FINAL REPORT

PREPARED FOR TIMBERFISH
WESTFIELD, NEW YORK

"EVALUATION OF TIMBERFISH'S PROPRIETARY SYSTEM FOR PRODUCING FISH USING WOOD CHIPS AT THE CONSERVATION FUND"

The Conservation Fund Freshwater Institute
Shepherdstown, WV 25443

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1.0 Overview

The Timberfish Technology culture system was constructed according to the configuration described in the Scope of Work and is illustrated in Figure 1. A 3.7 m^3 (980 gallon) polyethylene tank measuring 2.3 m (7.5 ft) in diameter and 0.9 m (3 ft) in depth and partially-buried in the ground to provide structural support was used as the culture tank. The trickling filter was constructed of cedar 4x4's (vertical supporting posts) and 2x6's (horizontal bracing) and measured 2.0 m (6.5 ft) tall by 1.1 m (3.5 ft) square. The trickling filter was elevated 1.2 m (4 ft) on a timber-framed platform and filled with wood chips. A 4.8 m^3 (1260 gal) rectangular fiberglass tank measuring 3.6 m (11.75 ft) in length, 1.2 m (4 ft) in width, and 1.2 m (4 ft) in depth was used as the submerged filter. Four U-shaped, PVC-framed wood chip bins were housed inside the submerged filter. Minor additions / modifications were made to ensure capture of culture tank discharge.

Three pumping loops were installed to recirculate water through the woodchip filters. One submersible pump was located in the center of the culture tank and was controlled by a timer to operate 1 minute of every hour, pumping approximately 95 L/min (25 gal/min) to the top of the trickling filter. A second pump (end-suction centrifugal) was located at the side of the culture tank, which pumped 11-19 L/min (3-5 gal/min) of water continuously to the submerged filter. A third pump (submersible) was installed in the selector tray dewatering sump and continuously pumped approximately 19 L/min (5 gal/min) to the top of the trickling filter.

Initially, the Timberfish system was filled with spring water and afterward operated with make-up water (76-95 L [20-25 gallons] per day) obtained from the greenhouse drum filter wastewater discharge sump, which provided the initial carbon substrate needed to encourage heterotrophic bacteria growth in the trickling and submerged filters. Subsequent to stocking fish into the culture tank, the make-up water source was switched to screened facility discharge. Thereafter, culture tank fish waste provided the carbon source for any heterotrophic microbial growth.

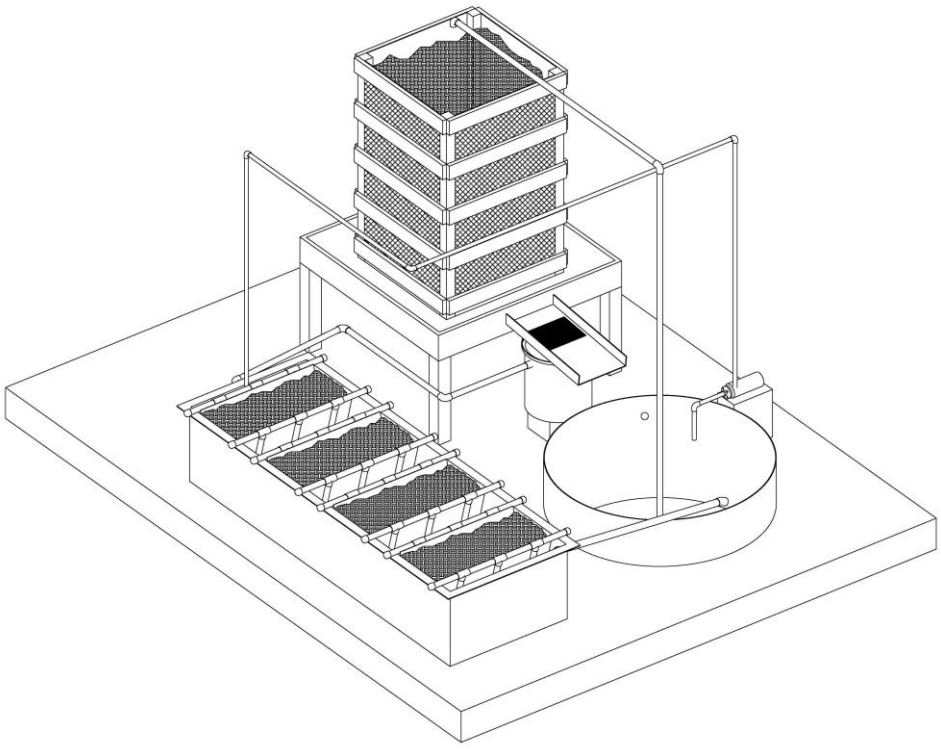

Figure 1 – Drawing indicates culture tank, trickling filter, selector tray, selector tray sump and submerged filter orientation as well as piping inlet and discharge positions.

2.0 Fish Growth

One hundred rainbow trout (*Oncorhynchus mykiss*) fingerling were stocked into the Timberfish culture tank on 10/7/2010 at a mean weight of 25.9 g / fish resulting in a stocking density of 0.7 kg / m^3. Over the entire study, one fish mortality was recorded on 11/17/2010. The study ended on 3/24/2011 when 51 of the remaining 99 rainbow trout were harvested for data collection and taste analysis. Mean weight was 396 ± 13 g and mean length was 29.7 ± 0.4 cm. Biomass density in the culture tank at the end of the experiment was 10.5 kg / m^3. The fish were hand-fed during the 169 days of the study. Feed conversion ratio (FCR) was calculated at a value of 0.7 indicating that the fish were likely feeding on either invertebrates or suspended / attached microbial floc as a supplemental food source. In comparison, rainbow trout from the same cohort were maintained in a single-pass, circular tank and fed at regular intervals using an automated feeding system. A comparison of bulk weights performed on 3/10/11 showed that the Timberfish trout weighed 340 g and the control trout weighed 382 g. The bulk weight data suggests that a regular diet of fish feed and a more consistent temperature in the control fish system led to slightly faster growth.

The Timberfish system rainbow trout collected at the end of the study had an excellent appearance. The fish were colorful and fins were in excellent condition. A condition factor (K) calculated on the mean length and weight data indicate a value of K = 1.51, which suggests good-to-excellent fish condition for a salmonid. Six rainbow trout were sampled

from both the Timberfish culture system and the flow-through control system. The fish were filleted (skin removed) and assessed for proximate analysis (moisture, crude fat, protein, and ash) and fatty acid composition at the Division of Animal and Nutrition Sciences laboratory (West Virginia University, Morgantown, WV).

3.0 Water quality

3.1 General water quality parameters

Water quality sampling occurred on an approximately monthly basis subsequent to rainbow trout stocking in the culture system. Continuous aeration was provided with a ½ hp regenerative blower and course bubble air-stones resulting in a mean dissolved oxygen concentration (DO) of 10.5 ± 0.4 (mg/L). Due to a relatively low fish biomass density, DO supplementation through coarse bubble aeration was sufficient.

Hourly pumping events from the culture tank to the trickling filter, continuous pumping from the selector tray dewatering sump, and diffused aeration within the culture tank resulted in carbon dioxide (CO_2) stripping and subsequent elevation in water pH according to acid-base equilibrium. The resulting elevated pH can increase the proportion of un-ionized ammonia (NH_3), which is toxic to fish, relative to the proportion of ionized ammonia (NH_4^+). The acid-base equilibrium relationships for estimating un-ionized ammonia and carbon dioxide concentrations from measurements of pH and total ammonia nitrogen (TAN) and alkalinity (Alk), respectively, are shown below:

$$CO_2(\text{mg/L}) = 44{,}000\left\{\frac{\text{Alk}(\text{mg/L as CaCO}_3)}{50{,}000} - 10^{(\text{pH}-\text{pK}_w)} - 10^{(-\text{pH})}\right\} \cdot\left\{\frac{1}{10^{(\text{pH}-\text{p}(K_0K_1))} + 2\cdot 10^{(2\cdot\text{pH}-\text{p}(K_0K_1)-\text{pK}_2)}}\right\}$$

$$NH_3(\text{mg/L as N}) = \frac{\text{TAN}(\text{mg/L})}{\left\{1+10^{(\text{pK}_{NH_3}-\text{pH})}\right\}}$$

As a result, in order to lessen potential un-ionized ammonia toxicity, CO_2 supplementation (recarbonation) was necessary to maintain the pH at < 7.90. Mean culture tank pH was 7.86 ± 0.06.

Temperature varied significantly over the course of the study resulting in a mean of 11.5 ± 1.3 ºC with minimum and maximum temperatures of 8.9 ºC and 20 °C while fish were cultured within the system. Fluctuating ambient air temperatures in the greenhouse facility combined with apparent heat exchange across the trickling filter promoted diurnal and long-term temperature oscillation.

Culture tank mean salinity was 0.5 ± 0.0 ppt, indicating that some salt accumulation was apparent compared to the 0.3 ppt spring water supply. Alkalinity also built up within the system. Inlet mean alkalinity was 268 ± 7 (as $CaCO_3$) during the period when facility discharge was used as system make-up water. Culture system mean alkalinity was 435 ± 31 mg/L (as $CaCO_3$). Potential sources of alkalinity include the fish feed into the system or from wood chip degradation, which were used in the trickling and submerged biofilters.

	Dissolved Oxygen (mg/L)	ph (SU)	Temperature (ºC)	ORP (mV)	Salinity (ppt)	Alkalinity (mg/L)
Culture Tank	10.5 ± 0.4	7.86 ± 0.06	11.5 ± 1.3	84 ± 3	0.5 ± 0.0	435 ± 31

Table 1 – Indicates general water quality conditions (mean ± s.e.) in the Timberfish culture tank measured on a monthly basis from 11/2/2010 – 3/3/2011.

3.2 Solids, $cBOD_5$, and COD

Suspended solids concentration in the Timberfish system process water was remarkably low. And, other than in the first void space in the submerged filter (below the filter inlet pipe), little solids settling in quiescent areas was observed. Inlet water loaded to the trickling filter contained a mean TSS concentration of 2.3 ± 0.9 mg/L resulting in mean trickling filter effluent TSS of 3.2 ± 1.0 mg/L. Solids capture in the trickling filter was likely, but particulates and fines emanating from the wood chips was evident. Also, some sloughing of microbial biomass in the wood chip filter was likely. Mean TSS from the submerged filter effluent was 5.5 ± 2.9 mg/L. Periodic sloughing of microbial biomass from the submerged filter into the culture tank was observed. A mean TSS concentration of 7.7 ± 3.2 was measured in the culture tank (Table 2). Microbial floc growth on the culture tank and other vessel surfaces was apparent, however the system was managed to prevent disturbance and suspension of this growth in the process water, i.e., no cleaning or brushing of culture tank or vessel surfaces was performed. Culture tank mean total volatile solids concentration was 5.4 ± 2.6 mg/L (Table 2), suggesting suspended solids were likely comprised of detached microbial floc or suspended fish waste.

	TSS (mg/L)	TVS (mg/L)	$cBOD_5$ (mg/L)	COD (mg/L)
Inlet	2.3 ± 0.9		3.3 ± 0.9	
Trickling Filter Effluent	3.2 ± 1.0		2.2 ± 1.1	
Submerged Filter Effluent	5.5 ± 2.9		3.0 ± 0.9	
Culture Tank	7.7 ± 3.2	5.4 ± 2.6	4.0 ± 0.9	63 ± 11

Table 2 – Table indicates total suspended solids, total volatile solids, 5-day carbonaceous biological oxygen demand, and chemical oxygen at the sampling locations in the Timberfish system.

Culture system $cBOD_5$ concentrations were also quite low and fairly similar between the four sampling sites (Table 2). Biodegradable organic compounds were probably quickly consumed by heterotrophic bacteria in the trickling and submerged filters. Chemical

oxygen demand measured in the culture tank was 63 ± 11 mg/L (Table 2) and probably reflects inorganic and non-biodegradable compounds associated with the wood chips that accumulated into the process water. Although culture system true color was not measured, obvious color was imparted to the system. Initially, tannic compounds leached from the fresh wood chips turned the system water a dark amber color. By the time fish were stocked into the system, the dark amber appearance abated to a relatively clear, yellow shade. However, the COD values observed probably reflected the presence of the remaining non-biologically degradable compounds.

3.3 Nitrogen and Phosphorus

Screened facility discharge used as inlet make-up water (inlet) and formulated feed added when fish were present provided the nitrogen sources in the Timberfish system. However, total nitrogen (TN) values in the culture system were low at all sampling locations (Table 3). The lowest TN value was observed in the trickling filter discharge suggesting microbial assimilation occurring within the filter (Table 3). A culture tank total kjeldahl nitrogen (TKN) value of 3.1 ± 0.5 mg/L was observed, indicating that the large proportion of nitrogen within the culture tank was comprised of either organic nitrogen or total ammonia nitrogen (TAN).

	Total Nitrogen (mg/L)	Total Kjeldahl Nitrogen (mg/L)	Total Ammonia Nitrogen (mg/L as N)	Nitrite (mg/L as N)	Nitrate (mg/L as N)	Total Phosphorus (mg/L as P)	Dissolved Reactive Phosphorus (mg/L as P)
Inlet	2.5 ± 0.7		0.3 ± 0.2			0.4 ± 0.4	0.1 ± 0.0
Trickling Filter Effluent	1.9 ± 0.5		0.4 ± 0.1			2.3 ± 0.6	3.5 ± 0.7
Submerged Filter Effluent	2.5 ± 0.8		0.6 ± 0.2			2.9 ± 0.8	4.0 ± 0.9
Culture Tank	3.3 ± 0.5	3.1 ± 0.5	0.7 ± 0.2	0.22 ± 0.10	0.8 ± 0.4	2.7 ± 0.7	4.2 ± 1.0

Table 3 – Indicates nitrogen and phosphorus concentrations (mean ± s.e.) at the sampling locations in the Timberfish system

The culture tank contained the highest TAN concentration at 0.7 ± 0.2 mg/L, suggesting ammonia excretion by the fish. Other than at the inlet sampling location, the lowest concentrations of TAN was observed in the trickling filter effluent at 0.4 ± 0.1 mg/L (Table 3) indicating heterotrophic assimilation of nitrogen (as NH_4^+). Fish were stocked into the system on 10/7/10, and resulting TAN concentrations in the culture tank were steadily reduced during the first 90 days of system operation (Figure 2). However, an increasing trend in tank TAN concentration occurred over the last 90 days of operation (Figure 2), which suggests that as tank biomass increased ammonia excretion by the fish exceeded that rate of heterotrophic assimilation in the trickling filter. Alternatively, or perhaps contemporaneously, decreased temperature (data not shown) during this same time period probably reduced heterotrophic bacteria metabolic activity.

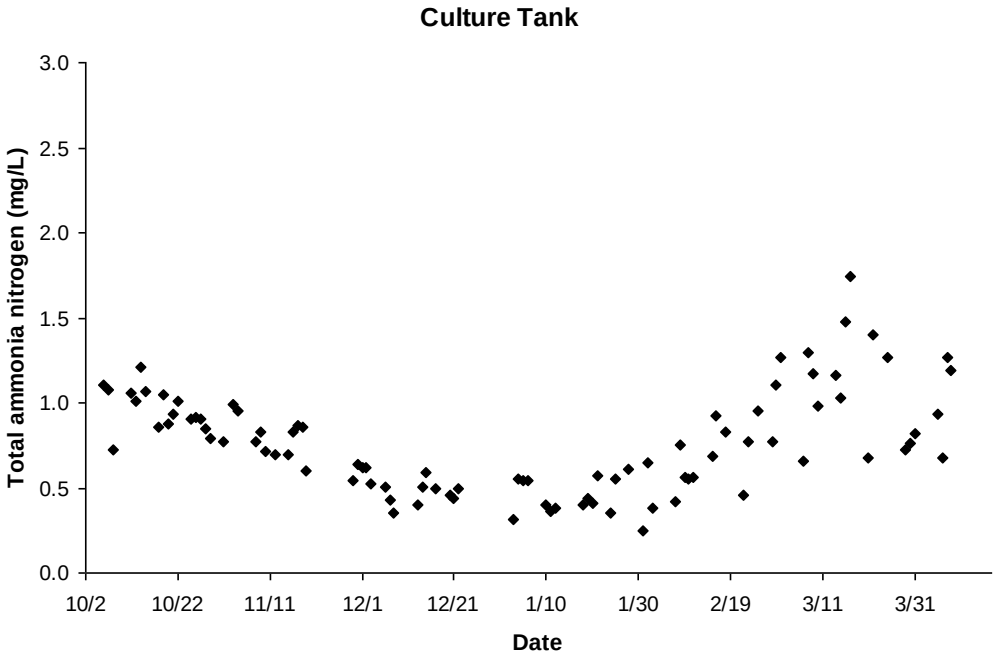

Figure 2 – Plot indicates total ammonia nitrogen concentrations over time in the Timberfish system culture tank.

Nitrite accumulation in a freshwater fish culture system is of concern because of its toxic effects. Specifically, when nitrite enters the bloodstream, it hinders the capacity for hemoglobin to carry oxygen resulting in methemoglobinemia or 'brown blood disease'. Nitrite concentration in the culture tank was 0.22 ± 0.10 mg/L, which is not considered high for a freshwater rainbow trout culture system with hard water. In addition, calcium and chloride ions can reduce the effect of nitrite toxicity. Salinity averaged 0.5 ± 0.0 ppt over the duration of the study, which would contribute more than sufficient calcium and chloride ions to moderate the effects of nitrite toxicity. Nitrate nitrogen concentration in the culture tank was quite low at 0.8 ± 0.4 mg/L (Table 3). These results suggest that total ammonia nitrogen was taken up directly by heterotrophic bacteria and that the two wood chip filters did not develop more traditional nitrification microbial activity, which would have produced nitrate as an end product. There is also a chance that some low level nitrification occurred and that the presence of heterotrophic bacteria capable of denitrification in anaerobic micro-zones resulted in low culture system nitrate concentrations.

Inlet phosphorus concentrations were quite low at 0.4 ± 0.4 mg/L total phosphorus (Table 3). However, exogenous inputs from the formulated feed and plant material increased the culture system total phosphorus to a range of 2.3 – 2.9 mg/L at the other three sampling sites (Table 3). The moderately high levels of dissolved reactive phosphorus (Table 3) suggest that denitrifying heterotrophic bacteria presence in any anaerobic micro-zones in the trickling and submerged filters was low.

4.0 Invertebrate Growth

Invertebrate growth in the Timberfish system was evident. Snails, earthworms, round worms, planarians, and fly larvae were observed in the trickling filter and in the platform / filter catchment used to elevate the filter. Although invertebrate activity was apparent in the trickling filter, few organisms were found collected on the selector tray. As a result, invertebrate quantification in the selector tray was unfeasible. However, during the final two weeks of the system trial, elevated ambient temperatures provided an environmental cue that prompted earthworm movement out of the wood chips and into the selector tray (2-3 dozen were observed). Yet, earthworm migration from the selector and into the tank was not observed, but the worms were manually moved into the culture tank. Snails, planarians, and tubifex worms were observed in the submerged filter.

5.0 Proximate Analysis and Fatty Acid Profile

Timberfish and control fish fillets were assessed by proximate analysis and differences in moisture, fat, protein, and ash were determined (Table 4). Specifically, crude fat content was 3.9 ± 0.4 % for the Timberfish and 6.8 ± 0.3 % for the control fish, which suggests that the Timberfish system provided conditions resulting in a leaner fish with slightly more protein content when compared to the control system fish (Table 4). It is likely that the environment created in the Timberfish system, which places less reliance on formulated feed dispensed at regular intervals, promotes consumption of invertebrates and biological floc growing and migrating through the culture system and resulted in a less fatty fish when compared to control fish.

	Moisture (%)	Crude Fat (%)	Crude Protein (%)	Ash (%)
Timberfish	74.7 ± 0.3	3.9 ± 0.4	20.7 ± 0.1	1.5 ± 0.1
Control	72.9 ± 0.2	6.8 ± 0.3	19.9 ± 0.2	1.4 ± 0.1

Table 4 –Indicates proximate analysis (moisture, fat, protein, and ash) of both Timberfish and control fish fillets.

A fatty acid profile comparison indicated some key differences in fatty acid compounds when comparing the Timberfish and control fish (Table 5). From a nutritional standpoint, the benefit of incorporating fish into the human diet is the presence of elevated concentrations of PUFA compounds in fish as compared to other protein sources. In this analysis, noteworthy differences were apparent regarding the PUFA:MUFA ratio when comparing Timberfish to control fish. Specifically, the Timberfish fillets had a 0.77 PUFA:MUFA ratio and the control fish had a 0.71 PUFA:MUFA ratio indicating that the Timberfish were likely consuming organisms (e.g.: invertebrates, biological floc) that imparted higher concentrations of PUFA compounds to the fish compared to the control fish. Further, the Timberfish omega-3:omega-6 fatty acid compound ratio was 2.6 compared to a ratio of 2.0 for the control fish, which is also considered nutritionally advantageous. And of particular note, the omega-3 fatty acid compound cis-4,7,10,13,16,19-

Docosahexaenoic (DHA) was found to be at a concentration of 17.80 ± 1.24 g/100g of tissue in the Timberfish fillets and at 13.02 ± 0.40 g/100g of tissue in the control fish fillets. It is considered by nutritional health professionals that incorporation of foods high in DHA results in multiple health benefits. And despite the fact that the Timberfish fillets had lower a concentration of crude fat than the control fish, the Timberfish fillets had a notably higher concentration of DHA.

Fatty Acid	Compound Shorthand	SFA / MUFA / PUFA	Timberfish (g/100g - tissue)	Control (g/100g - tissue)
Dodecanoic	C12:0	SFA	0.08 ± 0.01	0.07 ± 0.00
Myristic	C14:0	SFA	4.87 ± 0.25	4.66 ± 0.14
Margaric	C17:0	SFA	0.21 ± 0.01	0.19 ± 0.01
Stearic	C18:0	SFA	4.41 ± 0.05	4.25 ± 0.06
n-pentadecanoic	C15:0	SFA	0.34 ± 0.01	0.31 ± 0.01
Palmitic	C16:0	SFA	23.66 ± 0.39	23.55 ± 0.29
Plamitoleic	C16:1	MUFA	8.79 ± 0.35	10.58 ± 0.24
Erucic	C22:1n9	MUFA	0.64 ± 0.07	0.54 ± 0.08
Elaidic	C18:1n9t	MUFA	0.24 ± 0.01	0.25 ± 0.01
Oleic	C18:1n9c	MUFA	20.75 ± 0.64	25.33 ± 0.36
Myristoleic	C14:1	MUFA	0.10 ± 0.01	0.12 ± 0.00
Gadoleic	C20:1	MUFA	1.98 ± 0.13	2.25 ± 0.07
Linoleic	C18:2n6c	PUFA[1]	8.12 ± 0.20	7.80 ± 0.16
γ-Linolenic	C18:3n6	PUFA[1]	0.35 ± 0.05	0.31 ± 0.02
α-Linolenic	C18:3n3	PUFA[2]	1.44 ± 0.04	1.25 ± 0.07
cis-11,14-Eicosadienoic	C20:2	PUFA	1.60 ± 0.02	1.53 ± 0.02
cis-8,11,14-Eicosatrienoic	C20:3n6	PUFA[1]	0.60 ± 0.05	0.56 ± 0.03
cis-11,14,17-Eicosatrienoic	C20:3n3	PUFA[2]	0.14 ± 0.02	0.10 ± 0.01
cis-5,8,11,14,17-Eicosapentaenoic	C20:5n3	PUFA[2]	3.88 ± 0.20	3.32 ± 0.10
cis-4,7,10,13,16,19-Docosahexaenoic	C22:6n3	PUFA[2]	17.80 ± 1.24	13.02 ± 0.40

Table 5 – Indicates the concentration of each saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) in the Timberfish and control fish fillets (mean ± s.e.).
[1] Omega-6 fatty acid [2] Omega-3 fatty acid

6.0 Conclusions

The Timberfish system successfully cultured rainbow trout from fingerling (26 g) to pan size (396 ± 13 g) in 169 days (24 weeks) with only 1% mortality. Rainbow trout growth rate in the Timberfish system was nearly as fast as control fish fed to near satiation while

grown at the same time in a single-pass system at 12.5-13ºC. In addition, the Timberfish system successfully maintained good water quality for rainbow trout culture, even with a system hydraulic retention time of between 50-100 days. While few organisms were found in the selector tray, invertebrates such as snails, earthworms, round worms, planarians, and fly larvae were observed in the system. Although we had no clear indication how much of the rainbow trout diet consisted of these invertebrates or periphytic growth, overall feed conversion in the rainbow trout was excellent at approximately 0.7 pound of feed per pound of gain. Thus, potentially 1/3 of the rainbow trout food intake may have come from organisms and periphytic growth that was produced within the Timberfish system. For these reasons, the Timberfish system appears to have considerable potential for rainbow trout production with little water or wastewater footprint and with reduced feed input.

Moving forward, we recommend designing systems that do not use aeration diffusers within the circular culture tank. One potential solution would be to incorporate a sidewall box airlift pump to provide aeration for the fish, while keeping the diffused aeration outside of the culture tank. This type of design can be built in either a circular tank or square tank configuration (Figure 3).

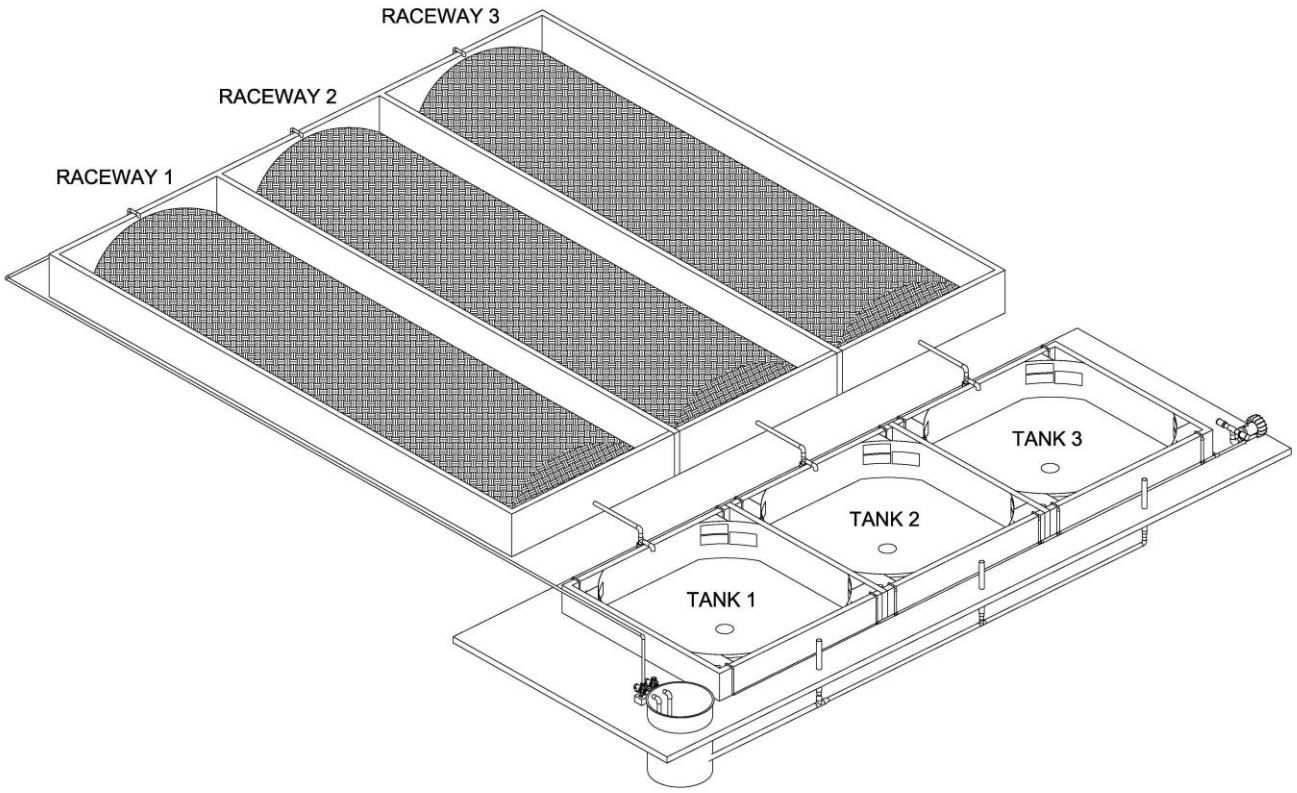

Figure 3. An illustration of a conceptual Timberfish system that uses three culture tanks (each with four sidewall box airlift pumps) in a recycle system using raceway-type windrow woodchip piles.