter systems were prepared in triplicate sets. The four types were as follows: CR + Aquamat™ (Aq), CR + Seaweed (Swd), CR + Aq + Swd, and CR alone (Control). Each of the recirculating systems was stocked with 55 juveniles of *Lates calcarifer*, (MW = 1.06 ± 0.41 g) also known as barramundi. The water flow rate averaged 0.05 ± 0.01 L/sec in each recirculating tank. A series of intensive samplings of dissolved inorganic nitrogen (NH_3-N, NO_2-N and NO_3-N) and *in situ* water quality (temperature, dissolved oxygen, pH, salinity, oxidation reduction potential (ORP) and conductivity) were carried out every four hours for 36 hours. After that, the sampling was repeated once daily (between 0900-1000 h) for one week.

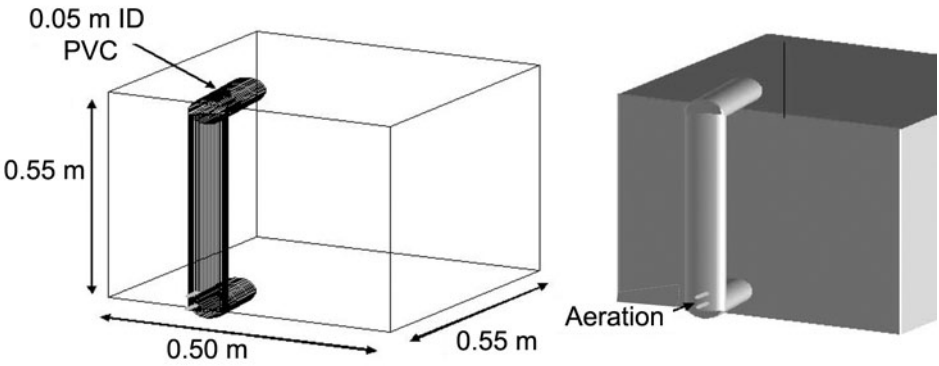


Figure 1. Layout of the recirculating tank in Experiment 1.

Three Different Varieties of Seaweeds in Recirculating Systems

The second experiment was conducted over 56 days in duplicate recirculating systems with and without seaweed (Figure 2). Each recirculating system consisted of one circular tank (1000 L) and two rectangular fiberglass tanks (100 L). In the circular tank, Aquamat™ (with surface area of 31.28 m^2) was installed and stocked with 150 *L. calcarifer* (mean weight = 0.94 ± 0.24 g). In the first 100 L rectangular tank, eight kg CR was added. The other 100 L rectangular tank was

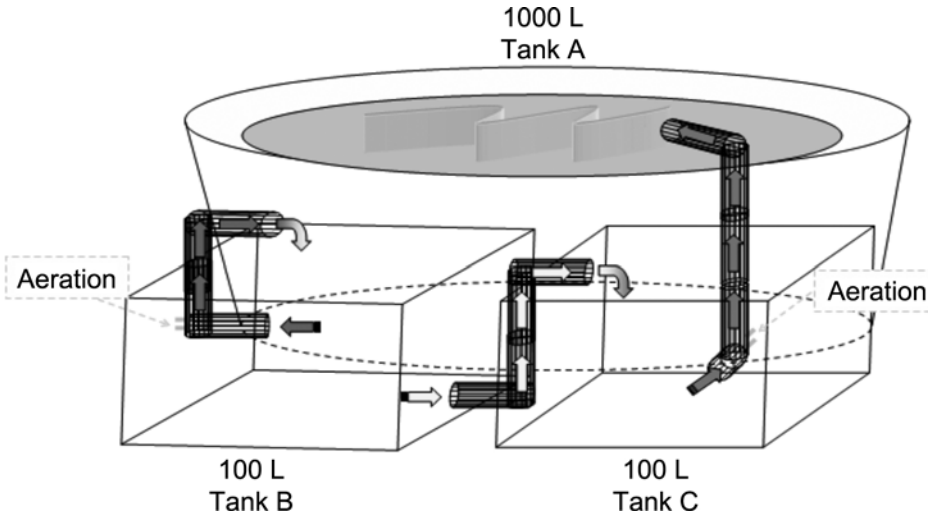


Figure 2. Layout of the recirculating systems of CR+Aquamat™ in Experiment 2.

planted with three varieties of seaweeds (Figure 3). The three different seaweeds were *Eucheuma cottonii* and two varieties of *Eucheuma spinosum* (brown and green varieties). Each seaweed cutting had an initial mean weight of 20.13 ± 6.55 g for *E. cottonii*, 18.07 ± 2.60 g for brown *E. spinosum* and 18.52 ± 2.96 g for the green *E. spinosum*. A water flow rate of 0.16 ± 0.04 L/sec was maintained in each recirculating system.

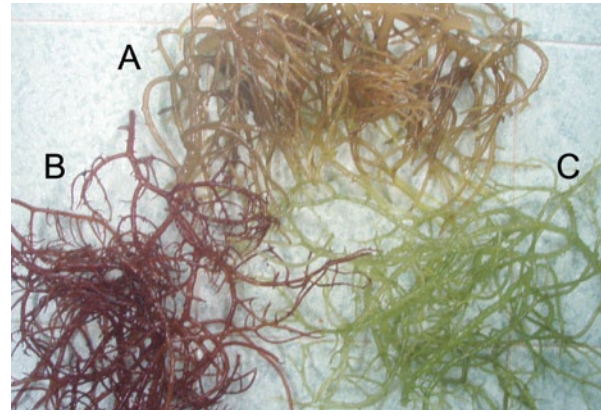


Figure 3. Three varieties of seaweeds. E. cottonii (A – light brown) E. spinosum (B – dark red) and E. spinosum (C – green).

The seaweed samples were collected from a seaweed farm in Bangi Island, North Borneo (7°06'46.60" N; 117°05'57.17" E) and transported in a styrofoam box as described by Mysua and Neori (2002). In each treatment tank, a pre-weighed seaweed biomass was stocked to the initial density for the study. Seaweed was harvested every seven days, drained to eliminate the superficial water then weighed using a digital balance. Specific seaweed growth rates (SSGR) were calculated as

SSGR=[(Ln W_t – Ln W_o)/*t*) x 100], where W_o is the initial weight or initial biomass, and W_t is the biomass at *t* culture days. The biomass yield (fresh weight) was calculated as the difference between the initial and the final weights and expressed in units of g/m^2/day, based on the areas of the culture tanks. The seaweed weight gain (SWG) was determined as SWG=[[(W_f – W_i)/W_i] x 100], where W_i and W_f are the initial and the final weight or wet biomass, respectively.

Water Quality

Dissolved inorganic nitrogen concentrations were analyzed using colorimetric methods as described by Parsons et al. (1984). The *in situ* water quality parameters [pH, temperature, oxidation reduction potential (ORP), conductivity and salinity] were monitored using a Cyberscan™ data logger (Eutech/Thermo Fisher Scientific, Ayer Rajah Crescent, Singapore). In the intensive experiment, seawater samples were collected every four hours initially, but later once a day between 0900-1000 for a week. Each time, after the seawater samples were collected from the recirculating tank, new seawater was added to maintain the volume and flow rate in each of the recirculating tanks. For the experiment involving the three varieties of seaweeds, water samples were collected from each tank every two days between 0900 and 1000 h. All seawater samples were filtered through GF/C Whatman filters (Whatman PLC, Maidstone, UK) with pore size of 0.45 µm. The light intensity in the culture set-up was measured with a digital light meter (TENMA® model 72-6693, Premier Farnell PLC, Bristol, UK) and was between 10.89 and 22.74 µmol/m^2/sec on cloudy days; and 35.21 to 68.06 µmol/m^2/sec on sunny days. Fish weight gain, specific growth rate and survival rate were determined.

Data Analysis

All data were analyzed by ANOVA to determine the statistical significance of the different treatments. All the tests were conducted after the confirmation of homogeneity of variance (Levene's test). To satisfy the assumptions of normality and homogeneity of variance, data of dissolved inorganic nutrient concentrations were transformed by Ln (NH_3-N and NO_2-N), Cos (NO_3-N) and Log_{10} for the DO concentrations prior to the statistical analysis. Multiple post-hoc comparisons among mean values were tested by Duncan test. In all cases, the null hypotheses were rejected at the five percent significance level.

RESULTS

Aquamat™, Aquaponic Algae, and Coral Rubble in Recirculating Systems

The four recirculating systems were not significantly different (P>0.05) in seawater temperature, DO, pH, salinity, ORP, and conductivity levels. Water temperature ranged from 25.99 ± 0.82 to 26.05 ± 0.82 °C, DO ranged from 5.64 ± 0.37 to 5.95 ± 0.24 mg/L, pH ranged from 8.06 ± 0.09 to 8.11 ± 0.05, salinity ranged from 31.14 ± 2.24 to 31.71 ± 0.45 ppt, ORP ranged from 41.4 ± 6.8 to 43.6 ± 6.7 mV, and conductivity ranged from 48.57 ± 0.55 to 48.63 ± 0.60 µS/cm (Table 1).

Changes in NH_3-N, NO_2-N and NO_3-N concentrations in the four recirculating tanks during the experiment are shown in Figure 4 and Figure 5. The variance analysis showed that the four recirculating tanks had significantly different ($p<0.05$) values of NH_3-N and NO_3-N concentrations, but no significant difference in NO_2-N concentration (Table 1). The mean NH_3-N concentrations were 0.85 ± 0.76 mg/L in the CR tank, 0.72 ± 0.71 mg/L in the Swd + CR tank, 0.35 ± 0.23 mg/L in the Aq + Swd + CR tank, and 0.31 ± 0.20 mg/L in the Aq + CR tank. The mean NO_3-N concentrations were 10.24 ± 4.22 mg/L in the Aq + CR tank, 5.06 ± 3.76 mg/L in the Aq + Swd + CR tank, 3.79 ± 2.58 mg/L in the CR tank and 2.45 ± 1.22 mg/L in the Swd + CR tank. The mean NO_2-N concentrations ranged from 0.20 ± 0.04 mg/L to 0.80 ± 0.21 mg/L in the four recirculating tanks (Table 1).

Table 1. Means (±SD) of in situ water quality, NH_3-N, NO_2-N and NO_3-N concentrations in the four recirculating systems.

	n	Control (CR)	Aq + CR	Swd + CR	Aq + Swd + CR
Temperature (°C)	39	25.99 ± 0.82	26.03 ± 0.85	26.05 ± 0.82	26.04 ± 0.82
DO (mg/L)	39	5.95 ± 0.24	5.64 ± 0.37	5.66 ± 0.24	5.71 ± 0.29
pH	39	8.11 ± 0.05	8.07 ± 0.09	8.08 ± 0.07	8.06 ± 0.09
Salinity (ppt)	39	31.7 ± 0.4	31.1 ± 2.2	31.7 ± 0.4	31.4 ± 1.6
ORP (mV)	39	41.4 ± 6.8	42.2 ± 6.7	43.2 ± 6.6	43.6 ± 6.7
Conductivity (uS/cm)	39	48.63 ± 0.60	48.58 ± 0.57	48.57 ± 0.55	48.59 ± 0.56
NH_3-N (mg/L)	39	0.85 ± 0.76 a	0.31 ± 0.20 c	0.72 ± 0.71 ab	0.35 ± 0.23 bc
NO_2-N (ug/L)	39	0.80 ± 0.21	0.55 ± 0.15	0.20 ± 0.04	0.32 ± 0.10
NO_3-N (mg/L)	39	3.79 ± 2.58 ab	10.24 ± 4.22 c	2.45 ± 1.22 a	5.06 ± 3.76 b

Values with different superscripts within row are significantly different (P<0.05)

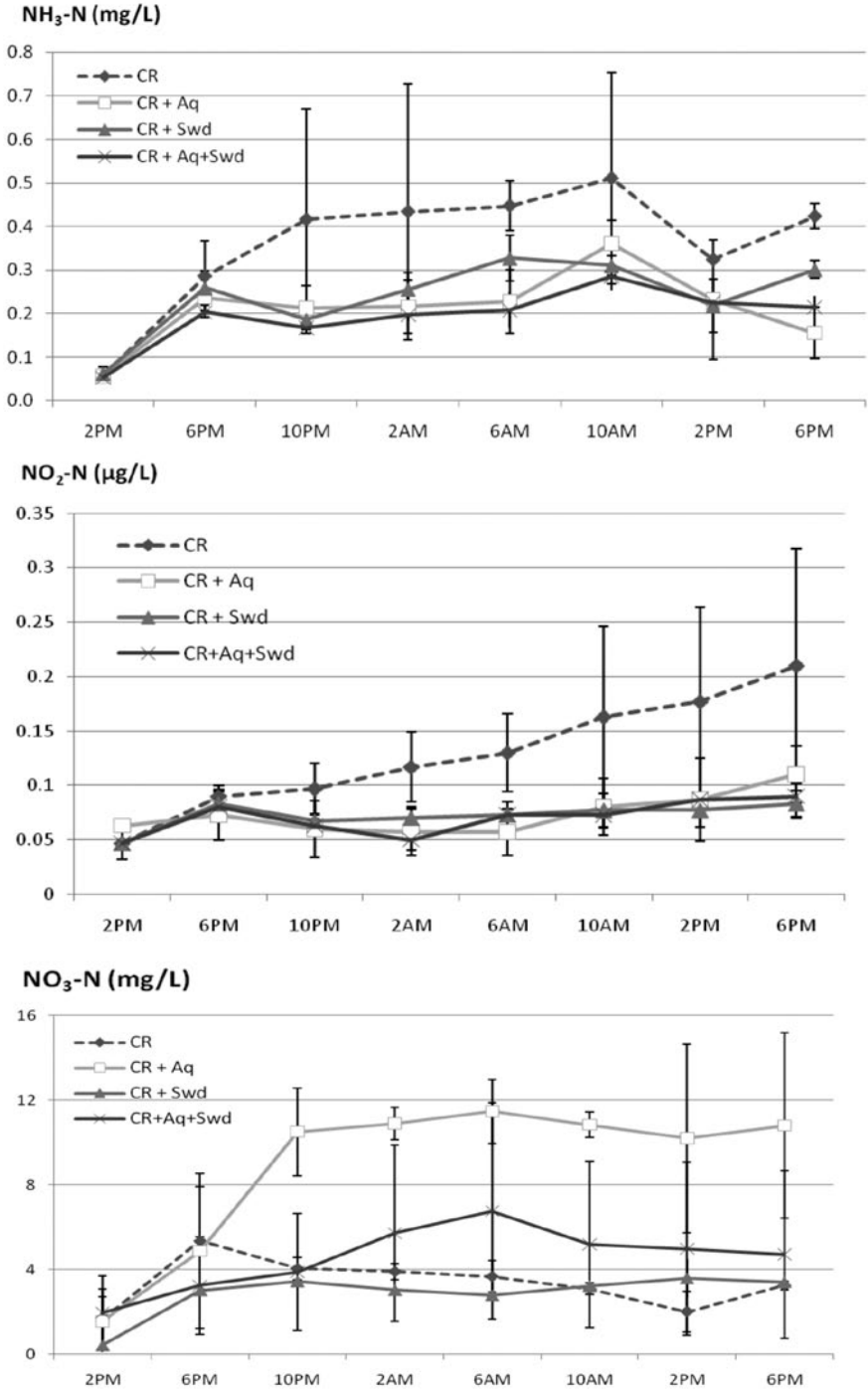


Figure 4. Changes (hours) in NH_3-N, NO_2-N and NO_3-N concentrations (mean ± SD) in the four recirculating tanks.

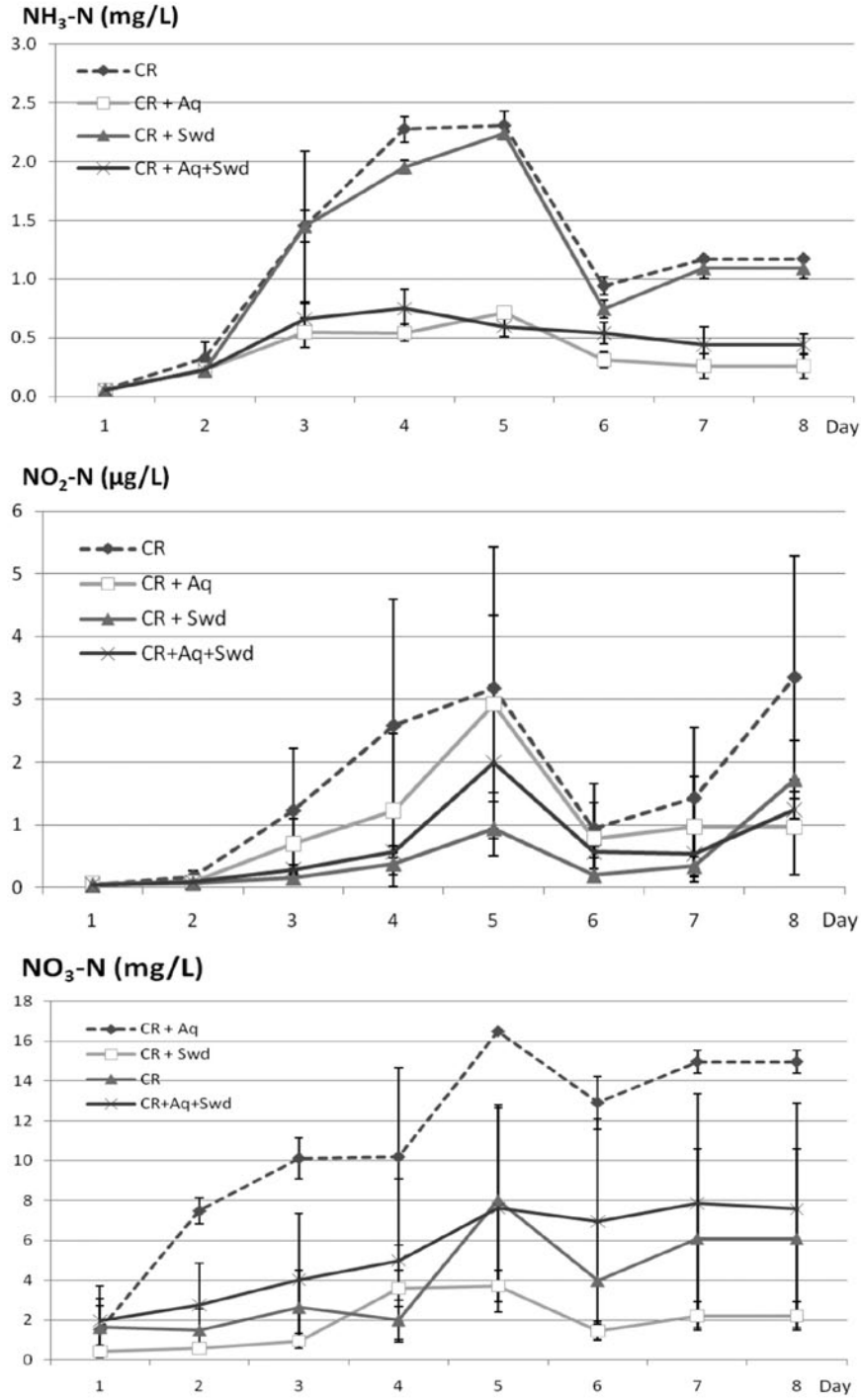


Figure 5. Changes (day) in NH_3-N, NO_2-N and NO_3-N concentrations (mean ± SD) in the four recirculating tanks.

The mean fish weight gain and survival rate in the Aq + Swd + CR tank were 96.4 ± 53.4 % and 96.4 ± 4.8 %, respectively (Figure 6). The values for the Aq + CR tank were 77.7 ± 28.8 % and 95.2 ± 2.1 %, respectively; for the Swd + CR tank they were 58.8 ± 18.1% and 92.1 ± 3.8 %, respectively; for the CR tank they were 51.3 ± 5.70 % and 90.9 ± 1.8 %, respectively (Table 1). It appeared that the fish weight gains and survival rates in the four treatment tanks were different (Figure 6). However,

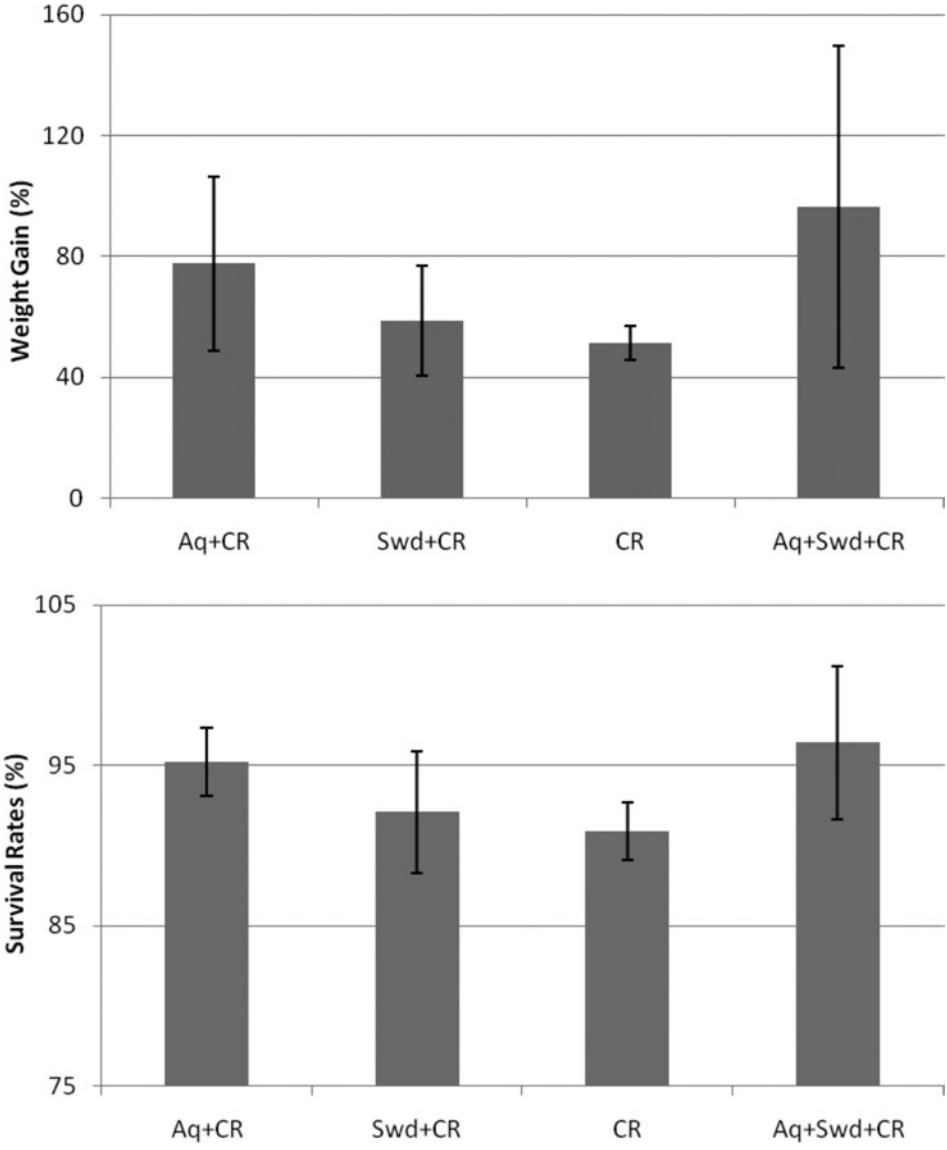


Figure 6. Means (± SD) of fish weight gain (%) and survival rate (%) in the four recirculating systems at the end of the experiment (7 days).

variance analysis showed that there was no significant difference ($p<0.05$) in fish weight gain or survival rate in the four tanks.

Three Varieties of Seaweeds in Fish Recirculating System

Comparisons between the culture systems with and without seaweeds were not significantly different in temperature, pH, DO, and salinity levels. The seawater temperature averages in the culture tanks with and without seaweeds were 26.75 ± 0.51 °C and 26.77 ± 0.50 °C, respectively. The pH averaged 8.06 ± 0.40 in the culture tank without seaweeds and 8.22 ± 1.84 in the culture tank with seaweeds. The mean values of DO in the culture tanks with and without seaweeds were 6.56 ± 0.49 mg/L and 6.77 ± 2.21 mg/L, respectively. Salinities decreased from 31.1 to 23.4 ppt in both recirculating systems, due to the influence of rain after five, seven, 15, 21, and 26 days of the experiment. Once the salinity recorded dropped below 27 ppt in both recirculating systems, the water was exchanged with 75 % new seawater (Table 2).

The analysis of variance indicated no significant difference in NH_3-N, NO_2-N, and NO_3-N concentrations in recirculating systems with and without seaweeds. The NH_3-N averaged 0.44 ± 0.24 mg/L in the culture tank without seaweeds and 0.44 ± 0.25 mg/L in the culture tank with seaweeds. NO_2-N and NO_3-N concentrations averaged 0.0406 ± 0.0066 mg/L and 109.0 ± 113.9 mg/L, respectively in the culture tank without seaweeds and 0.0409 ± 0.0060 mg/L and 112.1 ± 112.4 mg/L, respectively in the culture tank with seaweeds (Table 2).

Table 2. Means (±SD) of in situ water quality in the recirculating systems with and without seaweed.

Treatment Tanks	**Without seaweed**	**With seaweed**	**p<0.05**
n	96	96	df=1; N=190
Temp.(°C)	26.78 ± 0.50	26.75 ± 0.51	F=0.754; MS=0.025
pH	8.06 ± 0.39	8.22 ± 1.84	F=0.701; MS=1.234
DO (mg/L)	6.55 ± 0.49	6.77 ± 2.02	F=0.962; MS=2.083
Salinity (ppt)	27.42 ± 2.50	27.52 ± 2.41	F=0.085; MS=0.510
NH_3-N (mg/L)	0.43 ± 0.23	0.44 ± 0.25	F=0.082; MS=0.005
NO_2-N (mg/L)	0.0406 ± 0.0066	0.0409 ± 0.0060	F=0.119; MS=0.000
NO_3-N (mg/L)	109.0 ± 113.9	112.4 ± 112.4	F=0.035; MS=452.702

Two varieties of *E. spinosum* were grown in the recirculating system, however, after 35 days, both varieties showed signs of decay. The *E. cottonii* decayed in the first week of the experiment (Figure 7). The average specific growth rates of brown and green varieties of *E. spinosum* during the 35 days were 0.329 ± 0.129 % per day and 0.317 ± 0.178 % per day, respectively. Variance analysis proved that these two varieties did not differ significantly in terms of specific growth rates. The average yield per unit area of the brown and green varieties was 1.555 g/m^2/day and 1.476 g/m^2/day, respectively.

Table 2 shows that the fish growth rates and survival rates were not significantly different in both recirculating systems. The specific growth rates of *L. calcarifer* in the recirculating systems with and without seaweeds were 1.96 ± 0.90 % per day and 1.90 ± 0.90 % per day, respectively. *Lates calcarifer* survival rate was 94 % in the recirculating system with seaweeds and 86 % in the recirculating system without seaweeds.

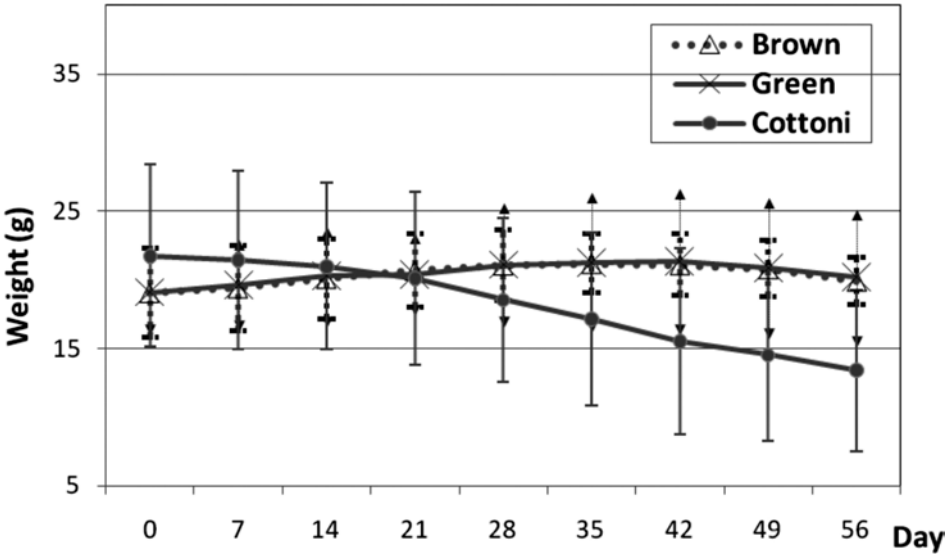


Figure 7. Weight (g) of three varieties of seaweeds (Eucheuma cottonii, brown E. spinosum, and green E. spinosum) in a fish recirculating system.

DISCUSSION

The preliminary experiment showed that the nitrification process occurred in all recirculating tanks (Figure 4 and 5). This was evident from the observed increase in NO_2-N and NO_3-N concentrations in

the four recirculating tanks. The nitrification process is the biological oxidation of ammonia into nitrite, then into nitrate, which requires oxygen and bacteria. Nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*) in the production system utilize ammonia-nitrogen as an energy source for growth and produce nitrite and nitrate as a by-product. Ammonia is a by-product of protein metabolism, which is excreted from the gills of fish as they assimilate feed, and is also produced when bacteria decompose organic waste solids within the system.

However, the rates of nitrification in the four recirculating systems were significantly different. Table 1 shows the four recirculating systems had significantly different ($p<0.05$) in NH_3-N and NO_3-N concentrations. The ammonia mean concentrations recorded in the Aq + CR and Aq + Swd + CR recirculating tanks were lower compared to the other two recirculating tanks (Swd + CR, and CR alone). This suggested that the nitrification process occurred faster inside the recirculating tanks of Aq + CR and Aq + Swd + CR compared to the other recirculating tanks. Aquamat™ and CR provided a substantial surface area for microbes to grow and enhance the nitrification process in recirculating systems. In biological filtration, a substrate with a large surface area is required for nitrifying bacteria to attach and grow (Stehr et al. 1995, Losordo et al. 1999, Estim et al. 2009). The rate of the nitrification reaction is highly dependent on a number of environmental factors. These include the substrate and oxygen concentration, temperature, pH, and the presence of toxic or inhibiting substances. Stehr et al. (1995) added that an increase in the surface area available in the oxygenated water column may also promote growth of specific bacterial groups such as nitrifiers, which are more likely to inhabit surfaces than to be free-floating. Previous studies showed that the bacteria colonies were, in fact, more numerous on the surface of Aquamat™ than in the water column in the culture system (Estim et al. 2009). Aquamat™ alone is still not sufficient to remove the dissolved inorganic nitrogen in a recirculating system (Figure 4 and 5), where the ammonia by-product, namely nitrate, also accumulates in the culture system. For aquatic animals, nitrate is the least toxic of the inorganic nitrogen compounds. However, if nitrate is released into the environment, it can stimulate harmful algal blooms (Estim et al. 2001). Some of the negative impacts attributed to aquaculture are due to the release of nitrogen and phosphorus into the surrounding environment; an excess of these nutrients can cause eutrophication and deteriorate the

environment (Camargo and Alonso 2006). Van Rijn (1996) explained that accumulation of other inorganic nutrients such as nitrate and phosphate have received little attention, but deserve increasing consideration.

The dissolved inorganic nitrogen concentration is lower in recirculating tanks with a combination biofilter using Aq + Swd + CR (Figures 4 and 5); this system also supported a marginally higher fish weight gain and survival rate over the other recirculating systems (Table 1). The inclusion of seaweed significantly reduced the load of dissolved nutrients that are returned to the environment (Neori et al. 1996, Msyua and Neori 2002, Shpigel and Neori 2007, Estim and Mustafa 2010, Troell et al. 1999). The methods for using seaweed to treat effluents from enclosed mariculture systems were initiated in the mid 1970s, and have recently garnered new interest, now that it has been shown that waste water from intensive and semi-intensive mariculture is suitable as a nutrient source for seaweed production.

In the second experiment, the three varieties of *Eucheuma sp.* were not seen to grow steadily and produced no noticeable effects on NH_3-N, NO_2-N, and NO_3-N concentrations. It was noted that *E. cottonii* decayed in the early days, while the two varieties of *E. spinosum* decayed after 35 days. Qian et al. (1996) reported that *Kappaphycus alvarezii* in a co-culture system grows faster and removes nitrogenous waste released by pearl oysters. Besides, Msuya and Neori (2002) reported that *Eucheuma denticulatum* (also known as *E. spinosum*) did not survive after 10 days. They explained that the algae started to lighten in color, and then white lesions were observed at the tips, which is a typical sign of stress (peroxide formation). The specimens finally rotted and died. Those observations were also made on the *E. cottonii* in this experiment. Although Msuya and Neori (2002) reported that *E. denticulatum* died after 10 days, they also observed that pieces of *E. denticulatum* planted in the fishpond effluent channels survived until the fourth week. It was also observed that the new thallus of *E. spinosum* is slightly small and thin as reported before (Estim and Mustafa 2010).

During the study, fresh water (rain) influenced salinity inside the culture systems, which decreased from 31.1 to 23.4 ppt. This change most likely caused the early decay of *E. cottonii*. In addition, low temperatures during the experimental period may also have contributed to this process. Environmental conditions have to be optimal for stocked

species to give highest production (Qian et al. 1996). Therefore, when conditions are suboptimal, the co-culture system can produce negative results. Anggadirehja et al. (2002) explained that the suitable salinity for *Eucheuma spp.* was in the range of 28 to 34 ppt and that light played an important role in the photosynthetic activity and overall survival of the algae. The lower temperatures and decrease in light exposure may have resulted in setbacks to growth as well as biofiltration capacity (Schuenhoff et al. 2006). As detailed in Yan et al. (1998), the key elements in the successful management of this systemic photosynthesis are control over the respiration ratio and recycling of nutrients. Other factors affecting growth and survival are the concentration of dissolved oxygen, pH, temperature, and the concentration of ammonia and nitrite.

A concept and qualitative experimental results for integrated waste-recycling marine polyculture systems were described in the early 1970's (Yan et al. 1998, Shpigel and Neori 2007). In these studies, the source of nutrients was domestic effluents that were mixed with seawater to obtain brackish water for phytoplankton culture. In turn, the microalgae were fed to filter feeders (oysters and clams) as well as additional organisms that consumed the solid waste particles. Dissolved nutrients in the final effluent were biofiltered by seaweed. Replacement of the sewage water with effluents from fish culture and use of the seaweed for macroalgivore (abalone) culture were subsequently proposed (Shpigel and Neori 2007). In this study, it can be concluded that the *Eucheuma sp.* cannot survive for long under the conditions provided, and once dead, water quality impairment follows. While seaweeds carry out a degree of water quality remediation, they themselves require a good environment to perform the role. When the conditions are not optimal for the stocked organisms, the co-culture system can produce negative results. Follow-up investigations are necessary to determine the suitability of this type of integrated recirculating aquatic system for large-scale fish production. In fact, the variable costs of producing fish in recirculating systems (feed, fingerling, electricity, labor) are not much different than that of other production methods. The authors agree with conclusion of Yan *et al.* (1998) that although many forms of wastewater aquaculture are successful, they are not always universally applicable, and must be adapted to the local environmental, economic, social conditions. The integrated production of marine fish and seaweed has the potential to be ecologically, economically and socially more sustainable than current practices.

This will reduce environmental impact of fish farming, produce extra income for farmers and create additional jobs while helping to improve the public image of intensive aquaculture. Many developed countries have identified recirculating aquaculture as an area for research and development. Asia is lagging behind in this field. However, if as a result of intensive research, a feasible technology emerges, that technology will have a better chance of widespread application in the current climate, where environmental concerns are taking center stage in all industrial-scale operations, including seafood production.

ACKNOWLEDGEMENTS

Special thanks to the Malaysia Ministry of Higher Education and the Malaysia Ministry of Science, Technology and Innovation for providing financial support under research grants of Fundamental FRG0041-ST-001 and Escience, SCF05-01-10-SF0053, respectively. I also would like to thank the staff of Borneo Marine Research Institute fish hatchery especially Prof. Dr. Shigeharu Senoo and Prof. Dr. Ridzwan A. Rahman.

REFERENCES

Anggadirehja, J.T., Zatnika, A., Purwoto, H. and Istini, S. **2002**. *Rumput Laut, Pembudidayaan, Pengolahan & Pemasaran Komoditas Perikanan Potensial.* Indonesia. Penebar Swadaya.

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

Camargo, J.A. and Alonso, A. Ecological and Toxicological Effects of Inorganic Nitrogen Pollution in Aquatic Ecosystems: A Global Assessment. *Environment International* **2006**, 32:831-849.

Estim, A., Anton, A., Ridzwan, A.R. and Alexander, J. **2001**. NO_3-N dan PO_4-P Merangsang Peningkatan Populasi Alga Beracun (*Pyrodinium bahamense* var *compressum*) di Perairan Marin Sabah. *Proceedings Malaysian Science and Technology Congress 2001*. 24 – 26 September. Kota Kinabalu, Sabah.

Estim, A., Mustafa, S. and Anton, A. **2009**. Dissolved Inorganic Nitrogen and Aquamat™ Application in Marine Fish Hatchery System. In Workshop on Protect Diversity of Bioresources in East and South-East Asia, 10 August 2009. JSPS Exchange Program for East Asian Young Researchers, Kagoshima University: Kagoshima, Japan.

Estim, A. and Mustafa, S. Aquaponic Application in a Marine Hatchery System. *Aquaponics Journal* **2010,** 57:26-34.

Gutierrez-Wing, M.T. and Malone, R.F. Biological Filters in Aquaculture: Trends and Research Directions for Freshwater and Marine Applications. *Aquacultural Engineering* **2006**, 34:163-171

Losordo, T.M., Masser, M.P., and Rakocy, J. **1999**. Recirculating Aquaculture Tank Production Systems, A Review of Component Options. SRAC (Southern Regional Aquaculture Center) Publication No. 453.

Msuya, F.E. and A. Neori. *Ulva reticulata* and *Gracilaria crassa*: Macroalgae that can Biofilter Effluent from Tidal Fishponds in Tanzania. *Western Indian Ocean Journal Marine Science* **2002**, 2:117-126.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H. and Troell, M. Effect of Aquaculture on World Fish Supplies. *Nature* **2000**, 405:1017-1024.

Neori, A., Krom, M.D., Ellner, S.P., Boyd, C.E., Popper, D., Rabinovitch, R., Davidson, P.J., Dvir, O., Zuber, D., Ucko, M., Angel, D. and Gordin, H. Seaweed Biofilters as Regulators of Water Quality in Integrated Fish-Seaweed Culture Units. *Aquaculture* **1996**, 141:183-199.

Parsons, T.R., Maita, Y. and Lali, C.M. **1984**. *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergumon Press: Oxford, UK.

Qian, P.Y., Wu, C.Y., Wu, M. and Xie, Y.K. Integrated Cultivation of the Red Algae *Kappaphycus alvarezii* and the Pearl Oyster *Pinctada martensi*. *Aquaculture* **1996**, 147:21-35.

Schuenhoff, A., Mata, L. and Santos, R. The Tetrasporophyte of *Asparagopsis armata* as a Novel Seaweed Biofilter. *Aquaculture* **2006**, 252:3-11.

Scott, S. and McNeil, R. Aquamats: How to Provide Habitat, Feed and Biofiltration for Hatchery and Nursery Tanks. *Hatchery International* **2001**, 2:19-21.

Shpigel, M. and Neori, A. **2007**. Microalgae, Macroalgae and Bivalves as Biofilters in Land-Based Mariculture in Israel. In Ecological and Genetic Implications of Aquaculture Activities. Bert, T.M. (Ed.). Springer: New York, NY, USA, 433-446.

Stehr, G., Böttcher, B., Dittberner, P. and Rath, G. The Ammonia-Oxidizing Nitrifying Population of the River Elbe Estuary. *FEMS Microbiology Ecology* **1995**, 17:177–186.

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

Van Rijn, J. The Potential for Integrated Biological Treatment Systems in Recirculating Fish Culture - A Review. *Aquaculture* **1996**, 139:181-201.

Wu, R.S.S. The Environmental Impact of Marine Fish Culture: Towards a Sustainable Future. *Marine Pollution Bulletin* **1995**, 31:159-166.

Yan, J., Wang, R. and Wang, M. The Fundamental Principles and Ecotechniques of Wastewater Aquaculture. *Ecological Engineering* **1998**, 10:191-208.

Production of Microbial Flocs Using Laboratory-scale Sequencing Batch Reactors and Tilapia Wastewater

D.D. Kuhn[1]*, G.D. Boardman[2], G.J. Flick[1]

[1]Department of Food Science and Technology
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061, USA

[2]Department of Civil and Environmental Engineering
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061, USA

*Corresponding author: *davekuhn@vt.edu*

Keywords: sequencing batch reactors, SBR, microbial flocs, recirculating systems, tilapia, effluent, carbon supplementation, alternative protein, aquaculture feed

ABSTRACT

Laboratory-scale studies using sequencing batch reactors (SBRs) were conducted to evaluate microbial floc production and treatability of fish effluent from a tilapia farm utilizing recirculating aquaculture systems (RAS). Several trials were conducted, both with and without carbon sucrose supplementation. Results from this project suggest that treatment with carbon supplementation improved nutrient removal from the fish effluent and increased microbial floc production. Successful treatment of effluent using bioreactors could accomplish two primary objectives. The first objective is improving water quality of effluent to maximize water reuse. Secondly, production of microbial flocs is a means of recycling nutrients from the effluent into a useable and alternative protein source for aquaculture diets. Ultimately, this option could offer a sustainable option for the aquaculture industry.

International Journal of Recirculating Aquaculture 11 (2010) 37-54.

INTRODUCTION

Overfishing of natural fisheries is a global issue that is becoming more urgent as the human population continues to increase. According to the Food and Agriculture Organization of the United Nations, approximately 47% of the natural fisheries are fully exploited and an additional 18% are overexploited (FAO 2002). The high demand for seafood protein will likely increase, because worldwide, one out of five people currently depend on fish for their principal source of protein (Koonse 2006).

To meet the growing demand for seafood, aquaculture production is on the rise, and is reportedly the fastest growing sector of agriculture worldwide. Traditional aquaculture practices use pond and flow-through systems, which are often responsible for discharging pollutants (e.g., nutrients and solids) into the environment. Furthermore, aquaculture feeds often contain high levels of fish or seafood protein, potentially increasing demand placed on wild fisheries. To mitigate these drawbacks, there is a significant movement towards more sustainable practices, especially in developed countries (Avnimelech 1999, Hargreaves 2006). For example, recirculating aquaculture systems (RAS) maximize reuse of culture water, which decreases water demand and minimizes pollutants discharged to the environment (Skjølstrup et al. 2000, Menasveta 2002, Timmons et al. 2002). Alternative proteins (e.g., yeast-based proteins) are also replacing fish and seafood proteins originally used in aquaculture diets (McLean et al. 2006, Lunger et al., 2007; Fraser and Davies, 2009). Implementing these alternative proteins could ease pressures on wild fisheries and often leads to high quality and less expensive feeds. The research described in this paper focuses on maximizing the reuse of freshwater fish effluent in the culture of marine shrimp. More specifically, this reuse is accomplished by using suspended-growth biological reactors to treat tilapia effluent, generating microbial flocs that could be used as an alternative feed to support shrimp culture.

Previous research investigated using nutrients in effluents from a commercial tilapia farm as supplemental feed to *L. vannamei* directly, in the form of microbial flocs generated from biological treatment of the effluents. Microbial flocs generated in bioreactors, and offered as a supplemental feed, significantly ($P < 0.05$) improved shrimp growth and specific growth rates (SGRs) in shrimp fed a restricted ration of commercial shrimp feed (Kuhn et al. 2008). Further studies

demonstrated that microbial flocs produced in sequencing batch reactors (SBRs) were a useful ingredient in replacing fishmeal. In fact, inclusion of microbial floc increased shrimp growth rates by over 65% (Kuhn et al. 2009).

Since this previous research demonstrated the potential benefits of implementing suspended growth biological treatment to aid in the co-culture of shrimp, it is important to understand how to best treat the effluent while producing microbial floc that can be utilized by the shrimp as a supplemental feed. Therefore, this project was focused on the treatability of effluents from the tilapia farm using SBRs. Treatments with and without carbon supplementation were evaluated and compared. Biological kinetic data and nutritional properties of SBR produced microbial floc were also determined.

MATERIALS AND METHODS

Effluent Handling and Storage

Tilapia effluent was collected from a local commercial RAS tilapia facility (Blue Ridge Aquaculture Inc., Martinsville, VA, USA). Fish densities at harvest were approximately 0.2 kg per L of water and each growout tank was outfitted with a settling basin, rotating biological contactors, and oxygenation via U-tubes. The effluent was collected from settling basins at the farm while they were drained as part of normal operations. Variability of constituents in this effluent was minimal because the settling basins were only flushed after 230 kg of feed were provided to the tilapia. During trial one, effluent was stored at -20°C in 19 L buckets until needed. For trials two through four, approximately 950 L of effluent was stored in the laboratory in a 1,100 L storage tank. Untreated solids, collected directly from tilapia effluent after a 45 min settling period, were characterized for protein and organic matter content and compared against microbial flocs from SBRs.

Bioreactor Operation (Trials One Through Three)

Trial 1 setup consisted of twelve 1 L Beakers in a 29°C water bath (Table 1). These beakers were operated as SBRs with a hydraulic residence time (HRT) of 24 hours and no carbon supplementation. Effluent was stored in 19 L buckets in a -20°C freezer. Every 24 hours a bucket was removed

and thawed so a fresh source of effluent could be manually fed to the SBRs. Sludge was wasted at specific rates to evaluate biological solids residence times (SRTs) of 3, 6, 10, and 15 days in triplicate. Sludge was wasted by removing a known volume of well-mixed suspended solids from the reactor with a known suspended solids concentration. These SBRs were operated manually with the following periods: well-mixed aeration, 23 h; settling, 45 min; decant/idle/fill, 15 min. This trial lasted for 50 d.

Trials 2 and 3 (Table 1) were conducted in three SBRs (Figure 1) maintained at 28°C. Dissolved oxygen (DO) levels were greater than 5 mg/L during the aeration cycle. These 5 L SBRs were operated in triplicate using the following sequence: 4 h well-mixed aeration, 1 h settling, 45 min draw (water decantation/removal), and 15 min idle/fill periods. Water was pumped every 24 h from the storage tank (at room temperature) into a well-mixed 76 L equalization (EQ) tank. Microbial floc was wasted at a rate that provided a SRT of 10 d. Trial two was

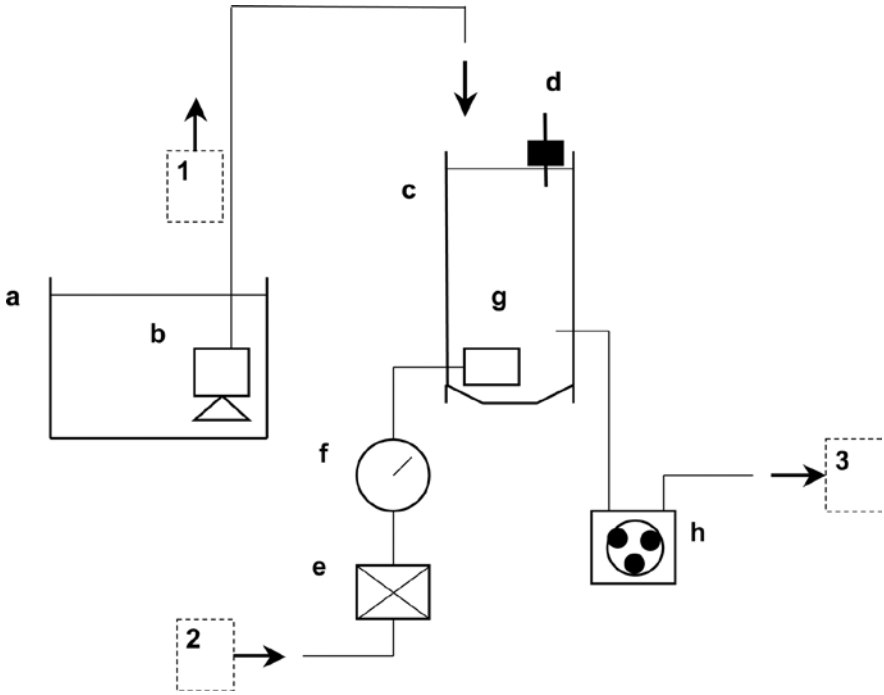


Figure 1. Diagram of SBRs used for trials 2, 3, and 4: a) Anaerobic equalization tank, b) submersible pump on float switch, c) aerobic SBR, d) float switch, e) solenoid valve, f) air flow meter, g) air stone, h) peristaltic pump, 1) tilapia effluent, 2) compressed air, 3) treated effluent.

conducted for 45 d with no carbon supplementation. In trial three, 500 mg/L (210 mg of carbon/L) of sucrose (Granulated white sugar, Kroger Co., Cincinnati, OH, USA) was added directly into the SBRs 5 min after each aeration cycle began, using peristaltic dosing pumps (Reefdoser RD4 Quadro, Aqua Medic©, Bissendorf, Denmark). Trial three was conducted for 30 to 35 d until the reactors became infested with fungi and were no longer operational.

Bioreactor Operation (Trial Four)

Every 24 h, the 76 L EQ tank was cleaned using pressurized well water. The EQ tank was well-mixed without aeration using a submersible Rio® 200 pump (TAAM Inc., Camarillo, CA, USA) and was maintained at 29°C. Sucrose was added directly to the EQ tank (500 mg/L sucrose, 210 mg of carbon/L) to promote denitrification and an increase in heterotrophic microbial floc. The resulting calculated food to microorganism ratio (F:M) over the stabilized period from day 30 to 50 was 0.15 ± 0.01.

Three 5 L SBRs were operated with 4 h well-mixed aeration, 1 h settling, 45 min draw (water decantation/removal), and 15 min idle/fill periods (Figure 1). The target SRT was 10 d. The temperature in the SBRs was maintained at 28.7 ± 0.2°C (mean ± standard error) using a water bath, and DO levels were always greater than 5 mg/L. Effluent was collected in 19 L buckets, and volumetric measurements of treated water were determined every 24 h for each reactor to ensure proper operation. Two independent batch trials were performed on stabilized SBRs on day 50 to determine kinetic coefficients from concentrations of microbial floc (mixed liquor volatile suspended solids, MLVSS), soluble total organic carbon (sTOC), and soluble chemical oxygen demand (sCOD) versus time (n = 17). Initial levels of MLVSS and sucrose spike concentrations to initiate the kinetic batch experiments were similar to levels used during the 50 day trial. The initial F:Ms for the two kinetic trials were, 0.14 and 0.17, respectively.

Laboratory analysis

After samples were filtered through a 1.5 µm filter, the filtrate was analyzed for nitrite-N, nitrate-N, orthophosphate (OP), and total ammonia-N (TAN) in accordance with HACH (2007) spectrophotometric methods 8507, 8039, 8048, and 8038, respectively. Sludge volume index (SVI), sCOD, sTOC, total solids (TS), total suspended solids (TSS) and

volatile suspended solids (VSS) were determined using methods 2710D, 5310B, 5220D, 2540B, 2540D, and 2540E, respectively (APHA 2005). Crude protein levels were determined in accordance with AOAC (2003). Temperature and DO were determined with a YSI 85 probe (Yellow Springs Inc., Yellow Springs, OH, USA). A HI 9024 pH meter (HANNA Instruments, Woonsocket, RI, USA) was used to determine pH.

Statistical Analysis

Statistical analysis, t-test, was performed using SAS v9.1 for Windows (SAS Institute Inc., Cary, NC, USA) on composition data regarding microbial floc versus untreated solids.

RESULTS

Trials One through Three

Results for trials one to three are summarized in Table 1. For trial one, reduction of sCOD and TAN ranged from 58 to 72% and 79 to 83%, respectively, and both increased with increasing SRT. Volatile suspended solids ranged from 100 to 200 mg/L and increased with increasing SRT. Trial two resulted in highly variable treatment, ranging from 18 to 80% removals for sCOD while MLVSS concentrations remained less than 200 mg/L. Trial three reactors generated levels of MLVSS greater than 1,000 mg/L. Removals of sCOD and TAN were both greater than 80%. However, fungi became dominant starting between days 30 and 35 (Figure 2). Although fungi was present during trial 3, it was not detected during trials one and two.

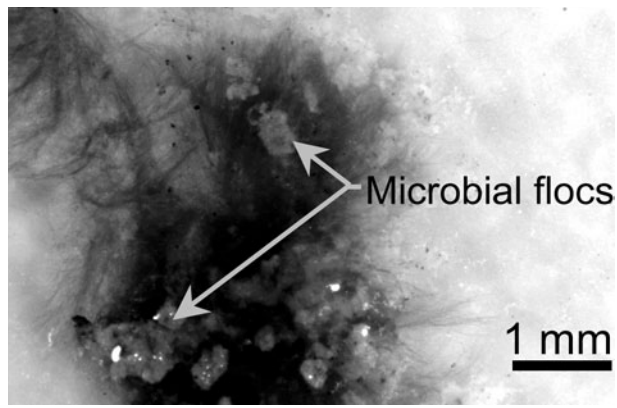


Figure 2. Macro-photograph of fungi (filamentous shape) and a few microbial flocs (spherical shape).

Table 1. Comparison of various treatment and operation schemes performed at the laboratory scale.

Trial	Operation/input	Treatment	Microbial floc production	Fungi production	Comments
One: Aerobic (Beaker SBR)	HRT = 24 hours SRT = 3,6,10,15 days CS = no	Moderate 58-72% sCOD 79-83% TAN	Insufficient <200 mg/L	None	Fresh wastewater from freezer every 24 hours
Two: Aerobic (SBR)	HRT = 6 hours SRT = 10 days CS = no	Highly variable (e.g., 18 to 80% sCOD treatment)	Insufficient <200 mg/L	None	Up to 7 day old wastewater
Three: Aerobic (SBR)	HRT = 6 hours SRT = 10 days CS = yes	Sufficient > 80% sCOD > 80% TAN	Sufficient >1,000 mg/L	Excessive	Up to 7 day old wastewater
Four: anoxic/ aerobic (EQ tank/SBR)	HRT = 6 hours SRT = 10 days CS = yes	Sufficient > 80% sCOD > 80% TAN	Sufficient >1,000 mg/L	Limited	Up to 7 day old wastewater

Note: CS = carbon supplementation (sucrose)

Trial Four

A strong linear correlation (R^2 of 0.9930) was observed between sCOD and sTOC (Figure 3). This function yielded a slope of 2.26 (mg sCOD)/(mg sTOC) and was determined over a range of sTOC (11-230 mg/L) and sCOD (12-510 mg/L), which was reflective of the range observed during this 50 day study. Similarly, ratios of COD to TOC were 2.33 ± 0.063 (mean ± standard error) when removal of sTOC, or sCOD, was less than 85% (Figure 4). However, for treatment levels greater than 85%, this ratio was significantly ($P < 0.05$) reduced to 1.36 ± 0.099.

During the stabilized period from day 30 to 50 (Figure 5), the overall mean concentration of MLVSS in the three SBRs was 1,383 ± 151 mg/L. No significant differences ($P > 0.05$) were observed between the mean MLVSS concentrations on the different days. During this stabilized period, removal of sTOC was always greater than 89% with an average reduction of 93.0 ± 0.8%. Furthermore, the mean effluent concentration of sTOC was 14.7 ± 1.7 mg/L. Figure 6 illustrates the changes in various constituents between the storage tank, equalization tank, and treatment from the SBRs. Overall, the percent difference in TAN, NO_2, pH, NO_3, and OP from influent to effluent were, respectively, –91, 0, +9, -60, and –23 % during the aforementioned stabilized period.

Table 2. Trial four normalized kinetic coefficients based on two independent kinetic trials, except for yield coefficients for anoxic/oxic cycles which were determined from 8 data points from day 30 to 50. Mean values with standard errors.

	Substrate	
Kinetic Coefficients	**sTOC**	**sCOD**
$Y_{anoxic/oxic}$ [g microbial floc/g substrate]	1.54 ± 0.11	0.68 ± 0.05
Y_{oxic} [g microbial floc/g substrate]	1.60 ± 0.07	0.69 ± 0.02
μ [1/h]	0.27 ± 0.028 (0.9225)	
Zero-order rate [g substrate/ (g microbial floc*h)]	0.17 ± 0.01 (0.9964)	0.39 ± 0.03 (0.9759)
First-order rate [(1/hr)/gVSS]	1.59 ± 0.39 (0.9650)	1.72 ± 0.64 (0.9656)

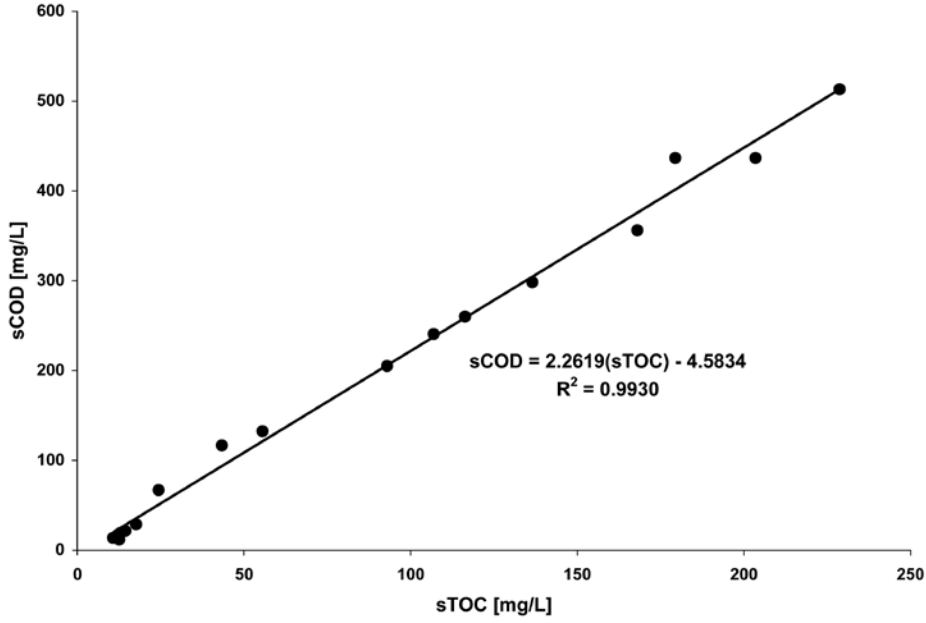


Figure 3. Correlation relationship between sCOD and sTOC.

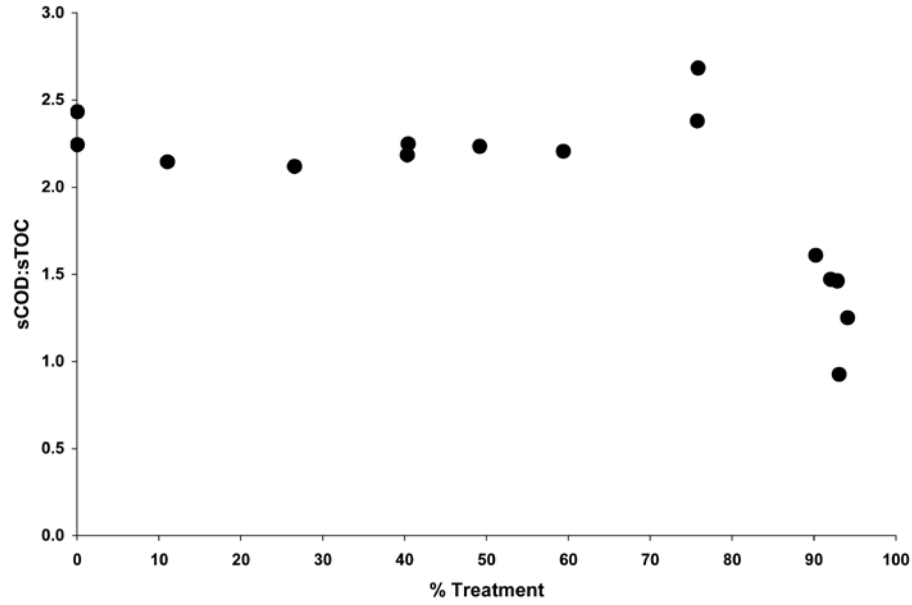


Figure 4. Oxidation state versus % treatment as sTOC.

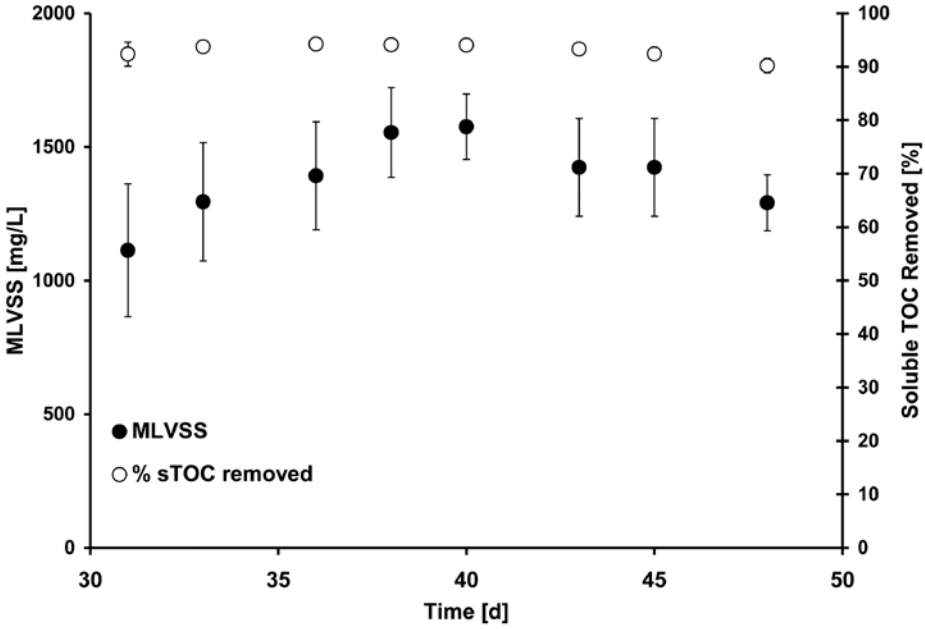


Figure 5. Microbial floc concentration and % soluble TOC treated (mean values ± standard errors) for the three SBRs used in trial four.

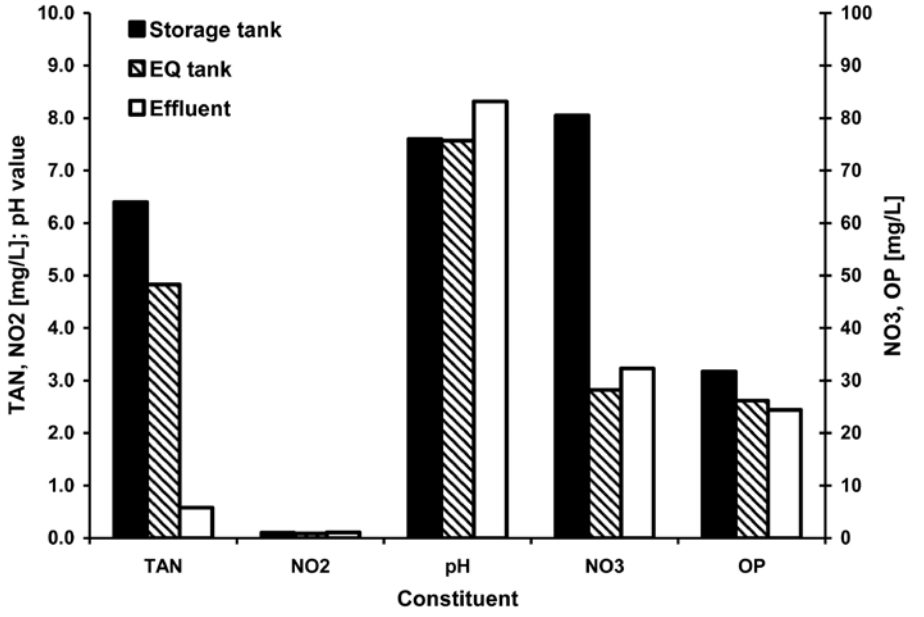


Figure 6. Mean constituent levels determined in storage tank, equalization tank, and effluent after SBR treatment in trial four.

Kinetic coefficients are reported in Table 2. No significant differences ($P > 0.05$) were reported between the anoxic/oxic and oxic yield coefficients values based on TOC and COD measurements. Correlations were strong for all determined normalized rate values; R^2 values were never less than 0.92. Even though the correlation rates were good for both first and second order rates, zero order rates exhibited slightly better fits.

Microbial floc characterization for microbial flocs and untreated solids are compared in Table 3. Protein levels, determined as crude protein or Lowry protein, were significantly higher ($P < 0.01$) in microbial flocs as compared to untreated solids. More specifically, crude protein and Lowry protein values of microbial flocs were, respectively, 95% and 69% greater than untreated solids. The organic fraction of microbial flocs was significantly greater ($P < 0.01$) than that of untreated solids. Some fungal growth was observed in the SBRs, but the amount was insufficient as to interfere with bioreactor operations.

Table 3. Characteristics of SBR microbial floc versus untreated solids.

	Biomass	**Untreated Solids**
SVI [ml/g]	129 ± 10.7	-
Crude protein [%]	54.4 ± 0.3	27.9 ± 1.5
Lowry protein [mg/g TSS]	40.2 ± 1.5	23.8 ± 2.5
Organic fraction [%]	89.1 ± 0.3	84.4 ± 0.1

DISCUSSION

The results suggest that operational inputs significantly influenced removal/treatment efficiencies, microbial floc production, and fungal development. Trial one treatment performance was likely limited by the low microbial floc concentration in the SBRs. Furthermore, the HRT of 24 h was perhaps too long and could have also contributed to these low efficiency levels. The microbial floc concentration could have theoretically been four times greater if the HRT was decreased to the levels (HRT of 6 h) used in trials two to four. This is based on mathematical relationships presented in Metcalf and Eddy (2003). Trial two was conducted to test this theory. This trial yielded similar results in terms of MLVSS concentrations. However, during this trial, the low microbial floc levels and highly variable treatment efficiencies observed could have been due to: (1) the tilapia wastewater not being fresh (it was used over the course of 7 days until a new batch was transported from

Blue Ridge Aquaculture, 130 km away), and/or (2) a low biodegradable fraction of sCOD that would need carbon supplementation (Metcalf and Eddy 2003, Avnimelech 1999, Ebeling et al. 2006). For this reason, trial three was conducted with carbon supplementation. This trial resulted in better treatment of sCOD and TAN than was seen in trials one and two. Microbial floc concentrations greater than 1,000 mg/L were also achieved. Even though this trial yielded desirable levels of treatment and microbial floc, fungi (Figure 2) populations began to proliferate on day 30 and eventually interfered with the decant cycle. This type of filamentous organism is not uncommon in aerobic systems when a readily degradable substance, such as a simple sugar, is being treated (Eckenfelder 2000, Elmaslar et al. 2004). Trials one through three were informative, but were not completely successful. However, trial four was more effective by improving nutrient removal and microbial floc production; this is a good foundation for future work.

Since there was a strong correlation between sCOD and sTOC (Figure 3), one constituent can be accurately estimated by measuring the other. Typically, a higher COD:TOC, means that more carbon is available for oxidation via heterotrophic microorganisms (Metcalf and Eddy 2003; Kleerebezem and Van Loosdrecht 2006). Plotting sCOD:sTOC versus percent treatment of sCOD (Figure 4) demonstrated the importance of this ratio, because this ratio was significantly reduced ($P < 0.05$) when the treatment was greater than 85%.

From personal experiences, bioreactors become stable when the reactor has been operated for a period of time, typically three to five times its average SRT. Therefore, during trial four, it was assumed that the three SBRs were stable after 30 days. This was verified by measuring the MLVSS concentrations and treatment performance from days 30 to 50 (Figure 5). As expected, there were no significant differences between MLVSS concentrations during this time period, and treatment of sTOC was consistently greater than 90%. Effluent concentrations of sCOD were calculated to be 20.6 ± 2.2 mg/L.

Total ammonia nitrogen is typically reduced in SBRs via assimilation by heterotrophic microorganisms as well as via oxidation by autotrophic microorganisms (Metcalf and Eddy 2003, Ebeling et al. 2006). Nitrite remained low, less than 0.11 mg/L in all stages. The pH increased after treatment in the SBRs. As expected, denitrification was only

accomplished during the anoxic portion of the treatment sequence because in the absence of oxygen, nitrate becomes the electron acceptor for microbial metabolism (Metcalf and Eddy 2003, Boopathy et al. 2005). Nitrate was reduced by 65% during the anoxic stage and increased by 5% during the aerobic phase. This increase in nitrate is due to oxidation of reduced nitrogen by autotrophic microorganisms.

The kinetic coefficients of microbial floc production and substrate removal presented in Table 2 are important because they help the operator understand how best to manage the systems as well as any additions of supplemental carbon. Yield coefficients represent the amount of microbial floc produced per unit of substrate consumed. Typically, operators should prefer low yield coefficients because they have to dispose of this sludge, which can be time consuming and expensive. However, in this case, a high yield coefficient is beneficial because the microbial floc can be used as a supplemental feed for shrimp culture, reducing the total amount of commercial feed required (Kuhn et al. 2008), or reducing fishmeal requirements in the diets (Kuhn et al. 2009). The anoxic/oxic yield coefficients in this study were not significantly different ($P > 0.05$) from the oxic yield coefficients. Typically, anoxic yield coefficients are significantly lower than aerobic yield coefficients (Metcalf and Eddy 2003).

Microbial floc growth rates (μ) of 0.27 ± 0.028 h^{-1} observed in this study (Table 2) were higher than those observed for treating aquaculture wastewater using molasses (0.10-0.12 h^{-1}, Schneider et al. 2006). This is because the granulated sucrose used in this study is readily biodegradable, while molasses is a more complex polysaccharide that is not as biodegradable (Najafpour and Shan 2003, Quan et al. 2005). Even though fungi (Figure 2) were observed in low numbers during trial four, they did not adversely affect treatment performance or reactor operation. Although uptake rates for both substrates related well to zero-order and first-order rate equations (Table 2), zero-order rates represented the data sets more accurately.

Microbial floc generated in the SBRs had significantly higher ($P < 0.01$) protein values compared to untreated solids. Furthermore, these microbial flocs are a combination of microorganisms and exocellular biopolymers. Biopolymers are a conglomerate of multivalent cations, polysaccharides, and proteins (Higgins and Novak 1997). Even though

the sludge volume indices were relatively high, they were not indicative of bulking because they weighed less than 150 ml/g microbial floc (Eckenfelder 2000).

CONCLUSION

Without carbon supplementation, removal of nutrients and production of microbial flocs were neither adequate nor sufficient. However, carbon supplementation with sucrose significantly improved nutrient removal and microbial floc production under these laboratory-scale conditions. Since the cost of marine and plant proteins have more than doubled since the 1990s (FAO 2007), developing a high quality, alternative ingredient for inclusion in shrimp feed is becoming increasingly important. Furthermore, the production of microbial flocs yields additional environmental benefits, in that using SBRs to treat a fish waste stream offers farmers a means to mitigate the cost and environmental impact of farm effluents.

ACKNOWLEDGEMENTS

The authors would like to acknowledge that funding for this study was provided in part by the United States Department of Agriculture Cooperative State Research Education and Extension Services (USDA-CSREES) and the Commercial Fish and Shellfish Technologies (CFAST) program at Virginia Polytechnic Institute and Sate University. The authors would also like to thank Blue Ridge Aquaculture Inc. (Martinsville, VA) for their support.

REFERENCES

AOAC **2003**. Official Methods of Analysis of AOAC International, 17th edition, 2nd revision. AOAC International, Gaithersburg, MD, USA.

APHA. **2005**. Standard Methods for the Examination of Water and Wastewater, 21st ed. Edited by Clesceri, Greenberg and Trussell. Washington, D.C., USA.

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

Boopathy, R., Fontenot, Q., Kilgen, M.B. Biological Treatment of Sludge from a Recirculating Aquaculture System using a Sequencing Batch Reactor. *Journal of the World Aquaculture Society* **2005,** 36:542-545.

Ebeling, J.M., Timmons, M.B., Bisogni, J.J. Engineering Analysis of the Stoichiometry of Photoautotrophic, Autotrophic, and Heterotrophic Removal of Ammonia-Nitrogen in Aquaculture Systems. *Aquaculture* **2006**, 257:346-358.

Eckenfelder, W.W. **2000**. Industrial Water Pollution Control, 3rd ed. Edited by Tchobanoglous. McGraw Hill. New York, NY, USA.

Elmaslar, E., Tufekci, N., Ovez, S. Aerobic Treatability of Fruit Juice Industry Effluents in Sequencing Batch and Activated Sludge Reactors. *Fresenius Environmental Bulletin* **2004,** 13:985-988.

Fraser, T.W.K, Davies, S.J. Nutritional Requirements of Cobia, *Rachycentron canadum* (Linnaeus). *Aquaculture Research* **2009** 40:1219-1234.

FAO **2002**. The State of World Fisheries and Aquaculture 2002. Food and Agriculture Organization of the United Nations. Rome, Italy.

FAO **2007**. Fishmeal Market Report – December 2007. By: Helga Josupeit, FAO Globefish. Food and Agriculture Organization of the United Nations.

URL: *http://www.thefishsite.com/articles/370/fishmeal-market-report-december-2007*

HACH **2007**. From *http://www.hach.com/*

Hargreaves, J.A. Photosynthetic Suspended-Growth Systems in Aquaculture. *Aquacultural Engineering* **2006**, 34:344-363.

Higgins, M.J., Novak, J.T. Characterization of Exocellular Protein and its Role in Bioflocculation. *Journal of Environmental Engineering - American Society of Chemical Engineers* **1997**, 123:479-485.

Kleerebezem, R., Van Loosdrecht, M.C.M. Waste Characterization for Implementation in ADM1. *Water Science and Technology* **2006**, 54:167-174.

Koonse, B. Seafood Safety: Down on the Farm (regulatory report). *Food Safety Magazine,* October/November **2006**, p. 5-8.

Kuhn, D.D., Boardman, G.D., Craig, S.R., Flick, G.J., McLean, E. Use of Microbial Flocs Generated from Tilapia Effluent as a Nutritional Supplement for Shrimp, *Litopenaeus vannamei*, in Recirculating Aquaculture Systems. *Journal of the World Aquaculture Society* **2008**, 39:72-82.

Kuhn, D.D., Boardman, G.D., Lawrence, A.L., Marsh, L., Flick, G.J. Microbial Floc Meal as a Replacement Ingredient for Fish Meal and Soybean Protein in Shrimp Feed. *Aquaculture* **2009**, 296:51-57.

Lunger, A.N., McClean, E., Gaylord, T.G., Kuhn, D., Craig, S.R. Taurine Supplementation to Alternative Dietary Proteins used in Fish Meal Replacement Enhances Growth of Juvenile Cobia (*Rachycentron canadum*). *Aquaculture* **2007**, 271:401-410.

McLean, E., Reid, B., Fegan, D., Kuhn D., Craig, S. Total Replacement of Fishmeal with an Organically Certified Yeast-Based Protein in Pacific White Shrimp (*Litopenaeus vannamei)* Diets: Laboratory and Field Trials. *Ribarstvo* **2006**, 64:47-58.

Menasveta, P. Improved Shrimp Growout Systems for Disease Prevention and Environmental Sustainability in Asia. *Reviews in Fisheries Science* **2002**, 10:391-402.

Metcalf, Eddy. **2003**. Wastewater Engineering: Treatment and Reuse, 4th ed. Edited by Tchobanoglous, Burton, and Stensel. McGraw Hill. New York, NY, USA.

Najafpour, G.D., Shan, C.P. Enzymatic Hydrolysis of Molasses. *Bioresource Technology* **2003**, 86:91-94.

Quan, Z-X., Jin, Y-S., Yin, C-R., Lee, J.J., Lee, S-T. Hydrolyzed Molasses as an External Carbon Source in Biological Nitrogen Removal. *Bioresource Technology* **2005**, 96:1690-1695.

Schneider, O., Sereti, V., Eding, E.H. Molasses as C Source for Heterotrophic Bacteria Production on Solid Fish Waste. *Aquaculture* **2006**, 261:1239-1248.

Skjølstrup, J., McLean, E., Nielson, P.H., Frier, J-O. The Influence of Dietary Oxolinic Acid on Fluidised Bed Biofilter Performance in a Recirculation System For Rainbow Trout, *Oncorhynchus mykiss*. Aquaculture **2000**, 183:255-268.

Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T., Vinci, B.J. **2002**. Recirculating Aquaculture Systems, 2nd ed. Cayuga Aqua Ventures. New York, NY, USA.

Water Quality in Identical Recirculating Systems Managed by Different Aquaculturists

K. Hanna[2], F. Wheaton[1], A. Lazur[3], S. VanKeuren[2]

[1]Environmental Science and Technology Department
University of Maryland
College Park, MD 20742, USA

[2] Biological Resources Engineering Department
University of Maryland
College Park, MD 20742, USA

[3] Center for Environmental Science
University of Maryland
Cambridge, MD 21613, USA

*Corresponding author: *fwheaton@umd.edu*

Keywords: Recirculating systems, system management, tilapia culture, water quality, management records

ABSTRACT

Water quality in recirculating aquaculture systems is a function of many variables including system design, loading, and management; temperature; feeding rate, and other variables. This research attempted to determine how different managers' management practices affected system water quality when the managers were using identical production systems. Water quality was monitored in two tanks on each of three farms, and an attempt was made to correlate management practices with the resulting tank water quality. The investigators worked with farm managers to collect as much data as possible about the management practices of each manager, economic data, when fish were placed into the tanks and when they were harvested, growth rates and other information. The resulting analysis proved there is great variation in water quality parameters in individual tanks both between farms and within a farm.

International Journal of Recirculating Aquaculture 11 (2010) 55-74.

The study showed that management of aquaculture systems had a strong influence on tank water quality. Operational data on economics, filter cleanings, fish growth and other information proved to be difficult to obtain as the managers did not keep detailed records of many of these variables. As a result, it was not possible to relate water quality to economics of the farm. It was apparent that good records are necessary for an aquaculture production facility if the operation is to be successful.

INTRODUCTION

Recirculating aquaculture systems are used throughout the United States and the world. Although economic considerations are a concern with recirculating systems, interest has remained high because of their potential benefits. System benefits include: 1) minimum water use that enables aquaculturists to raise salt water fish inland or increase the carrying capacity of a fixed water flow rate, 2) control of market timing and product size; 3) higher quality and/or more consistent quality of the product; 4) ability to produce aquatic products free from contamination by heavy metals, toxic organic compounds, and other potential toxins, 5) year-round production, and 6) the ability to satisfy markets requiring a continuous supply. Tremendous emphasis has been placed on the engineering aspects of these systems including; bio and mechanical filtration, circulation, oxygenation, heating and the like, in order to maintain high stocking densities, and make efficient use of energy and material inputs. However, a critical parameter whose importance has often been overlooked, is system management. It is likely that management practices are as important in determining the profitability of a recirculating aquaculture venture as the system design and equipment. Studies have shown how water flushing rates affect fish health (Davidson et al. 2009), and explored the effect of feed quality or feed content on water quality (Jisa et al. 1997). Unfortunately, there is little documentation on the qualitative and economic effects of various management practices on recirculating aquaculture system performance.

The study described herein attempted to determine the effects that three different management strategies (e.g. biomass or stocking densities, feed inputs, and water exchanges), have on recirculating system operation, maintenance, and profitability. These three parameters play a significant role in determining the profitability of an aquaculture operation and the overall water quality in the system.

Efficient use of the systems necessitates that biomass levels are kept at or near system capacity. Operating systems below their biomass capacity limits output and distributes capital (and in some cases, operating costs) over a smaller number of production units (fish). Production costs per unit (by weight) increase and profitability drops. Maintaining optimal biomass levels requires constant harvesting or transfer of fish from tank to tank as the fish grow. Handling increases the risk of injury, stress, and bacterial and fungal infections in the livestock; factors that can increase the risk of high mortalities and reduce growth rates. Lower biomass levels make it easier to maintain high water quality levels and fish health, and thereby reduce the risk of system failure. These factors must be continually balanced in management of recirculating systems.

Controlling the feed rate is an important management practice as it directly affects water quality and fish growth. The recommended feed rate varies between 1.5 and 15% of biomass weight per day depending on the stage of growth and the species of fish cultured (Losordo et al. 1992). Feed rates are maximized to maintain high growth rates, however waste production is directly proportional to feeding rates and feed quality. Higher waste production leads to lower water quality, which can impair growth.

The third management practice of importance in this study is that of water exchange frequency. Recirculating aquaculture systems are most often used when water supply is limited (Losordo et al. 1992). Recirculating systems offer an alternative to pond systems, typically using less than 10% of the water required in pond operations at an equivalent production level. Therefore, the conservation of water is one of the primary advantages of recirculating systems. Most recirculating systems are designed to replace no more than 5-10% of the system volume each day (Masser et al. 1999). These systems require constant filtration to maintain the high water quality standards needed for proper fish health. Higher water exchange rates reduce the need for filtration, however, the trade off is lower water use efficiency.

Each of these three management components (stocking density, feed rationing, and frequency of water exchange) have direct economic consequences. The costs of these management variables should be weighed against the resulting economic profitability. Unfortunately, clear cost-benefit analysis is often difficult to perform due to a lack of concrete

data. This study looked at the effects of these three management factors on a wide range of measurable water quality variables, which have a direct impact on the health and growth of the fish and the quality of the fish produced.

METHODS AND MATERIALS

Aquaculture system

The aquaculture systems used at the three facilities involved in this study were engineered and manufactured by the same manufacturer, to the same specifications. The system was designed by Rick Sheriff (formerly of Opposing Flow Technology, Inc.) and is often referred to as the 'Sheriff Tank' (Figure 1).

Figure 1. Sheriff tank in operation at an aquaculture production facility.

Although the tanks can be constructed of aluminum or fiberglass, all tanks used in this study were aluminum and were operated by a regenerative blower air source. Air is introduced along the bottom of both long sides of the tank causing a flow upward along the outer tank walls, horizontally across the top of the tank, downward near the center of the tank, and outward along the bottom of the tank, enabling solids to migrate to openings positioned along the tank side and bottom juncture for collection in the biofilter section of the system. Thus, there are two circular flows across the cross section of the tank. In addition, water is drawn from one end of the tank, pumped through the filter on the other end of the tank, and returned to the tank on the end opposite the outlet. This causes a slow flow along the primary tank axis. The result of these two flow systems is two side-by-side helical flows in the tank with a slow movement along the axis of the helix and a more rapid flow around the two helixes. The tanks are thus completely and continuously mixed.

Air lift pumps are used to drive flow through the filters. The filters consist of a settling system and a biofilter. Many materials could be used for the filter media, but the Sheriff design uses PVC shavings such as are produced when turning a circular piece of PVC in a lath. The primary maintenance of the filters is to drain the filter section of the tank, wash it down to flush out the solids, and refill it with water. The tank and filter hold about 37,800 L (10,000 gallons) of water with the filter containing about 7,560 L (2,000 gallons) depending on the water depth in the tank. Due to incomplete draining of the filter during cleaning the system requires about 3,780 L (1,000 gallons) of replacement water after each cleaning. Design biomass for a fully loaded tank is about 2272 kg (5,000 pounds) of fish.

All farms included in this study grew tilapia, and each relied on ambient temperatures to regulate tank water temperature. Each farm used solid commercial feed pellets from different manufacturers, and included aquaculture as a part of their larger farm production. Because all farms used the same system hardware, any variation in water quality and economic profitability is attributable to differences in management practices at each of the recirculating aquaculture facilities. It was hoped, therefore, that a close examination of the operation of each of these facilities would shed light on critical management practices that make or break recirculating aquaculture production facilities, or alternatively show which practices had little effect on the economic viability of the operation.

Data collection

The study began with two commercial facilities, one of which ended production and went out of business halfway through the study. As a result, a third farm under different management was added to the study. Water quality parameters were measured and recorded on a weekly basis, but records of the daily management practices maintained by the farm managers were sparse and insufficient to meet the needs of the study. This "daily management" data included the time, frequency and volume of water exchange; daily feeding rate over time; addition of pH adjustment inputs; fish harvest quantities and dates, and biomass of the fish in the tanks over time; sale prices of the harvested product; cost and number of fingerlings added; and operational costs.[1]

[1] Operating costs were often combined with other operations on the farm.

Biomass data was available only periodically resulting in insufficient data being available to carry out an analysis. Thus, biomass values were estimated assuming a linear growth rate of 0.25 lbs/mo after a size of 0.5 lbs had been reached. The fish were purchased as fingerlings. It was assumed that the fish reached a size of 0.5 lbs after five months from time of purchase. The farm periodically recorded dates and quantities of fish harvested and the size of the fish, which allowed us to estimate the total biomass in each tank at that point in time. The data allowed the predicted growth rates to be checked against real data to ensure they were reasonable. These checks showed that the predicted growth rates were reasonable but considerable variation between predicted and actual weight data was apparent from tank to tank. The variation could have been due to incorrect weight data being reported or model prediction error. Daily feed rates were recorded by the farm manager as well as the number of filter cleanings involving water exchange. The amount of water exchanged with each filter cleaning was not always the same, but limited data required this assumption in order to get water exchange data.

Feeding, biomass and growth rates were collected from the farm mangers when the data was available. Weekly water quality parameters were measured by the project team from two tanks from each farm on a weekly basis. Measured water quality parameters included dissolved oxygen (DO), total solids (TS), ammonia, nitrate, nitrite, phosphate, pH and conductivity. Samples were taken from two tanks at each of three farms, for a total of six tanks. The sampling period for Farm 1 was between June 13, 2003 and October 30, 2003; for Farm 2 between February 13, 2004 and May 21, 2004; and for Farm 3 between July 24, 2003 and May 21, 2004.

Water quality

Alkalinity measurements followed the titration method outlined in Method 2320 (APHA 1995). Dissolved concentration of ammonia was measured using a Hach spectrophotometer model 4000, following Hach standard method 8038 for ammonia NH_3-N, which used the Nessler reagent, a corrosive oxidizer. Nitrite concentrations were measured using a Hach 4000 spectrophotometer (Hach, Loveland, CO, USA), following the Hach method 8507 (Hach 2000). Nitrate concentrations were measured using a Hach 4000 spectrophotometer, following Hach method 8039 (Hach 2000). Phosphate concentrations were measured

using a Hach spectrophotometer (model 4000). Phosphorous values were measured in mg/L of phosphate (PO_4^{-3}). For samples made between the beginning of the study and August 28, 2003, Hach method 8048 was used. From September 5, 2003 until the end of the study, the method was changed to Hach method 8114, using molybdovanadate as a reagent (Hach 2003). Nearly all of the dissolved forms of phosphorous exist in solution as phosphates (APHA 1995). As with the nitrogen sample measurements, samples had to be diluted, as phosphorous levels were out of range for the Hach method employed.

When feasible, dissolved oxygen was measured promptly after the sample was taken. When not feasible, the sample bottle was filled completely and dissolved oxygen was measured within a few hours using a YSI® Model 55 Dissolved Oxygen Meter (YSI, Inc., Yellow Springs, OH, USA). All conductivity measurements were made using the YSI® Model 55, multi-meter. All sample pH readings were obtained directly using a Jenco® (Model 6071, Jenco Instruments, San Diego, CA, USA) pH meter and electrode. Total solids concentrations were determined using the method prescribed in the Section 2540B of Standard Methods (APHA 1995). Turbidity was measured using a Hach Portable Turbidity Meter (Model 2100P, Hach, Loveland, CO, USA) using a 'Ratio Optical System' (Hach 1998).

Statistical Analysis

The water quality parameters listed above were compared between each farm to determine if a qualitative difference existed between them that could be attributed to management practices. A regression was performed between each of these water quality parameters and the three independently measured management practices. These management practices include biomass, feed rate and water exchange rate. This analysis was conducted only on data obtained from Farm 3, as this was the only farm in the study that supplied sufficient information to conduct this analysis. Farm 2 and Farm 3 had shortened data collection periods that did not provide sufficient data due to Farm 1 going out of business. Regression analysis was conducted using the statistical analysis software package SAS version 8.0, using the MIXED procedure for ANOVA in SAS.

A second analysis of variance was performed comparing data between tanks. This analysis was to evaluate the variation in the different water quality parameters across all of the tanks sampled on the three farms.

RESULTS AND DISCUSSION

Table 1 gives the ranges of water quality parameters recorded for this study and the range of mean water quality parameters in individual tanks. These ranges were quite wide and reflected the lack of control of water quality parameters in the systems.

Oxygen, usually the most critical factor in recirculating culture systems, ranged from 1.8 to 9 mg/L in the tanks. Mean concentrations by tank ranged from 4.75 to 7.38 mg/L, while the standard error of the mean for the tanks ranged from 0.20 to 0.49. Rakocy (1989) recommends oxygen concentrations for tilapia remain above 5 mg/L; tilapia are well known to be able to tolerate lower oxygen concentrations. In those few instances where oxygen concentrations dropped below 4 mg/L in the tanks, the fish could have experienced some stress. Because there were no mass mortalities in any of the tanks monitored, the low oxygen did not appear to be fatal but could have caused some stress in the fish.

The pH values ranged from a low of 6.3 to a high of 8.5. The mean tank pH ranged from 7.03 to 7.51 while the standard error of the mean varied from 0.0615 to 0.0978. All pH values were within the tolerance range for tilapia and thus were not considered to be causing significant stress for the fish.

Total ammonia concentrations (TAN) are an important consideration because the unionized fraction (NH_3) is toxic to fish. Total ammonia concentrations in the tanks varied from essentially zero to a high of

Table 1. Variation in range of water quality parameters analyzed in this study.

Water Quality Parameter	Variation Range	Mean Tank Range
Total Ammonia Concentration (TAN)	0 – 17 mg/L	1.9-3.6 mg/L
Nitrate Concentration (NO_3)	0 – 180 mg/L	97 – 180 mg/L
Nitrite Concentration ($NO_{2)}$	0 – 7 mg/L	0.38 – 2.4 mg/L
Phosphate Concentration (PO_4)	0 – 180 mg/L	34 – 84 mg/L
Dissolved Oxygen Concentration	2 – 9 mg/L	4.75- 7.38 mg/L
Total Solids Concentration	300 – 3100 mg/L	670 – 1500 mg/L
pH	6.0 – 8.5	7.03 – 7.51

17 mg/L. Mean values varied from 1.9 to 3.6 mg/L while the standard error of the mean varied from 0.18 to 0.64. This suggests that the very high ammonia concentrations were of short duration and were not generally a continuing problem. However, even short duration spikes can create stress and reduce growth rates and/or lead to disease outbreaks a few days after exposure. Rakocy (1989) gives the upper ammonia tolerance for tilapia as 2 mg/L of NH_3-N, but Chapman (1992) suggests a limit of 1 mg/L of TAN (total ammonia nitrogen) as the upper limit for the culture of tilapia. Using Rakocy's values and converting this 2 mg/L of NH_3–N to total ammonia at a pH of 7.5 and 23°C gives a limit of approximately 115 mg/L total ammonia (TAN). At a pH of 8.0 and the same temperature the equivalent total ammonia is 37 mg/L and at a pH of 8.5 it is 13 mg/L. For the ammonia conditions measured in the tanks, high stress would only be caused when pH values approaching 8.5 were accompanied by some of the higher ammonia levels recorded. However, if Chapman's suggested limit is used, the fish experienced considerable stress throughout the data collection period. Insufficient data are available to determine if the fish in this study were stressed or not.

Nitrite concentrations (NO_2) in the tanks generally remained below 2.5 mg/L except in two cases when nitrite concentrations reached 7 and 4 mg/L, respectively. The mean nitrite concentrations in the tanks ranged from 0.38 to 2.4 mg/L with the standard error of the mean ranging from 0.038 to 0.65. Rakocy (1989) states that tilapia begin to die when nitrite concentrations reach 5 mg/L as NO_2-N. Because there were no die offs in the two tanks having 7 and 4 mg/L of nitrite, the fish appear to be able to tolerate higher nitrite concentrations, at least for short time periods and at the pH experienced in the tanks. There is a good chance that the fish experienced stress at these high levels, but there was no negative result measured in the data collected.

Nitrate (NO_3) is relatively less toxic than nitrites to fish, but can be toxic at higher concentrations (e.g. 400 mg/L or higher, Timmons et al. 2001). Nitrate concentrations in the tanks ranged from essentially zero to 320 mg/L. The mean values of nitrate concentrations for the tanks ranged from 97 to 180 mg/L, while the standard error of the mean ranged from 7.3 to 14. None of these concentrations should create fish stress. Water changes were used by the aquaculturists to limit nitrate concentrations.

Phosphate (PO_4) concentrations are not normally considered to be toxic to fish in recirculating systems. It was monitored in this study primarily to determine the phosphate concentrations in wastewater from these systems. Because there was no usable method of measuring the solids lost during filter washing, it was not possible to develop either a nitrogen or a phosphorous balance for the systems. Thus, the phosphate concentrations measured were concentrations in the culture water. Considerable variation in the phosphate concentrations in the water were observed varying from 1 or 2 to over 170 mg/L of phosphate. Mean concentrations in the tanks varied from 34 to 84 mg/L while the standard error of the means varied from 3.2 to 10.

System alkalinity was controlled by the aquaculturists, usually by adding sodium bicarbonate or some other base. The base was added manually and periodically, and one system used a slow injection that was manually controlled. Alkalinity varied from 25 to over 360 mg/L as $CaCO_3$. The mean values for the various tanks varied from 88 to 220 mg/L as $CaCO_3$ while the standard error of the means varied from 12 to 22. Most authors recommend alkalinity in recirculating systems should be maintained above 50 to 100 mg/L as $CaCO_3$. Chapman (1992) gives an acceptable alkalinity for tilapia as 50 to 700 mg/L. Although the alkalinity was relative low at times in some tanks it does not appear to be a major problem in the systems as pH did not suddenly drop.

Turbidity values ranged from 1 to 79 NTU with the mean values varying from 7.80 to 43.3 NTU. The standard error of the mean for turbidity varied from 0.668 to 5.31. Although this is considerable variability, it is within the acceptable range for tilapia.

Conductivity data is not normally a consideration in fish culture, except as an indirect measure of salinity. Conductivity values over the course of the study did not appear to be out of the reasonable range for these freshwater fish. Thus, salinity was not a limiting factor in these studies.

Total solids ranged from 3,100 to a low of about 300 mg/L. The mean values for total solids for the tanks varied from 670 to 1,500 mg/L while the standard error of the mean varied from 39 to 200. Chapman (2000) suggests that total solids be maintained between 25 and 100 mg/L. However, this recommendation is based on what is desirable and may not reflect the acceptable tolerance limits for tilapia. The effect of solids

on fish is mostly related to negative consequences resulting from gill irritation. The type of solids (e.g. silt or organic material) and several other variables affect the concentration of solids the fish can tolerate. In this study, no obvious negative effects were evident from high solids concentrations, and no gill tissues were assayed.

The weekly water quality values varied widely. The analysis of variance results verified this observation, showing a significant difference (at the 0.05 level) between tanks in the values obtained for all water quality parameters measured with the exception of ammonia (Table 2). This indicates the significant impact that management practices have on water quality, given that each tank was identical. Each aquaculturist managed his individual tanks approximately the same. However, each of the farmers had different management methods, most of which varied with time. The ultimate result is an understanding that the management of a recirculating system may be every bit as important as good system design, and possibly more so.

Regression curves were drawn plotting measured water quality parameters with each of the three management practices emphasized in this study: biomass, feed rate, and water exchange rate. Some trends were observed, although the high variability produced relatively low

Table 2. ANOVA ('MIXED' procedure) results on water quality parameters for the three farms operated using the same tanks but different managers.

ANOVA:	Numerator DF	Denominator DF	f-value	Probability	Significance
pH	5	144	4.43	0.0009	Significant
Nitrate	5	138	7.04	<0.0001	Significant
Nitrite	5	142	8.95	<0.0001	Significant
Phosphate	5	137	5.63	<0.0001	Significant
Ammonia	5	137	2.19	0.0584	Not Significant
DO (mg/L)	5	108	6.95	<0.0001	Significant
Total Solids	5	118	7.88	<0.0001	Significant
Alkalinity	5	110	5.65	0.0001	Significant
Conductivity	5	119	5.41	0.0002	Significant
Turbidity	5	142	33.64	<0.0001	Significant

R^2 values, leaving few statistically significant regressions. Table 3 shows the significance of the regression coefficients for all regressions. A projected growth curve was used to estimate biomass data between measurements, however, limited or missing biomass removal data made this sort of assessment difficult. When comparing biomass data, where it was available, to the model growth rate, the model biomass values were slightly higher. An economic analysis was not attempted due to lack of sufficient economic data. The collection of this sort of data was complicated by the fact that in each case the aquaculture production was a part of a larger agricultural enterprise. Therefore, labor and operating cost could not be accurately separated for each of the components of the farm.

Table 3. Significance of regression parameters analysis of tanks 1 and 2 of Farm 3 for biomass, feed rate and water exchange rate. All values less than 0.05 are significant.

Regression Parameter	**Biomass**		**Feed**		**Water Change**	
	Tank 1	**Tank 2**	**Tank 1**	**Tank 2**	**Tank 1**	**Tank 2**
pH	0.0492	0.3023	0.1755	0.0007	0.9832	0.8175
Nitrate	0.2491	0.7930	0.0920	0.1413	0.6142	0.8714
Nitrite	0.0005	<0.0001	0.0003	0.2125	0.0878	0.6985
Phosphate	<0.0001	<0.0001	<0.0001	0.0158	0.1524	0.0923
Ammonia	0.0150	<0.0001	0.0323	0.3102	0.3101	0.8891
Dissolved Oxygen	0.0023	0.0096	<0.0001	0.5175	0.4915	0.3108
Total Solids	0.0006	0.0210	0.0001	0.0313	0.9832	0.8081

Figures 2-6 show the regression of each of the water quality parameters versus biomass, feed rate, and water exchange rate for the data for Farm 3, as this was the only farm in the study that supplied sufficient information on biomass levels, feeding rates, and water exchange rates to conduct this sort of analysis. Only those variables found to have a significant regression (slope greater than zero) against any of the three primary management indicators were plotted. Figure 2 presents a regression plot for the total solids versus feed; while Figures 3-6 present regression plots of nitrite, phosphate, dissolved oxygen and total solid concentrations versus biomass, respectively. The aquaculturist from

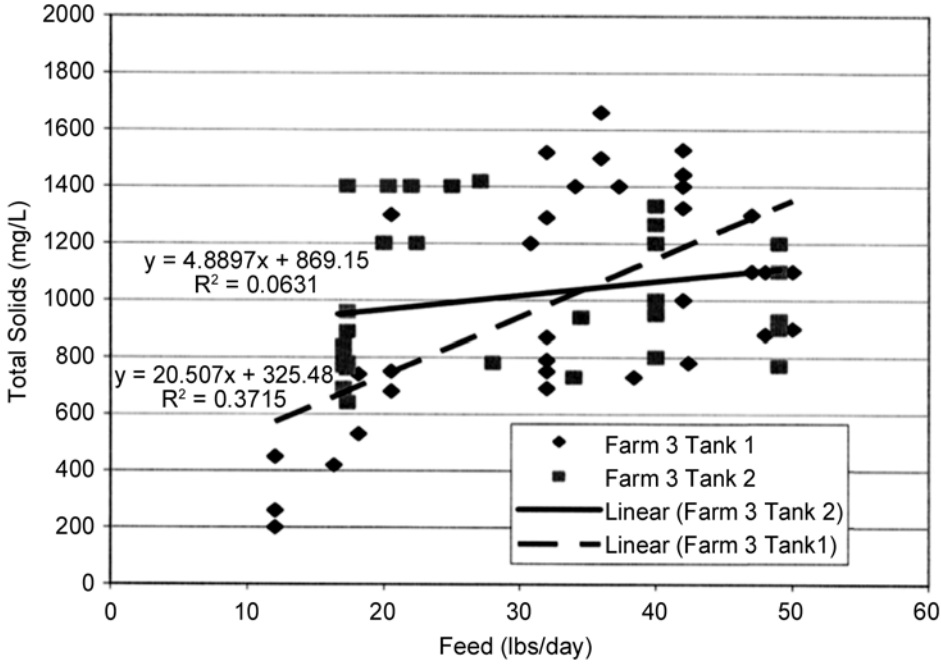


Figure 2. Regression of total solids versus feed for Farm 3.

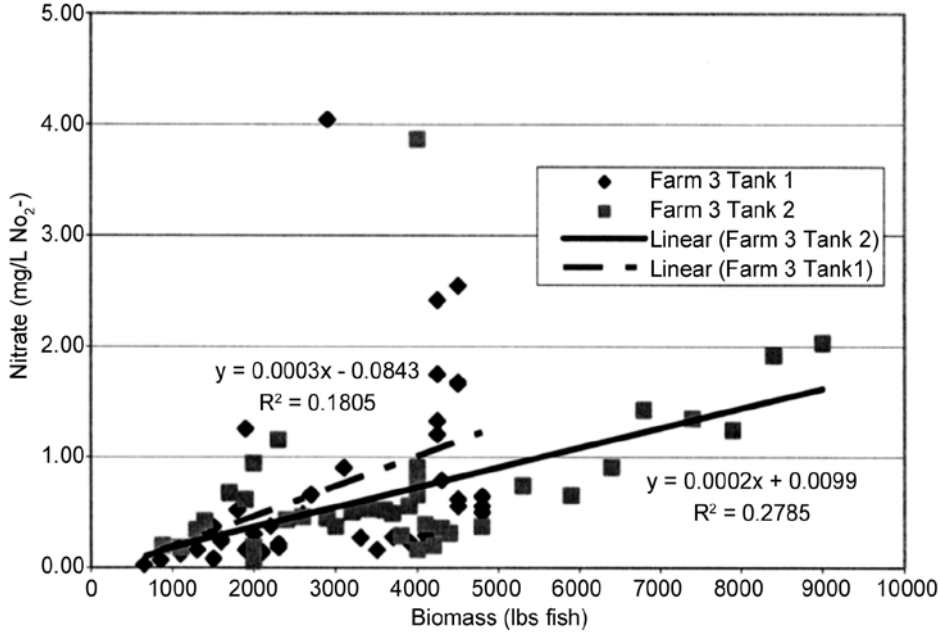


Figure 3. Regression of nitrite versus biomass for Farm 3.

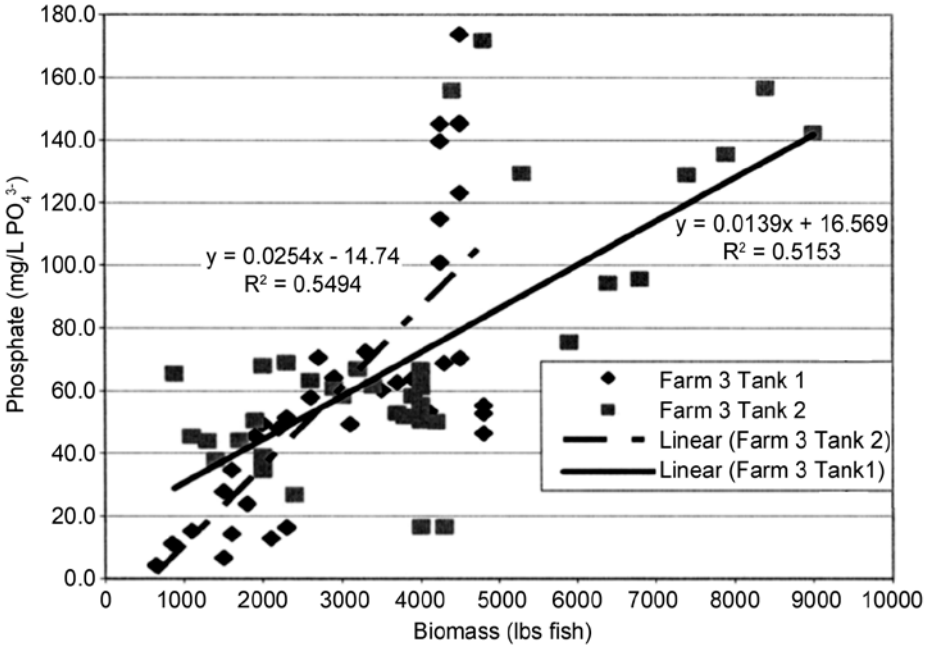


Figure 4. Regression of phosphate versus biomass for Farm 3.

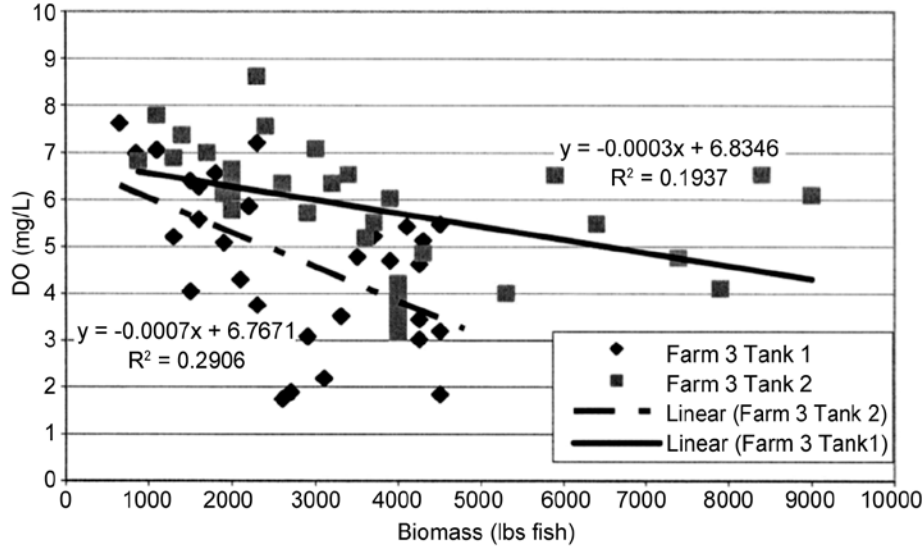


Figure 5. Regression of dissolved oxygen (DO) versus biomass for Farm 3.

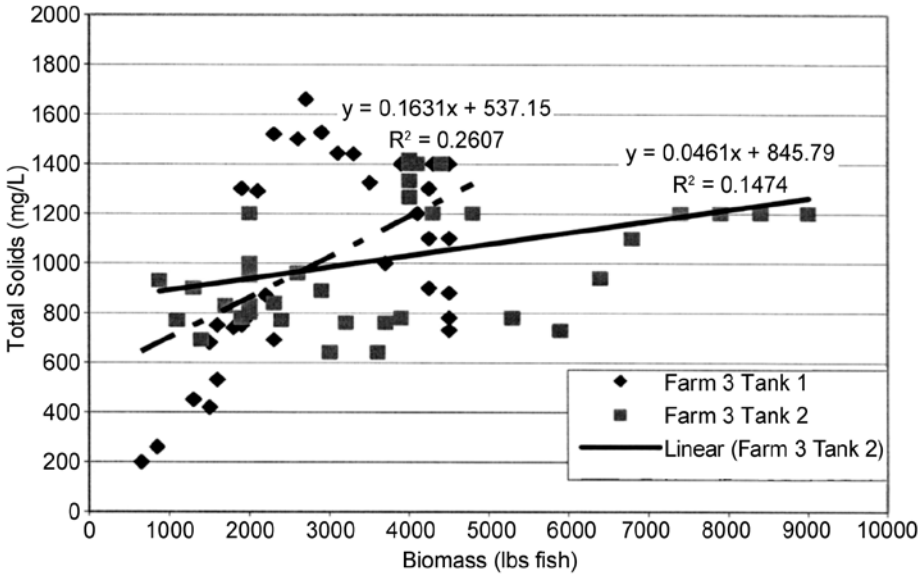


Figure 6. Regression of total solids versus biomass for Farm 3.

Farm 3 believed he was managing both tanks 1 and 2 in the same way. The data, however, suggested there were differences in what was happening in the two tanks, but it was not possible to quantitatively define these differences.

Biomass was shown to have a significant impact on all measured water quality parameters, with the exception of nitrate. Feed was shown to have a significant impact on all water quality parameters in at least one of the two tanks from Farm 3. This is not surprising when one considers that feed is the primary cause of water quality impairment, and is directly tied to the stocking density of each tank. The farms did manage biomass when it became too high, but management was more in response to necessity than following a systemic plan.

In contrast, water exchange rate was found to have no significant impact on any of the water quality parameters measured in this study. This may be due to the relatively consistent frequency and quantity in which water changes occurred resulting in a very narrow range of exchange volumes to which water quality parameters could be compared.

It has been emphasized above that profitability of recirculating systems depends on maintaining stocking densities as close to system capacity as possible, essentially 100 percent of the time. Tank biomass recorded

in this study varied from less than 454 to over 4090 kg (1,000 to over 9,000 pounds), with the upper end probably being an overestimate of production because the tanks' carrying capacity was between 2,272 and 2,727 kg (5,000 and 6,000 pounds). A highly variable biomass in the production tanks shows that the tanks were often operated well below capacity. Because tank depreciation and operating costs are virtually the same for both high and low stocking densities, it is most cost effective to operate tanks at or near capacity in order to lower per unit costs. Stocking densities beyond tank capacity will lead to higher waste production and oxygen consumption, ultimately leading to reduced fish health and growth and increased mortality, negatively affecting production.

Maintaining stocking densities at or near capacity throughout the year requires the farm manager to continually add or remove fish as the fish grow and are harvested. This is labor intensive and requires careful planning and record keeping. In addition, handling the fish also increases fish stress levels and increases the risk of disease and mortality. Ideally, data on biomass levels would be linked to production in order to determine the optimal biomass level based on economic considerations. However, in this study it was impossible to obtain adequate financial records, or distinguish the production costs of the aquaculture tanks from the rest of the farm facility.

Feed is closely tied to biomass but is distinguished from it in that feed levels must be balanced against the need to not feed excessively, resulting in higher waste loadings, and the need to maintain high growth rates. For both tanks on Farm 3, phosphate and total solids were significantly affected by feeding rate, increasing with increasing feeding levels, while pH, nitrate, nitrite, ammonia and dissolved oxygen were found to be significantly affected in one or the other of the two tanks studied. Dissolved ion levels were found to increase with increasing feed, while oxygen levels were lower when associated with higher feeding rates, as would be expected given the microbial degradation of suspended particles.

Throughout this study water exchange frequency (frequency of cleaning the filter) was not found to significantly impact any of the water quality variables measured. This may be due in part to two reasons. First, it is apparent that other factors, such as feed rate and biomass, had a more

significant impact on water quality, which may have overshadowed the effects of water exchange. Secondly, the water exchange frequency data was available for only one farm, or rather two tanks under the same farm manager. As a result, the frequency of water exchange was fairly consistent as determined by the habits, standards and practices of the farm manager. To more clearly define a regression for each of the water quality parameters and the water exchange frequency it would be necessary to compare systems with widely different water change frequencies in order to more easily define regression variables.

CONCLUSIONS

Overall, the study shed light on significant differences between water quality parameters and current management practices of various aquaculturists. Consistent with management practices and attitudes, it was also found that farm managers varied significantly in their approach to recordkeeping, as well as in the detail and reliability of the information contained therein. If these management variables are to be properly evaluated, it is necessary that similar studies be conducted under more controlled conditions with accurate and detailed records being maintained at all times. Likewise, it is critical that these studies be linked with actual production costs and the ultimate yield or profit from said production, under similar circumstances and market conditions. The three aquaculturists participating in this study did not keep detailed records of their production variables or of their costs and expenses. Therefore inadequate records prevented historical tracking of costs, income and profitability; or improvements in management practices of the enterprise.

Although much of the data needed to draw definitive conclusions regarding the role of management practices on specific water quality variables were sporadic, the study demonstrates the value and necessity of proper management practices. It was determined that nitrite, ammonia, and phosphate concentrations increase with increasing biomass levels within the fish tanks, which is directly correlated to feeding levels. Conversely, dissolved oxygen levels tend to decrease with increasing biomass or feeding levels. No statistically significant correlations were observed between water exchange volumes and the water quality variables measured in this study. With the single system type employed

in this study proper management appears to be as important, if not more important, than the system hardware itself, and is a must for any recirculating system to function properly and be economically viable.

Along with good management comes proper and accurate record keeping, which is an absolute must if any aquaculturist wishes to be successful and profitable. To achieve high productivity, recirculating aquaculture systems must be optimized. Optimization can be defined as the highest productivity attainable given the limitations of the system; therefore, an optimized system is, by definition, a system of checks and balances that can only be achieved through proper management and accurate recordkeeping.

ACKNOWLEDGEMENTS

This project was funded by the Maryland Agricultural Experiment Station and the University of Maryland. The authors wish to express their appreciation to the three commercial aquaculture farmers that generously allowed the research team to sample their systems. Without their cooperation this project would not have been possible.

REFERENCES

APHA **1995**. Standard Methods for the Examination of Water and Wastewater, 19th ed. American Public Health Association: Washington, D.C., USA.

Chapman, F.A. **2000**. Culture of Hybrid Tilapia: A Reference Profile. Circular 1051. Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL, USA.

Davidson, J., Good, C., Welsh, C., Brazil, B., and Summerfelt, S. **2009**. Heavy Metal and Waste Metabolite Accumulation and their Potential Effect on Rainbow Trout Performance in a Replicated Water Reuse System Operated at Low or High System Flushing Rates. *Aquacultural Engineering* 41:136-145.

Hach Company **1998**. Portable Turbidity Model 2100P Instrument and Procedure Manual. Publication Cat. No. 46500-88. Loveland, CO, USA.

Hach Company **2000**. DR/2010 Spectrophotometer Handbook. Loveland, CO, USA.

Hach Company **2003**. DR/4000 Spectrophotometer Handbook. Loveland, CO, USA.

Jirsa, D.O., Davis, D.A., and Arnold, C.R. Effects of Dietary Nutrient Density on Water Quality and Growth of Red Drum, *Sciaenops ocellatus,* in Closed Systems. *Journal of the World Aquaculture Society* **1997**, 28(1):68-78.

Losordo, T.M., Masser, M.P., and Rakocy, J. **1992**. Recirculating Aquaculture Tank Production Systems: An Overview of Critical Considerations. SRAC Publication No. 451. Southern Regional Aquaculture Center. Mississippi State, MI, USA.

Masser, M.P., Rakocy, J., and Losordo, T.M. **1999**. Recirculating Aquaculture Tank Production Systems Management of Recirculating Systems. SRAC Publication No. 452. Southern Regional Aquaculture Center. Mississippi State, MI, USA

Rakocy, J.E. **1989**. Tank Culture of Tilapia. SRAC Publication 282. Southern Regional Aquaculture Center, Mississippi State, MI, USA.

SAS **1999**. SAS Online Documentation: Version 8. http://v8doc.sas.com/sashtml. SAS Institute Inc. Cary, NC, USA.

Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T., and Vinci, B.J. **2002**. Recirculating Aquaculture Systems. Northeastern Regional Aquaculture Center Publication No. 01-002. Cayuga Aqua Ventures, Ithaca, NY, USA.

BOOK REVIEW

Species and System Selection for Sustainable Aquaculture

Edited by PingSun Leung, Cheng-Sheng Lee, Patricia J. O'Bryen

Wiley-Blackwell, Hoboken, NJ, USA (2007)
528 pages, hardcover, ISBN: 978-0-8138-2691-2

Reviewed by:
Michael H. Schwarz, Ph.D.
Adjunct Assistant Professor
Department of Fishery and Wildlife Sciences
Aquaculture Specialist, Virginia Seafood Agricultural Research and Extension Center
102 S. King Street
Hampton, VA 23669 USA

As the global demand for aquacultured products continues to rise, so does the need for social, economic, and environmental sustainability. As this expansion progresses, established species under production are augmented with new and emerging species providing some "perceived" enhanced value. Likewise, both established and new production systems and technologies are continually undergoing a process of review and optimization. Given the ongoing and predicted expansion of global aquaculture, sustainability in all its forms is of paramount concern.

This book is a synthesis from review papers presented at a NOAA-funded workshop held in October of 2005. This workshop was organized by the Aquaculture Interchange Program, and held at the Oceanic Institute in Hawaii. The book was published in cooperation with the United States Aquaculture Society. Focusing on socioeconomic perspectives, the book explores the ramifications of selecting both established and emerging aquaculture species, as well as the systems required for their production. The contributing authors and editors provide a breadth of experience and knowledge ranging from research and policy through industrial applications.

The book is divided into three parts: Principles, Practices, and Species-Specific Public Policies for Sustainable Development. Within *Principles*, sustainability is defined, followed by discussions on enabling regulatory policy, assessment, economic analysis, and farm modeling toward farm feasibility assessment. *Practices* focuses on examples of successes and failures and reviews relating to species and production system selection in aquaculture from various regions around the world as well as a discussion on the evolution of regulatory policy in the United States towards sustainable aquaculture development. Part 3 provides examples of species-specific public policy that promotes sustainable development around the world, along with several species-specific industry reviews.

Comprehensively, the book does a good job demonstrating differences between commercial production often associated with developed countries, rural production related more to developing countries, and the role of government in the development process. This book is a valuable reference for all stakeholders looking to advance sustainable aquaculture. This includes but is not limited to the production sector, economists, researchers, educators, and policy makers.

Subscribe

International Journal of Recirculating Aquaculture

A publication of Virginia Tech,
Commercial Fish and Shellfish Technologies Program

IJRA presents the latest developments in RAS-related research, focusing on the areas of:

** Food Quality * Food Safety * Fish Pathology **
** Fish/Shellfish Growth & Nutrition * Aquatic Medicine **
** Recirculating Systems Mgmt. * Breeding & Genetics **
** Marketing & Economics * Waste Management **
** Water Conservation **

Don't miss an issue of this informative peer-reviewed journal! Subscribe now by check or credit card; actual shipping charges will be added to your total cost.

Name: _____

Address: _____

Line 2: _____

City: _____ State: _____

Country/Postal Code:_____

Phone: _____ Fax: _____

e-mail: _____

---

check: ___ 2 years, $25 U.S. (plus actual shipping), payable to Treasurer, VT

credit card: ___ 2 years, $25 U.S. (plus actual shipping)

number: _____

expiration date: _____

International Journal of Recirculating Aquaculture
Food Science & Technology Building (0418)
Virginia Polytechnic Institute & State University
Blacksburg, VA 24061 USA

phone: (540) 231-2075 fax: (540) 231-9293 e-mail: IJRA@vt.edu

International Journal of Recirculating Aquaculture
A publication of Virginia Tech, Commercial Fish and Shellfish Technologies

INSTRUCTIONS FOR AUTHORS

The International Journal of Recirculating Aquaculture (IJRA) encourages authors to submit original research papers that present high-quality work on all aspects of recirculating aquaculture. Papers will be peer-reviewed and evaluated for scientific merit, relevance, and for their usefulness in promoting the advancement of recirculating aquaculture. Any related papers submitted together must be thoroughly cross-referenced.

All papers must be original, meaning that the data and the information presented must be the work of the authors, and cannot have been published elsewhere. Dual publication of a paper or data is possible only through the permission of the Editors of both journals. There are no page charges for IJRA submissions.

INITIAL MANUSCRIPT SUBMISSION

Manuscripts should be sent to Angela Correa, Managing Editor, International Journal of Recirculating Aquaculture. E-mail submission is preferred (ijra@vt.edu), but regular mail submissions are also accepted. Please send paper correspondence to:

27-D Food Science & Technology Building (0418)
Virginia Polytechnic Institute & State University
Blacksburg, VA 24061, USA

All manuscripts must be in English, and should be submitted both as a Microsoft Word document (.doc), and as an Adobe Acrobat document (.pdf). Mailed submissions should include the electronic files on a CD-ROM or USB 'thumb' drive. Please note that it is not possible to return media.

Each submission must include a cover letter stating that the paper contains original research that has not been published elsewhere, and the names and contact information for three suggested reviewers who are well-versed in the subject area covered by your manuscript. Please also include the names of colleagues who have already reviewed the work presented, as well as the names of any persons you would like to exclude as reviewers.

IJRA has three categories for submission:

1. Articles, which are complete in-depth scientific studies;
2. Notes, which are short papers of limited scope, and
3. Book Reviews, which are summaries and opinions of recently-published aquaculture texts, 500-750 words in length.

Articles and notes will normally be critically reviewed by two or three experts, as well as by the Managing Editor. Book reviews will normally be critically reviewed by both the Executive Editor and the Managing Editor. Submissions may be returned without peer review in cases where the Managing Editor determines that they are inappropriate for the journal, of poor quality, or fail to follow the journal's style format.

PREPARING the MANUSCRIPT

IJRA uses *www.dictionary.com* for standard spellings and word definitions. All spellings should be in "American English." Foreign or science-related terminology that is unfamiliar and does not appear on *dictionary.com* will require an accompanying definition. All foreign language words should be italicized in the manuscript.

Acceptable scientific and common names of fishes are listed in A List of Common and Scientific Names of Fishes from the United States and Canada published by the American Fisheries Society. Scientific names should be italicized (e.g., *Antigonia aurorosea*). Common names may be used throughout each paper, but must be accompanied by a full scientific name when they first appear in a paper. The author must use the full common name, e.g., "yellow perch" not "perch."

Paper manuscripts must be submitted on paper 22 x 28 cm (8.5 x 11 inches) in size. Only one side of the paper should be used. Number each page sequentially and include the senior author's name next to the page number (e.g., Page 4, Flick) on each page. Single-space all typed material, including references. Type size in the body of the text should be no larger than Times New Roman 12pt font, and no smaller than Times New Roman 10pt font.

example: Times New Roman 12pt *Italic* **BOLD**

Times New Roman 10pt *Italic* **BOLD**

Subsection headings should be italicized in the manuscript. Spell out one-digit numbers, except when they are used with units of measure – e.g., six ponds, 7 days. Spell out any number that begins a sentence.

When two numbers occur sequentially in the text, one of the numbers must be spelled out – e.g., In 2007, fifty fish were stocked. Use commas in numbers of 1,000 or more. Use the 24-hour clock to describe time – e.g., 1300, not 1:00 p.m. Always place a zero to the left of a decimal point if the number is less than one; this includes probability values – e.g., P = 0.05, not P = .05. All units of measurement must be reported via the metric system. Parts per million or milligrams per liter should be reported as either "ppm" or "mg/l," but not as "mg L^{-1}."

Consult the current Edition of the ACS Style Guide (http://portal.acs.org) for detailed information regarding the use of standard international units (SIU) and measurements not mentioned here. Standard scientific abbreviations and symbols may be used without definition. If the use of a symbol could cause confusion, define the symbol the first time it is used.

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

ORGANIZATION of MANUSCRIPTS

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

• **Title** – The title should accurately reflect the contents of the paper. Brief, concise titles are encouraged. The title page must include the name(s) of the author(s) and all titles and addresses. Use a separate page for the title page.

• **Keywords** – The author should place 5 or 6 key words within the abstract in bold text to be used for indexing.

• **Abstract** – The abstract should be a concise highlight of the results and conclusions. Methodology should not be abstracted unless it is necessary to explain the results or unless the paper describes a new technique. Abstracts should be less than 300 words. Use a separate page for the abstract.

• **Introduction** – The introduction should explain why the paper was written and why it is relevant. The introduction should condense the information or problems in the field that led the author to do the

research. The introduction is not a complete literature review; therefore only key references should be cited. The introduction should also contain a statement that describes the purpose or objective of the paper.

• **Materials and Methods** – The goal of this section is to clearly describe what was done so that others can repeat the experiment. If previously published papers described a technique that was used, citations can be used to prevent unnecessary repetition. If the technique or process was modified, the modification must be described so that others can repeat the process. Experimental designs can be explained by use of figures to help clarify what was done. If the experiment was a complicated one with many subparts, subsections may be used to describe each subpart.

• **Results** – This section describes the data. Proper use of tables and figures can simplify and help explain the results. Statistical analysis of data is necessary, unless differences are so obvious that statistical analysis is superfluous. Although probability values of 0.05 and 0.01 are traditionally used, authors can choose their preferred significance value.

• **Discussion** – This section should interpret the results and compare the results from the experiment to those found in similar research. The section can also be used to speculate about the results, provide reasons for the trends, and to suggest new ideas that can advance our understanding. This section should not be a literature review.

• **Results and Discussion** – The results and discussion sections can be combined. In most cases this leads to a better paper, because the integration of these sections leads to more meaningful interpretations of the data.

• **Conclusion** – This section should be used only when the results of an experiment lead to an unequivocal interpretation.

• **Acknowledgements** – This section should be used to thank organizations that provided monetary support for the research, as well as individuals who assisted in the research or preparation of the paper. This section includes manuscript number designations for those institutions that assign such numbers.

• **References** – Select references with great care. Unless your submission includes a literature review, there is no need to reference every paper covering a subject. Include only the most important ones.

• **Figures and Tables** – All tables and figures should follow the references, with figures before tables. Number figures and tables to simplify referencing (e.g., Figure 1, or Figure 1a).

IN-TEXT CITATIONS --

Within the text, references are cited in one of two ways:

1. Fisher (2006) evaluated the role of laboratories. or—
2. The role of laboratories was evaluated (Mwanga 2006).

When there are three or more authors, list only the first in a citation and follow that with et al.:

Watanabe et al. (2009) reviewed tilapia mortality statistics.

When several citations are used in a single entry, they should be listed chronologically. If you are citing two papers that were written by the same author or sets of authors, the name only has to appear once per citation, and the name is followed by the years that designate the different papers; separate the years with commas. If you are citing two authors, you must separate the citations with a semicolon: (Schenkel 1999, 2006; Rusch 2004).

If the name of an institutional author is long, it can be abbreviated in a citation, so long as the full name appears in the references (e.g., National Institute for Food and Agriculture may be abbreviated as NIFA).

In the reference section, the reference list is strictly alphabetical by last name of the first author. If an author has written more than one paper, the following format should be followed: All single-authored papers go first, followed by co-authored papers, and then papers that were written by three or more authors. If an author has written more than one single-authored paper, the papers are listed chronologically. If an author has written more than one co-authored paper, they are listed alphabetically by the second author's last name. If a tandem has authored two or more papers, the order is chronological. Papers written by the same group of three or more authors should be listed chronologically.

TABLES and FIGURES –

When creating a table, decide the purpose of the table and what points are important; then organize the table so that the main features can be easily understood. The table heading should provide sufficient information for the table to be understood as an entity. Keep footnotes to a minimum. Use the ACS Style Guide for examples of acceptable tables. Each table goes on a separate page. Authors should endeavor to make tables fit on a single page. List any captions together with the table or figure. When drawings or other artwork are part of a manuscript, they should be submitted in a separate file, in .tif or .jpg format at a resolution of not less than 300 dpi (dots per inch). If this is not possible, the original artwork must accompany the final revised manuscript.

All figures and images must be of high-quality. Faint or illegible markings or dot-matrix figures are not acceptable. Original artwork or high-contrast photographs are acceptable. Photographs should be on glossy paper. Do not glue pictures to cardboard. Color photographs will be changed to grayscale unless the color is a key element of the image.

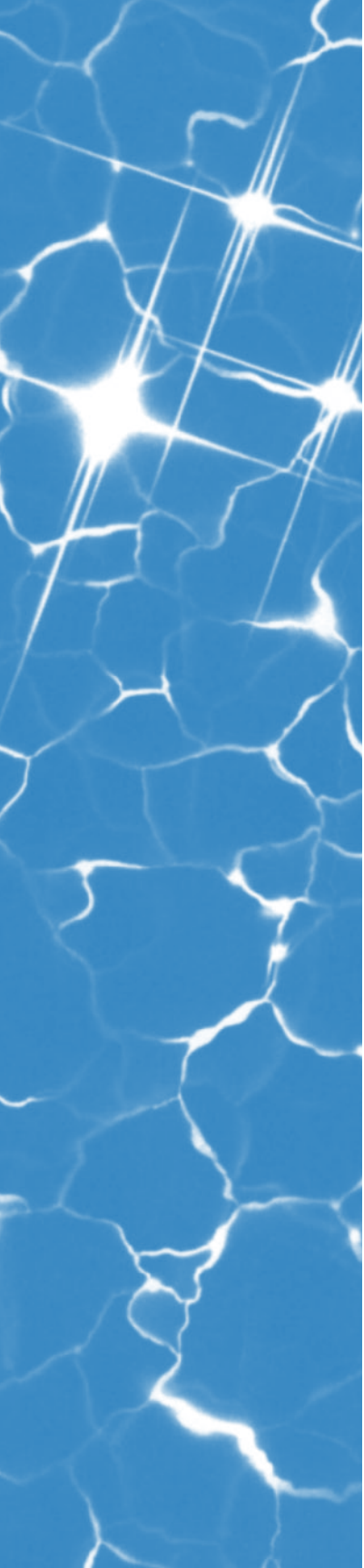
FINAL REVISED MANUSCRIPTS

Final manuscripts can be submitted electronically, preferably via e-mail, and should be submitted as a Microsoft Word document (.doc). If necessary, one set of original artwork (drawings or photographs) can be submitted via regular mail.

REPRINTS -- The senior author and each junior author will receive two copies of the journal containing his or her article, along with a .pdf file of his or her article. These will be sent several weeks after the journal is published and in circulation. Questions regarding reprints should be e-mailed to ijra@vt.edu, or sent to IJRA, 27-D Food Science & Technology Building (0418), Virginia Tech, Blacksburg, VA 24061, USA.

COPYRIGHT & PERMISSIONS -- Questions regarding permission to reprint articles that have appeared in IJRA should be e-mailed to ijra@vt.edu, or sent to IJRA, 27-D Food Science & Technology Building (0418), Virginia Tech, Blacksburg, VA 24061, USA. For, please contact: IJRA, Copyright & Permissions, 119 Food Science & Technology Building, Virginia Tech, Blacksburg, VA 24061-0418, USA.

COPYRIGHT TRANSFER --If your manuscript is accepted for publication, copyright ownership must be officially transferred to IJRA. The Editor's acceptance letter will include the necessary form. The copyright transfer form must be signed by all authors and the original returned to the Editor at this time. Failure to return the copyright form in a timely fashion will result in delays in publication.



VT/0611/NP0886