Improving sustainability of striped catfish (*Pangasianodon hypophthalmus*) farming in the Mekong Delta, Vietnam, through recirculation technology

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Abstract

The aim of this thesis was to document improvements in sustainability indicators of striped catfish (*Pangasianodon hypophthalmus*, Sauvage, *1878*) production through the application of recirculation and waste treatment techniques. To be able to document improvements in sustainability, in each system studied the same set of twenty sustainability indicators were measured. Indicators related to the use of fingerlings, water, diesel oil, electricity, labor, chemicals and antibiotics.

Also, indicators related to nutrient utilization efficiencies and waste discharge were monitored. In addition, a sampling scheme, allowing to calculate organic matter, nitrogen, phosphorous and chemical oxygen demand mass balances covering a full production cycle and applicable in different production systems, was developed. Overall, from a sustainability point of view, striped catfish culture in ponds compared well to other important aquaculture species.

Although favorable, it was concluded that water, chemicals and antibiotics use, survival, and the amounts of waste discharged could be further reduced through recirculation and treatment of solid wastes. The realized improvements through RAS technology and waste treatment technology were quantified in lab or pilot scale experiments. Large improvements were realized for water, antibiotic and chemical use, survival, waste discharge and color grade of striped catfish fillets at harvest. In addition, in RAS, utilization efficiencies of nutrients supplied through feeding were improved.

Solid wastes removed from ponds or RAS could be partially re-used by making compost or producing methane for generating electricity. Another approach tested was the integration of a denitrification reactor in the recirculation system, which allowed to decompose solid waste and reduce nitrogen discharge. Denitrification in RAS did not affect fish growth, nutrient retention efficiencies and the quality of the fish fillets produced, and thus also improved sustainability of striped catfish farming.

In conclusion, application of recirculation and waste treatment techniques tested in this thesis improved the sustainability for striped catfish culture. The challenge remains to scale up RAS and waste treatment technology for striped catfish to the production volumes handled in outdoor ponds without raising production costs.

Contents

CHAPTER 1

General introduction

1.1. Striped catfish culture

Striped catfish (*Pangasianodon hypophthalmus*, Sauvage, *1878*) is a facultative air breather (Lefevre et al., 2011c), endemic to the Mekong basin in Cambodia, Laos, Thailand and Vietnam and the Ayeyawady basin of Myanmar. It has been introduced to many other Asian countries, including India, China, Bangladesh, Indonesia and the Philippines (FAO, 2010). Today, Vietnam is the largest producer of striped catfish in the world, producing 1.1 million tonne per year in 5,000 ha of ponds. The product is exported to more than 130 countries (MARD, 2014a). Initially, different culture system models were used, including poly and mono culture ponds, cages and pens (Figure 1.1).

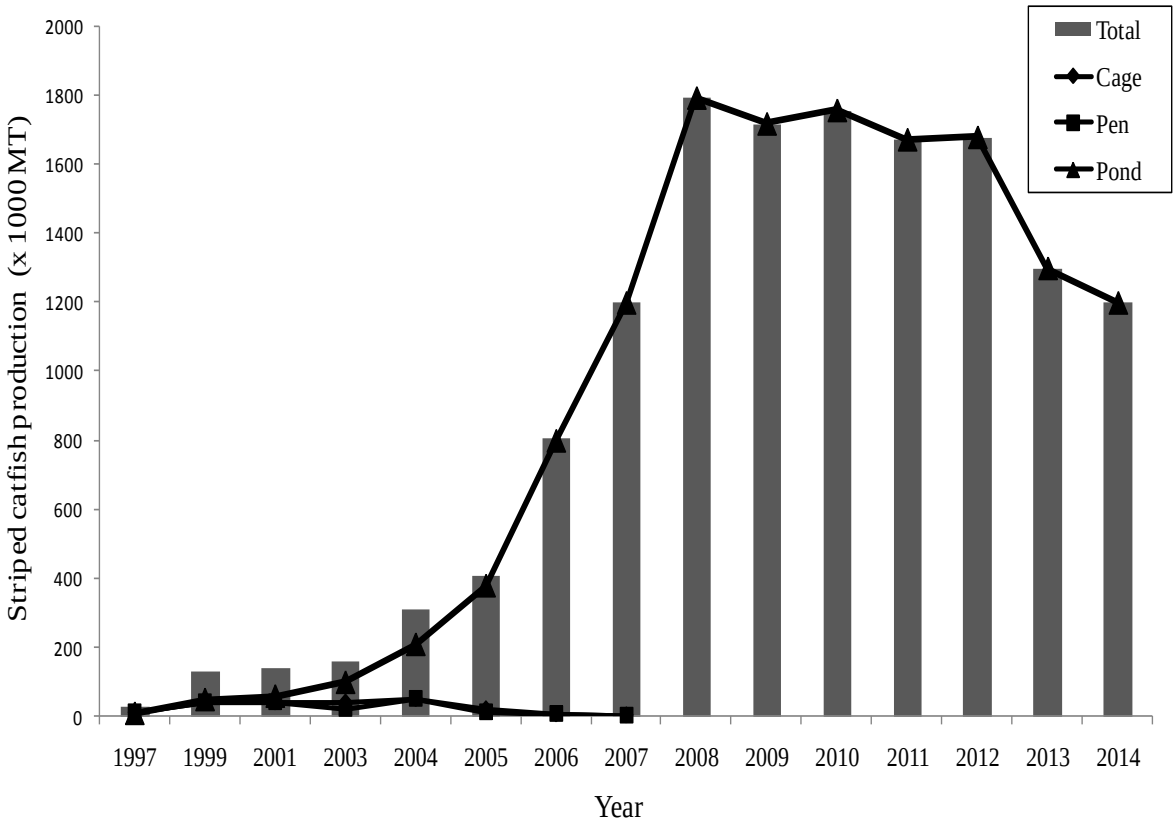

Figure 1.1: Striped catfish production by production system in Vietnam (MARD, 2014a)

Today, striped catfish is raised in none aerated 2 to 6 m deep ponds, realizing productions of 70 to 850 metric tonnes per ha per crop (Phan et al., 2009). Almost all ponds are located along tributaries of the Mekong river which is convenient to discharge nutrients to the river

and to transport feed, fingerling and market size fish to or from the farm. The amount of water used per kg fish produced ranges between 2.5 and 9.1 m^3 (Anh et al., 2010; Bosma et al., 2009; Phan et al., 2009). Commercial floating 22 – 30% protein pellets are fed, realizing a feed conversion ratio ranging between 1.5 and 1.8 (Bosma et al., 2009; Phan et al., 2009).

Per kg striped catfish produced, waste containing 46 g nitrogen (N) and 14 g phosphorous (P) is produced (De Silva et al., 2010). About 70% of striped catfish farmers discharge untreated effluents to the river. The remaining 30% of farmers discharge to rice fields or gardens (Phan et al., 2009), where part of the nutrients are reused. Discharging effluents pollute surface waters and increases the risk of horizontal disease transmission (Nguyen et al., 2007). Survival in striped catfish ponds is less than 70% mainly due to disease. More than 15 diseases/syndromes are commonly occurring in striped catfish farming (Phan et al., 2009). Diseases are treated with different antibiotics and chemicals (Bosma et al., 2009; Rico et al., 2013). However, consumers do not accept residues of antibiotics and chemicals in fish products leading to national and international laws and regulations that limit the number and amounts of chemicals and antibiotics farmers can use. When applying chemicals or antibiotics farmers are advised to ban application during the last 2 months before harvesting (BMP, 2009; MARD, 2011) .

Consumers prefer white fillets, but fillets can have grades of pink or yellow colour (Sang et al., 2009). Only white fillets are of export quality and fetch a higher price (Khoi, 2011). The fillet colour of striped catfish produced in deep ponds in the Mekong Delta is predominantly white. The high depth and water turnover rate of the pond farming system in the Mekong delta might contribute to more white fillets (Phu et al., 2014). Farmers will be willing to switch to less polluting farming systems, provided the colour grade of the fillet is maintained.

1.2. Aquaculture sustainability indicators

Sustainability includes environmental, economic and social dimensions (Verreth and Oberdieck, 2009), and to monitor and compare sustainability performance indicators are required that are measurable and easy to apply at farm level. Basic indicators used to assess sustainability of aquaculture production systems are listed in Table 1.1 (Boyd et al., 2007; Verreth and Oberdieck, 2009).

Comparisons of sustainability indicators across species, farming systems and countries or regions is important to develop standards for sustainable aquaculture. For example, trout production in Europe consumes more water in raceways than in recirculating aquaculture systems (RAS) (d'Orbcastel et al., 2009b), water use per kg fish produced being a sustainability indicator. Combining the different sustainability indicators, fish production in RAS showed better sustainability than production in raceways, cages or ponds (Eding et al., 2009). However, RAS technology has not been widely adopted due to high costs and the level of knowledge required (Ngoc et al., 2016a). Today, numerous fish farmers are willing to voluntarily adopt best management practices to reduce negative impacts from aquaculture on the environment and to better satisfy consumer demands for healthy and sustainable produced fish (Boyd et al., 2007; Ngoc et al., 2016a).

Table 1. 1: Some basic sustainability indicators at farm level (Boyd et al., 2007; Verreth and Oberdieck, 2009)

Parameter	Specific objective/criterion	Indicator	Unit
Utilisation resource efficiency:			
Fingerling use	Reducing fish mortality – high survival	Number of fingerling stocked per biomass harvested	# fingerling per kg fish produced
Feed use	Reducing feed input	Feed conversion ratio (FCR)	kg feed per kg fish produced
Energy use	Reducing energy input	Energy input per biomass produced	kWh per kg fish produced
Chemicals use	Reducing lime use if possible. No use of chemicals harmful to fish, animals, humans and environment	Chemicals input per biomass produced	g chemical per kg fish produced
Water use	Input: reducing amount of fresh water input to the production unit (reuse water as much as possible).	Input: Volume water consumed per biomass produced	l fresh water per kg fish produced
	Output: reducing amount of fresh water discharge (including nutrients, minerals and organic matter)	Output: Volume water discharged per biomass produced, not taking into account seepage and evaporation	

Table 1.1 (continued- 1): Some basic sustainability indicators at farm level (Boyd et al., 2007; Verreth and Oberdieck, 2009)

Parameter	Specific objective/criterion	Indicator	Unit
Land use	Reducing surface area use	Biomass produced per unit surface area.	kg fish produced per m^2
	Maximize percentage of input nutrients into harvested biomass.	Amount of input nutrient retained per amount of feed	g nutrient (N, P, COD, DM) retained per kg feed
Nutrients discharge:			
	Reducing discharge of nutrients.	Amount of input nutrient discharged per biomass produced.	g nutrient (N, P, COD, DM) discharged per kg feed.
Reuse of nutrient:			
	Optimizing percentage of input nutrient into secondary crops harvested on farm	Amount of input nutrient retained in biomass of secondary products per striped catfish biomass produced	g nutrient (N, P, COD, DM) in secondary product harvested per kg feed
Economy:			
Production costs/ labour use	Maximize biomass produced per unit of labour	Required labour time per biomass produced.	h per kg fish produced.
Reducing production losses	Reduce disease threats during a production cycle	Treatments per production cycle	Number of treatments per production cycle

DM: dry matter; COD: chemical oxygen demand; N: total nitrogen; P: total phosphorus.

1.3. Nutrient mass balances

By making nutrient mass balances, insight in the environmental impact of aquaculture is generated (Acosta-Nassar et al., 1994; Adhikari et al., 2014; Boyd, 1985; Funge-Smith and Briggs, 1998; Gross et al., 2000; Islam, 2005; Nhan et al., 2008; Papatryphon et al., 2005; Thakur and Lin, 2003; Thoman et al., 2001; Trépanier et al., 2002). In aquaculture, the main nutrients considered in mass balance studies are N, P, carbon (C), dry matter (DM) and chemical oxygen demand (COD) (Table 1.1) (Verreth and Oberdieck, 2009).

Nutrients enter production units with the feed, fertilizers, intake water (including infiltration water, rainfall and run-off water) and fingerlings stocked. Nutrients leave farms with harvested products, with effluents (including discharge water, seepage loss and sludge collection) and are lost through respiration. Nutrient mass balances can be approached either

focusing on the animal or on the farming system. At animal level, the principal nutrients entering the system are applied with the feed, while outputs include uneaten feed, faeces and branchial and urinary loss (Meriac et al., 2014a). In addition, nutrients are retained in newly produced fish biomass.

Nutrient mass balances are affected by culture system, feed composition, feeding practice, species or species combination and animal size (Acosta-Nassar et al., 1994; Adhikari et al., 2014; Boyd, 1985; Funge-Smith and Briggs, 1998; Gross et al., 2000; Islam, 2005; Nhan et al., 2008; Papatryphon et al., 2005; Thakur and Lin, 2003; Thoman et al., 2001; Trépanier et al., 2002). In feed driven production systems, at animal level, the main nutrient input is feed. The choice of feed ingredients influences nutrient utilization and the amount and composition of wastes produced (Meriac et al., 2014a). In outdoor systems, natural foods are also produced, which depending on culture species and culture intensity contribute to production. In extensively managed fed systems, natural foods can be the principal source to production (Bosma and Verdegem, 2011).

1.4. Recirculating aquaculture systems

In a typical RAS, fish tanks are linked to one water purification unit, in which solid removal and biofiltration are the principal processes managed. Commonly used types of solid waste removal units include swirl separators, drum filters and sedimentation tanks. The main biofiltration process is the conversion of ammonia into nitrate through nitrification, with trickling filters, moving bed and bead filters as commonly used types of biofilters. In addition, each RAS has minimum one reservoir which provides a buffer water volume for system operation and is also often used to take in new water to compensate water losses (Eding et al., 2006; Timmons and Ebeling, 2010) (Figure 1.2).

Fish excrete metabolic wastes (e.g. 50 – 80 % N of feed input) (Schneider et al., 2005) including non-faecal (34-53% N of feed) and faecal loss (30-39% N of feed input) (Bovendeur et al., 1987; Heinsbroek and Kamstra, 1990) in the fish tank. Part of the faecal loss is captured as solid waste in the solid removal unit. The efficiency of solid waste removal varies with the method applied (20 up to 90%), while the non-faecal loss is removed through biofiltration. Fine non settable organic substances can be decomposed, mostly aerobically but also partially anaerobically (Timmons and Ebeling, 2010).

Ammonia is highly toxic and is converted into more than 100 times less toxic nitrate in biofilters (Timmons and Ebeling, 2010). To avoid nitrate toxicity, 10 – 20% of the RAS water is daily replaced (Eding and Kamstra, 2002; Eding et al., 2009; Timmons and Ebeling, 2010). Converting one g ammonia nitrogen into 1 g nitrate nitrogen (NO_3-N) consumes 7.05 g $CaCO_3$ alkalinity. About 250 g sodium bicarbonate per kg feed is required to compensate the alkalinity loss resulting from nitrification, and preventing the pH to drop below 7 (Timmons and Ebeling, 2010).

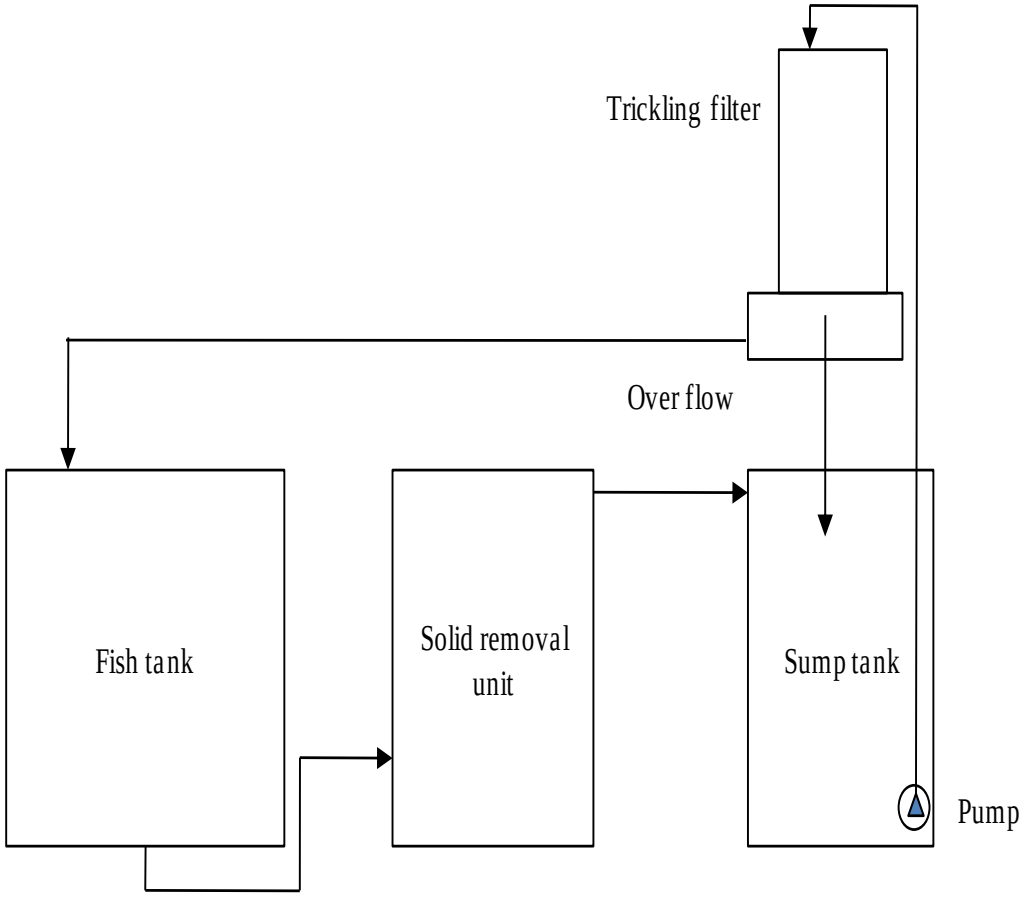

Figure 1. 2: Basic RAS design, a solid removal unit and a bio-filter (trickling filter).

Today, RAS technology is mainly applied indoors, but can also be applied outdoors with fresh, brackish or marine water (Timmons and Ebeling, 2010). Advantages include, year round operation, continuous maintenance of optimal water quality (d'Orbcastel et al., 2009b),

limited space and water requirements, efficient use of labour due to high culture densities, high bio-security, control of nutrient waste streams and daily control on animal welfare and product quality (Timmons and Ebeling, 2010) (Table 1.2). RAS requires 10 to 450 times less surface area and 80 to 4000 times less water than fish production in ponds or raceways (Timmons and Ebeling, 2010).

1.5. Anoxic (denitrification) and anaerobic sludge treatment in aquaculture

Denitrifying microorganisms use nitrate as oxidizing agent when free oxygen is very low or absent and nitrate is present. During denitrification, nitrate is converted into nitrogen gas, by using either an external (e.g. acetic acid, ethanol, glucose) or an endogenous carbon source coming from solid organic waste trapped in the solid removal unit (Henze et al., 1997). The denitrification rate is optimal when the COD/N ratio ranges between 3 and 6 (van Rijn et al., 2006). On average, when 1 g NO_3-N is removed, 2.86 g COD from organic matter is consumed (Henze et al., 1997) and 3.57 g $CaCO_3$ alkalinity is produced (Timmons and Ebeling, 2010).

In RAS, a high NO_3 concentration negatively influences growth, survival and osmoregulation (Bovendeur et al., 1987; Camargo et al., 2005; Davidson et al., 2011; Hamlin, 2006; Kamstra et al., 1998; Meriac et al., 2014a; Schram et al., 2014; van Bussel et al., 2012b; Westin, 1974). Furthermore, discharge of NO_3 rich water contributes to eutrophication of surface waters (Timmons and Ebeling, 2010). By integrating a denitrification reactor into RAS, NO_3 accumulation and discharge is reduced. This approach is effective (van Rijn et al., 2006), but also complex, explaining why few farmers apply denitrification. Nevertheless, in most RAS passive denitrification occurs accounting for 9 to 21% of the on-farm nitrogen loss (van Rijn et al., 2006). An additional advantage of denitrification in RAS is that the concentration of off-flavor producing microorganisms is reduced (Guttman and van Rijn, 2009).

1.5.1. Anaerobic sludge treatment

Under anaerobic conditions neither dissolved oxygen nor nitrate is present. Numerous microbial species working in symbiosis are involved in the anaerobic degradation of organic matter (Table 1.2).

Similar to agricultural solid waste, anaerobic decomposition of aquaculture sludge can be cost and energy efficient (Gebauer, 2004; Gebauer and Eikebrokk, 2006; Kuusik et al., 2014; Mirzoyan et al., 2012; Mirzoyan et al., 2008; Mirzoyan et al., 2010; van Rijn et al., 1995). Anaerobic breakdown is influenced by sludge composition (Mirzoyan et al., 2010), and runs best at temperatures between 24 and 35 ^{0}C. When targeting methane production, a higher methane yield is obtained at temperatures fluctuating between 11 and 30^{0}C, compared to a constant temperature of 30^{0}C (Mirzoyan, 2009). The retention time (RT) of sludge in an anaerobic digester (AD) ranges from 6 and 60 days. The dry matter fraction of sludge fed to an AD widely ranges between 0.07 and 12.3%. Sludge nutrient removal efficiencies during anaerobic breakdown of 80 – 100% for DM, 58 – 99.8% for OM, 34 – 99.6% for COD have been reported. Between 0.2 and 3.6 l CH_4 can be produced per g COD. The fraction of CH_4 in biogas varies from 4 to 80% (Gebauer, 2004; Gebauer and Eikebrokk, 2006; Kugelman and Van Gorder, 1991; Lanari and Franci, 1998; Mirzoyan, 2009; Mirzoyan et al., 2008).

Chen et al. (1997) reported that in aquaculture, per kg feed applied between 0.1 and 0.5 kg sludge DM can be efficiently broken down in an AD, resulting in a low nutrient discharge and the production of CH_4 as an energy source. However, in practice, collection and thickening of sludge before treatment is expensive. Therefore, broadly adopted use of anaerobic digestion in aquaculture remains a challenge.

Table 1. 2: Division of bacteria over two biological anaerobic processes (Henze et al., 1997).

Step	Name	Substrate(s)	End product
Acid production	Acid –forming bacteria	Carbohydrates, amino acids, lipids	butyric acid, propionic acid
Methane production	Acetoclastic bacteria	Acetic acid	Methane, carbon dioxide
	Methane bacteria	Hydrogen, carbon dioxide	methane

1.5.2. Composting

The end products of composting are CO_2, water, minerals and compost. Composting passes first through a decomposition stage, followed by a stabilization stage (Diaz et al., 2011). During composting, microorganisms first degrade easily degradable organic matter (OM). Subsequently, less easily degradable molecules and metabolites are broken down during the stabilization stage (Diaz et al., 2011). Overall, during composting of municipal waste, crop residues or animal wastes, 30 to 60% of N, C and OM are lost through volatilization and leakage (Diaz et al., 2011; Eghball et al., 1997; Eneji et al., 2003; Goyal et al., 2005; Leifeld et al., 2002; Li et al., 2008; Michel et al., 2004; Sánchez-Monedero et al., 2001; Sommer, 2001; Tran et al., 2011).

Physical and chemical properties of the substrate, including particle size, molecule tertiary structure and the C/N ratio, affect composting. A substrate C/N ratio between 25 and 30 is considered best. If the C/N ratio is below 20, ammonia volatilization becomes very high (Diaz et al., 2011; Goyal et al., 2005). Forty to 50% of the energy released during breakdown of OM is utilized by microorganism to synthesize adenosine triphosphate (ATP). The remaining energy is released as heat, causing the temperature in the substrate to rise to 70 – 90 ^{0}C. At lower temperatures of 30 to 45 ^{0}C, composting is more efficient, but then not all pathogens, potentially present in the wastes used as substrate for composting are pasteurized (Diaz et al., 2011).

During composting, initially the pH drops below 5, but subsequently raises to 8.0 - 8.5 at the end of composting). The optimal pH for compositing fluctuates between 5.5 and 8.0 (Diaz et al., 2011; Leifeld et al., 2002). To prevent anaerobic fermentation during composting about 0.15 m^3 air is supplied each minute for each metric tonne substrate composted (Diaz et al., 2011).

During composting the moisture content in the substrate must be maintained at 40 – 60 % (Diaz et al., 2011; Eghball et al., 1997; Eneji et al., 2003; Goyal et al., 2005; Iranzo et al., 2004; Michel et al., 2004). When the moisture content drops to a range between 8 and 12%, microbial activity becomes very low. However, when the moisture content is above 60%, then anaerobic breakdown or denitrification becomes the dominant process, reducing compost yield (Diaz et al., 2011).

Few studies report on the composting of aquaculture wastes (Adler and Sikora, 2004; Bui et al., 2015; James et al., 1998; Phung et al., 2009; Timmons and Ebeling, 2010). In RAS, where solid wastes are concentrated and removed, the dry matter content is low, ranging between 1.4 and 6% (Chen et al., 1997). Pond sludge is considered of low quality, due to a long residence time under mostly anaerobic conditions (Munsiri et al., 1996; Phu and Tinh, 2012). Nevertheless, compost not only is valuable, but also pollution is reduced, and part of nutrients in the sludge are recuperated (Timmons and Ebeling, 2010).

1.6. Thesis aim and content

In comparison to other aquaculture species grown in outdoor systems, striped catfish culture in ponds is dependent on high and daily water exchange with the Mekong River to maintain water quality and discharge nutrients. This creates negative environmental impacts and reduces biosecurity. Frequent disease outbreaks force farmers to apply antibiotics and chemicals to reduce disease related losses. Application of these products raises public concerns about product quality, development of drug resistance in bacteria and health risks to consumers.

Today, the Vietnamese government and western consumers aim to reduce these problems through regulations and certification, respectively. In this context, the aim of the thesis is to explore options to improve the sustainability of striped catfish culture by applying known environmental friendly production methods and waste treatment techniques and to qualify and quantify possible improvements made based on sustainability indicators as outlined in Table 1.1.

This thesis started with the detailed monitoring of striped catfish production in ponds in the Mekong delta. Upstream and downstream ponds were monitored during a full production cycles, giving a detailed description of culture practices while quantifying environmental impacts using pre-defined sustainability indicators (Chapter 2). Next, striped catfish was raised in RAS and flow-through tanks, recording the same sustainability indicators and comparing fish performance (Chapter 3).

In Chapter 4, compost quality and methane yield from solid waste collected in ponds and RAS was compared, while in Chapter 5, the effect of integration of a denitrification reactor in RAS to break down solid wastes on fish performance and sustainability indicators was investigated. In Chapter 6, considering study outcomes, options to turn striped catfish farming into the first fully sustainable aquaculture industry are discussed and placed in context.

CHAPTER 2

Nutrient mass balance and water use in striped catfish (*Pangasianodon hypophthalmus*, Sauvage, *1878*) pond culture: down-stream versus up-stream

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Abstract

Striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878) farming is the biggest aquaculture industry in the Mekong Delta in Vietnam, with an estimated production of 1.1 million metric tons from 5,500 ha of deep ponds. A key priority is to identify options to improve the sustainability of the industry. This study quantified sustainability indicators for two downstream and two upstream ponds along the Mekong River. The sustainability indicators considered were the use of fingerlings, water, diesel oil, electricity, labour, chemicals and antibiotics, and the utilization efficiency and discharge of dry matter, nitrogen and phosphorous per kg fish produced and per kg feed consumed. The results showed that in all ponds, the water quality remained favourable during the entire production period.

The sustainability indicators were (expressed per kg fish and listed as downstream vs. upstream): 2.8 vs. 7.1 m^3 water, 0.04 vs. 0.14 kWh energy, 0.06 vs. 0.11 hour human labour and 0.06 vs. 0.15 g antibiotics used ($P < 0.05$). The feed dry matter utilization efficiency was similar (28–30%, $P>0.05$) for both types of ponds. For nitrogen utilization efficiency was 44 vs. 40% ($P < 0.05$) and for phosphorous 17.6–17.7% ($P > 0.05$). The discharge was 357–415g dry matter, 19.8–20.1g of nitrogen and 17.0–17.7g kg $fish^{-1}$ ($P > 0.05$) of phosphorous. Through denitrification and fermentation in deep ponds removed 29–37% of dry matter and 30–34% of nitrogen feed input. Developing (semi)closed systems that adopt elements from recirculation technology will improve both the culture performance and sustainability.

Keywords*: Pangasius, striped catfish, nutrient budget, water quality, waste effluent, mass balance*

2.1. Introduction

Striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878) farming in the Mekong delta of Vietnam developed rapidly into one of the country's major aquaculture industries (Phuong and Oanh, 2010). Having started as recently as the mid-nineties, today striped catfish is the country's second most important export product (MoFi, 2005). In 2014, the total catfish production reached 1.1 million ton produced in 5,500 ha of ponds. Processed products have been exported to more than 150 countries (MARD, 2014a).

With 70–800 metric ton (MT) ha^{-1} $year^{-1}$ (Phan et al., 2009) striped catfish culture is intensive. Water quality is maintained through water exchange. Water use is an important sustainability indicator. The Aquaculture Stewardship Council (ASC) has set the not-to-exceed standard at 5,000 l kg^{-1} striped catfish (ASC, 2012). Water use values reported in literature are either 20–50% smaller (2,500–4,050 l kg^{-1} fish (Bosma et al., 2009; Phan et al., 2009)) or 82% larger (9,167 l kg^{-1} fish (Anh et al., 2010)). ASC indicators for nitrogen (N) and phosphorus (P) utilization are 27.5g N and 7.2g P discharge per kg fish (ASC, 2012). These ASC-indicator values are substantially lower than estimated values for nitrogen and phosphorus discharge reported in literature: 38–46g N kg^{-1}(Anh et al., 2010; De Silva et al., 2010) and 9.9–14.4 g P kg^{-1} fish produced (De Silva et al., 2010).

Today, common practice is to culture striped catfish in 2–6m deep earthen ponds close to the Mekong river (Phan et al., 2009). Striped catfish farming started upstream (US) before expanded to downstream (DS). Both US and DS farmers practice tidal water exchange. However, DS farmers have the advantage of (1) a larger tidal amplitude and a longer period of the day that tidal water exchange can be practiced and (2) less potential conflict with other types of land use (Bosma et al., 2009). In addition, DS farmers use less energy than US farmers, because in contrast to the latter, they must not pump to realize sufficient water exchange. However, higher water exchange might also result in more waste discharge. Thus, when analyzing the sustainability of current striped catfish farming technology, it is important to quantify the nutrient discharge related to water exchange. The objective of this study was to compare the effect of US and DS striped catfish farming (location) on water exchange, nutrient utilization efficiency and waste discharge. To improve the sustainability of striped catfish

production it is important to get insight in water and nutrient utilization over a complete culture period (ASC, 2012). Such a dataset, which to our best knowledge not yet available, is presented, differences between DS and US ponds are analyzed and options to improve sustainability of striped catfish farming are suggested.

2.2. Materials and methods

2.2.1. The experimental ponds

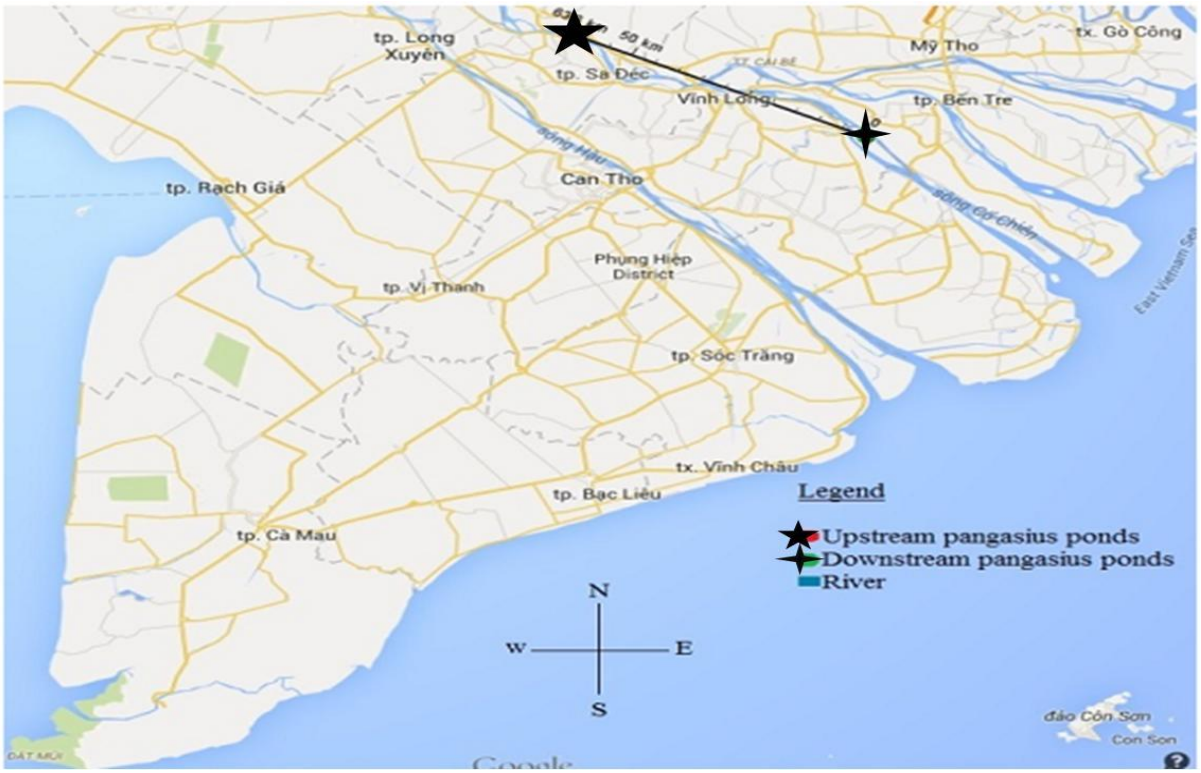


Figure 2. 1 : The locations of studied striped catfish downstream (DS) and upstream (US) ponds, Mekong Delta, Vietnam

Two DS ponds at the Phuoc Binh hamlet, Quoi Thien commune, Vung Liem district, Vĩnh Long province and two US ponds at the My Suong hamlet, Cao Lanh district, Dong Thap province, were monitored during a full production cycle. The DS and US ponds are 63.3 km apart in bird's eye view (Figure 2.1). The DS ponds have a surface area of 1.11±0.1 ha, the US ponds 1.08±0.1 ha (Table 2.1).

2.2.2. Pond management

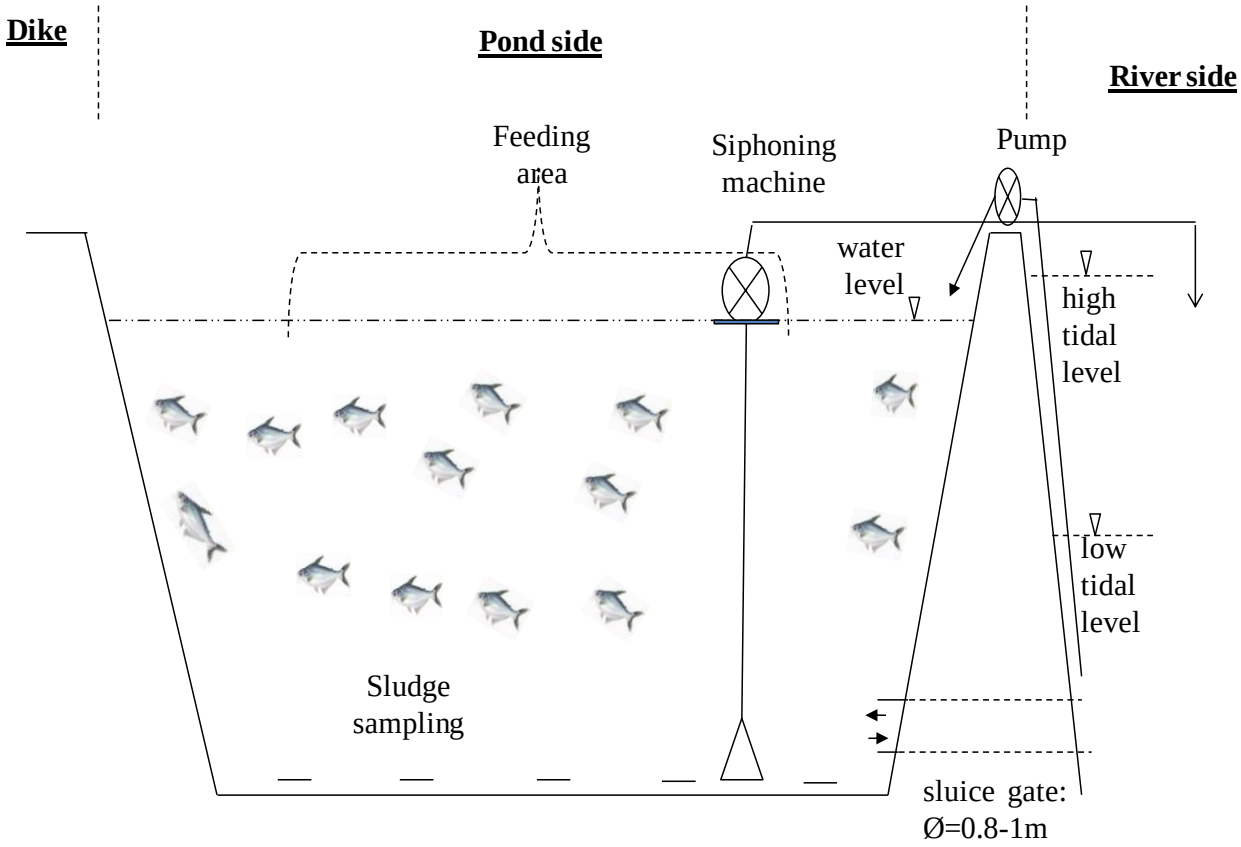


Figure 2. 2: Schematic view of experimental striped catfish ponds. The number of sample locations for sludge (fixed) and water column samples (random) are shown. Oxygen measurements were taken at fixed depths at random locations. Evaporation and rain volume measurements were taken on the dike. Position of the sluice gate was 0.5 m above the bottom (inner sluice valve not shown). Pumps for water exchange were positioned on top of the dam next to the river, and located in the corner away from the sluice gate. When sludge was siphoned from the bottom, 3 floating movable diesel pumps were used (see also Table 2.1).

Table 2. 1: Pond characteristics and management in downstream (DS) and upstream (US) ponds.

Parameter	Downstream	Upstream
Pond depth	3.4 ± 0.1 m	4.2 ± 0.1 m
Pond surface area	1.11 ± 0.1 ha	1.08 ± 0.1 ha
Pumping	Two diesel pumps (8 HP/pump) for emergency; no electrical pump.	Electricity powered pumping station (30 HP), running for 5-8 hours per day at neap tide dropping to 1-2 hours per day at spring tide.
Water exchange	Based on tidal regime: water discharge at low tide and water intake at high tide. Three first months, daily 0 – 10% water exchange, increasing to 10 – 40% water exchange at harvest. After discharge, it takes 8-12 hours to fill the pond. No water exchange possible during neap tide.	Based on tidal regime + active pumping: water discharge at low tide and water intake at high tide. Two first months, 0 – 10% daily water exchange, increasing to 12 – 50% at harvest. After discharge, it takes 6-8 hours to fill the pond. During neap tide, water exchange through pumping.
Liming	Use 1,000 kg ha^{-1} $CaCO_3$ for pond preparation and during culture liming to maintain pH and alkalinity (Figure 2. 2A).	Use 2,100 kg ha^{-1} $CaCO_3$ for pond preparation and during culture to maintain pH and alkalinity (Figure 2.2B).
Sludge removal	Three times 3,199 ± 842 m^3 sludge (2.1% ± 0.4 dry matter) was removed by 3 diesel suction pumps. Sludge was removed when sludge bed grew higher than 20 cm (checked by divers).	Once 3,200 ± 212 m^3 sludge (2.6% ± 0.6 dry matter) was removed by 3 diesel suction pumps. Sludge was removed when sludge bed grew higher than 20 cm (checked by divers).
Labor use	Per pond, 3 farm employees and one technician for monitoring. Farm activities include feeding, water exchange, fish health monitoring, water quality monitoring, sludge removal, fish transport, feed transport, log keeping, and accounting. The labor for transport, log keeping and accounting are classified as indirect labor.	
Chemical & antibiotic use	Iodine (10%) *(0.5-1 ppm)*, table salt (NaCl) *(10-15ppm)*, and antibiotics: doxycycline *(2.5-5 g kg^{-1} feed)*, oxytracycline *(2.5-5g kg^{-1} feed*) and florphenicol *(2-6g kg^{-1} feed)* during fish disease outbreaks. No antibiotics were used during 2 months before harvest (Figure 2.3A, 2.3B).	
Energy/fuel use	Electricity from public grid for light during nighttime, on farm lodges, office and operation. Diesel for sludge removal and sometimes for emergency pumping.	Electrical pumps for water exchange, light during nighttime, on farm lodges, office and operation. Diesel for sludge removal and sometimes for emergency pumping.

Four new ponds, two at a DS and 2 at a US farm, operated following the farm's protocol, were monitored during a full production cycle. Fish were stocked at a water depth between 2.5 and 3.0 m. The water depth was raised to 3.4–4.2 m one month after stocking. This depth was maintained until harvest. In DS ponds, tidal water exchange was practiced, in US ponds a combination of tidal exchange and active pumping was practiced. No aeration was applied during culture. Pond management included control of water exchange, liming and sludge removal (Table 2.1 and Figure 2.2).

2.2.3. Fish, feed and feeding

Table 2. 2: Diets (1-3) and pellet size (2.5, 4 and 8mm) used in culture period (1-3), of the striped catfish production cycle and analysed nutrient content on wet weight (ww) basis (g kg^{-1} feed).

		Period 1		Period 2		Period 3	
Parameter	Unit	downstream	upstream	downstream	upstream	downstream	upstream
Culture period	day	1-59	1-59	60-122	60-122	123- harvest	123-harvest
Pellet diameter	mm	2.5	2.5	4.0	4.0	8.0	8.0
Total feed	Kg*10^3	17.95	8.55	79.18	26.83	389.25	353.54
Dry matter	g kg^{-1}feed	889.1	891.9	894.0	894.0	892.0	894.0
Crude protein	g kg^{-1}feed	303.6	300.7	282.0	280.0	262.0	263.0
Crude fat	g kg^{-1}feed	52.0	52.0	53.0	53.0	52.0	54.0
Nitrogen-free extract (NFE)	g kg^{-1}feed	406.3	405.9	419.0	416.0	431.0	424.0
Fiber	g kg^{-1}feed	54.2	59.2	67.0	68.0	73.0	76.0
Ash	g kg^{-1}feed	73.0	74.1	73.0	77.0	74.0	77.0
Total-P	g kg^{-1}feed	13.3	13.2	13.2	13.5	14.0	13.4
COD(1)	g kg^{-1}feed	1,221.4	1,216.9	1,215.5	1,209.7	1,198.1	1,201.0
Energy(2)	Kcal kg^{-1}feed	4,157.7	4,137.5	4,132.8	4,112.9	4,073.7	4,083.5

(1) COD content of the diet was calculated as described in Dalsgaard and Pedersen (2011). COD_{feed} = crude protein (g kg^{-1}) * 1.77 + Crude fat (g kg^{-1}) * 2.88 + NFE (g kg^{-1}) * 1.16 + Fiber (g kg^{-1}) *1.16. (2) Energy content of the diet was calculated as 3.4 Kcal * g^{-1} COD according to Henken et al. (1986).

Fingerlings were stocked at a density of 51.0 individuals m^{-2} in DS ponds and at 51.3 in US ponds, with a mean individual weight of 46g and 31g, respectively. The same commercial extruded feeds were applied in all ponds (Viet Thang Company, Dong Thap province, Vietnam). During each of three distinct periods during the culture cycle (day 1–59, 60–122, 123–harvest), a diet with different composition and pellet size was fed (Table 2.2). The fish were hand-fed daily at 10 a.m. The farmers aimed to feed 2–3% of body weight d^{-1}. For each distinct period, feed samples from 5 randomly selected 25-kg feed bags were pooled to determine the proximate composition (Table 2.2).

2.2.4. Sampling and measurement

Sampling

During the production cycle, pond influent (river water) and pond water were sampled biweekly. Water in each pond was sampled at five locations, after which samples were mixed into one composite sample following the procedure described by Nhan et al. (2008). The influent water was sampled next to the inlet sluice gate in the river. Water samples were collected in a PVC pipe (5.8 cm inner diameter), with the length adjusted to the pond depth, that was lowered vertically at each sampling site, covering the full water column. Once the pipe was in position, the bottom opening was closed by a stopper pulled in place with a rope passing through the pipe. In the river, a 3-m long pipe with the same diameter was used to sample the water column between 1.5 and 4.5 m depth (Figure 2.2). Rhizon pore water samplers (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) were installed 0.3 m deep in the sediment at three randomly chosen locations in each pond to collect seepage water as described by Muendo et al. (2005). The collected rhizon water samples were mixed into one composite sample. Precipitation was measured daily at DS and US locations.

Every fortnight, per pond 50 fish were caught by casting net and batch weighed to determine the average individual weight. Of these 50 fish, fifteen were randomly taken to determine proximate composition; the remaining fish were returned to the pond. At harvest, all fish were removed by a processor, of which 15 fish per pond were processed to record fillet yield and fillet coloration, and another 15 fish was analyzed for proximate composition.

At three randomly selected locations in each pond, a 0.6-m^2 circular sludge trap and a 0.4-m^2 ceramic tile, put horizontally on the sediment surface, were installed. Sludge traps were emptied on a weekly basis, because they would spill over when sampled biweekly. The amount of sludge collected was however reported on a biweekly basis. The areas with tiles were marked and sediment accumulating above each tile was left undisturbed until harvest. On the harvest day, the accumulated sludge above the tiles was quantified and analyzed. Farmers decided when to remove sludge accumulated at the bottom in the pond (Table 2.1). Sludge removal was done using a diesel powered suction pump. When this happened, a sludge sample was collected and the total volume removed recorded. Thus, the total amount and composition of the sludge removed could be determined.

2.2.5. Measurements and analyses

Water

Dissolved oxygen and pH were measured daily at 6 a.m. at 3.5m depth in the river next to the inlet of the sluice gate (Figure 2.3). Inside each pond, water temperature (^oC), dissolved oxygen ($mg.l^{-1}$), pH (multi-parameter meter HI9828, Hanna Instruments, Rhode Islands, USA) and secchi disk transparency (cm) (Alamazan and Boyd, 1978) were measured daily at 6 a.m. and 2 p.m. at 5 randomly chosen locations at 1, 2, 3 and 4 m depth (Figure 2.3).

The collected water samples in river and ponds, including rain and seepage water were analyzed, all according to (APHA, 1999) for chemical oxygen demand (COD, dichromate reflux, 0.45 µm filter pore size), 5-day biological oxygen demand (BOD_5), total organic carbon (TOC, high temperature combustion method), total carbon (TC, high temperature combustion method), carbon dioxide (free CO_2 reacts with sodium hydroxide to form sodium bicarbonate), total alkalinity (titration with sulfuric acid and methyl orange indicator), total Kjeldahl nitrogen (Kj-N, Kjeldahl method), total ammonia nitrogen (TAN, colorimetric method), nitrite nitrogen (NO_2-N, colorimetric method with diazotized sulfanilamide), nitrate nitrogen (NO_3-N, cadmium reduction to nitrite and measurement nitrite), hydrogen sulfide (H_2S, photometric method), total suspended solid (TSS, dried to constant weight at 103–105 ^oC), chlorophyll-a (spectrophotometer method), orthophosphate (PO_4-P,ammonium molybdate and potassium antimonyl tartrate method) and total phosphorus (TP, photometric method).

For rainfall, the amount of rain water was measured daily by a 20-cm diameter according to Snowdon Rain Gauge (Mill, 1907) installed next to the ponds on each farm (Figure 2.2).

Fish, feed and sludge

Whole fish, feed and sludge were analyzed for DM, total Kj-N and TP. The DM was determined gravimetrically after drying at 105 ^oC for 24 hours (AOAC, 2000). Total Kj-N was analyzed by the Kjeldahl method (AOAC, 2000). The crude protein in feed was calculated from total Kj-N multiplied by 6.25.

TP in whole fish, feed and sludge was analyzed spectrophotometrically following Kitson and Mellon (1944). Dead fishes collected during culture were weighed and counted, and the

associated nutrient content was calculated based on the biweekly measured proximate composition.

The fifteen fish collected per pond at the end of the culture period were filleted manually by a professional expert from a processing company, and fillet percentage and color grade were determined according to Sang et al. (2012). The grade of fillet coloration was defined as white (score 1), pink (score 2) and yellow (score 3).

2.2.6. Calculations and statistics

Parameter units and calculation formulas are summarized in Table 2.3. Water use only considers pond-associated water use, not feed-associated water use (Verdegem and Bosma, 2009; Verdegem et al., 2006). Sludge includes the removed and accumulated fractions. Differences in water quality parameters, fish performance parameters, nutrient inputs or outputs, resource utilization parameters (consumption or use of fingerlings, water, diesel oil, electricity, labour, chemicals (lime, NaCl, $CuSO_4$ and Iodine), antibiotics, nutrients retained in fish (DM, P, N) and nutrient discharge (DM, P, N) between DS and US ponds were analyzed by One-Way ANOVA, followed by Tukey test in case of significant difference ($P < 0.05$).

All statistical comparisons were first done using the individual weight at stocking as co-variable. The latter was retained in the analysis when significant ($P < 0.05$). Daily and biweekly measurements were averaged over the culture period before ANOVA. Daily and biweekly measurements were used to make water and nutrient mass balances (Table 2.3).

Table 2. 3: Units and formulas

Parameter	Unit	Formula
Sustainability indicators*:		
Resource utilization efficiency:		
-Fingerling use (FU)	# kg^{-1}fish	$FU = (N_{tot\ Initial} - N_{tot\ Final}) / G_{tot}$
-Water use (WU)	m^3 kg^{-1}fish	$WU = V_{tot.inflow} / G_{tot}$
-Diesel use (DU)	ml kg^{-1}fish	$DU = V_{oil} / G_{tot}$
-Energy use (EU)	kWh kg^{-1}fish	$EU = E_{electricity} / G_{tot}$
-Labour use (LU)	hr kg^{-1}fish	$LU = L_{time} / G_{tot}$
-Chemical use (CU)	g kg^{-1}fish	$CU = M_{tot.chemical} / G_{tot}$
-Antibiotic use (AU)	g kg^{-1}fish	$AU = M_{tot.antibiotic} / G_{tot}$
Nutrient utilisation efficiency:		
-XR (X=DM or P or N)	g kg^{-1}fish	$XR = 1000 * X_{fish\ retained} / G_{tot}$
Nutrient discharge (XD):	g kg^{-1}fish	$XD = 1000 * X_{discharge} / G_{tot}$
		$X_{discharge} = X_{effluent} + X_{sludge} - X_{influent}$
*Sustainability indicators were also expressed per kg $feed^{-1}$ by dividing by FC instead of G_{tot}.		
Fish performance		
Initial individual body weight ($W_{Initial}$)	g	$W_{Initial} = W_{Initial\ sample} / n$
Final individual body weight (W_{Final})	g	$W_{Final} = W_{Final\ sample} / n$
Total fish stocked in pond ($N_{tot.\ Initial}$)	#	$N_{tot\ Initial} = 1000 * W_{tot\ Initial} / W_{Initial}$
Total fish harvested from pond ($N_{tot.\ Final}$)	#	$N_{tot\ Final} = 1000 * W_{tot.Final} / W_{Final}$
Survival (S)	%	$S = 100 * (N_{tot.Final} / N_{tot.Initial})$
Specific growth rate (SGR)	% bw d^{-1}	$SGR = 100 * (\ln W_{Final} - \ln W_{Initial}) / D$
Geometric mean body weight (Wg)	g	$W_g = e^{((\ln WFinal + \ln W\ Initial)/2)}$
Metabolic feeding rate (MFR)	g $kg^{-0.8}d^{-1}$	$MFR = 1000 * FC / [(N_{tot.Final} + N_{tot.Initial}) / 2] / D / (W_g/1000)^{0.8}$
Metabolic growth rate (MGR)	g $kg^{-0.8}d^{-1}$	$MGR = (W_{Final} - W_{Initial}) / D / (W_g/1000)^{0.8}$
Total biomass gain (G_{tot})	kg	$G_{tot} = W_{tot.Final} - W_{tot.Initial}$
Feed conversion ratio (FCR)	kg kg^{-1}	$FCR = FC / G_{tot}$
Biomass of fish mortality ($G_{Fish.mortality}$)	kg	Daily accumulation
Fillet yield (FY)	%	$FY = 100 * (FW / W_{Final})$
Fillet colour grade (FIG)		$FIG = (1*n_{white} + 2*n_{pink} + 3*n_{yellow}) / n$

Table 2.3 (continued- 1): Units and formulas.

Parameter	Unit	Formula
Nutrient mass balances		
At fish level		
Nutrients in feed (X_{feed})	kg	$X_{feed} = (C_{X.feed} /100) \times FC$
Nutrients in fish retained ($X_{fish\ retained}$)	kg	$X_{fish\ retained} = (C_{X.Final\ fish} * W_{tot.Final}) - (C_{X.\ Initial\ fish} * W_{tot.Initial})$
Nutrients in fish mortality ($X_{mortality}$)	kg	$X_{mortality} = C_{X.fish.mortality} * G_{fish.mortality}$
Metabolic waste production (X_{MW})	kg	$X_{MW} = X_{feed} - X_{fish\ retained} - X_{mortality}$
At pond level		
Nutrient input ($X_{input\ pond}$)	kg	$X_{input\ pond} = X_{MW} + X_{influent}$
Nutrient influent water ($X_{influent}$)	kg	$X_{influent} = [C_{X.inflow} * V_{tot.inflow}] / 1000$
Nutrient output ($X_{output\ pond}$)	kg	$X_{output\ pond} = X_{effluent} + X_{sludge} + X_{unaccounted}$
Nutrient in effluent ($X_{effluent}$)	kg	$X_{effluent} = [(C_{X.outflow} * V_{tot.outflow}) + (C_{X.seepage} * V_{tot.seepage})] / 1000$
Nutrient in sludge (X_{sludge})	kg	$X_{sludge} = [C_{X.sludge\ removal\ frequency} * V_{tot.sludge\ removal\ frequency}] /1000 + (C_{X.remained\ sludge} * M_{tot.sludge}) / 100$
Nutrient unaccounted ($X_{unaccounted}$)	kg	$X_{unaccounted} = X_{inputs} - (X_{effluent} + X_{sludge})$

$\mathbf{V_{tot,inflow}}$: total amount of inflow water culture cycle (m^3), $\mathbf{V_{oil}}$: total volume of diesel oil utilisation whole culture cycle (L), $\mathbf{E_{electricity}}$: total electricity consumption culture cycle (kWh), $\mathbf{L_{time}}$: total time use of labour whole culture cycle (hour), $\mathbf{M_{tot.chemical}}$**:** total amount of chemical (as limes, NaCl, $CuSO_4$ or Iodine) whole culture cycle (g), $\mathbf{M_{tot.antibiotic}}$: total antibiotics use in culture cycle (g), $\mathbf{X_{fish\ retained}}$: DM, N or P retained in fish (g), $\mathbf{X_{discharge}}$: DM, N or P discharge (kg), $\mathbf{W_{Initial\ sample}}$: initial fish biomass of sample (kg), $\mathbf{W_{Final\ sample}}$: fish biomass of sample at harvest (kg), $\mathbf{W_{tot.Initial}}$: total initial fish biomass at stocking day by weight (kg), $\mathbf{W_{tot.Final}}$: total fish biomass at harvest day by weight (kg), **n**: number of fish samples (#), **FW:** weight of complete skinless fillet after removing fat and red muscle following standard process for export market(g), $\mathbf{n_{white}}$: number of white fillet fish, $\mathbf{n_{pink}}$: number of pink fillet fish (#), $\mathbf{n_{yellow}}$: number of yellow fillet fish (#), $\mathbf{C_{X.feed}}$: Nutrient (DM, N or P) concentration (%), **FC**: cumulative feed (kg), $\mathbf{C_{X.Final\ fish}}$: Nutrient (DM, N or P) concentration in whole final fish body (%), $\mathbf{C_{X.Initial\ fish}}$: Nutrient (DM, N or P) concentration in whole initial fish body (%), $\mathbf{C_{X.inflow}}$: Nutrient (DM, N or P) concentration in inflow water (g m^{-3}), $\mathbf{C_{X.outflow}}$: Nutrient (DM, N or P) concentration in outflow water (g m^{-3}), $\mathbf{V_{tot.outflow}}$: total volume of outflow water (m^3), $\mathbf{C_{X.seepage}}$: Nutrient (DM, N or P) concentration in outflow water (g m^{-3}), $\mathbf{V_{tot.seepage}}$: total volume of seepage water (m^3), $\mathbf{C_{X.sludge\ removal\ frequency}}$: Nutrient (DM, N or P) concentration in sludge removal frequency during culture cycle (g m^{-3}), $\mathbf{V_{tot.sludge\ removal\ frequency}}$:total volume of sludge removal frequency during culture cycle (m^3), $\mathbf{C_{X.remained\ sludge}}$: Nutrient (DM, N or P) concentration in remained dry matter of sludge in pond at harvest day (%), $\mathbf{M_{tot.sludge}}$: total remained dry matter of sludge in pond at harvest day (kg).

2.3. Results

2.3.1. Water quality

Mean values per water quality parameter for the whole production cycle are summarized in Table 2.4.

Pond influent (river water). No significant differences ($P > 0.05$) were observed in pond influent between DS and US locations for pH, TAN, NH_3-N, NO_2-N, NO_3-N, PO_4-P, TP, alkalinity, CO_2, TC, TOC, TSS, Chlorophyll-a, COD and BOD_5 (Table 2.3). Significant differences ($P < 0.05$) were observed in pond influent water between DS and US ponds for oxygen at 2m depth (4.9 vs. 4.7 mg l^{-1}), total Kj-N (3.2 vs. 6.2 mg l^{-1}) and salinity (0.4 vs. 0.06 ppt).

Pond water. No significant differences ($P > 0.05$) were observed in pond water between DS and US ponds for morning pH and for Kj-N, TAN, NO_3-N, H_2S, PO_4-P, CO_2, TC, TOC, Chlorophyll-a, COD and BOD_5. However, pond morning and afternoon water temperature, NO_2-N, TP, alkalinity, TSS and salinity in DS ponds was significantly higher than in US ponds. The afternoon pH, morning oxygen concentrations at 1, 2 3 and 4m, afternoon oxygen concentrations at 1, 2 and 3m, morning and afternoon water transparency, and NH_3-N were lower in DS ponds than in US ponds.

Pond influent (river water) versus pond water. There were no significant differences between DS pond influent (river water) and corresponding pond water for NH_3-N, NO_3-N, PO_4-P, CO_2, and salinity and between US pond influent (van Rijn and Rivera) water and corresponding pond water for Kj-N, NH_3-N, NO_2-N, NO_3-N, PO_4-P, CO_2, TOC and salinity. Mean concentrations in pond influent (river water) were always lower than in the pond water for TAN, TP, alkalinity, TC, chlorophyll-a, COD and BOD_5 and higher for pH, oxygen and TSS ($P < 0.05$). At DS locations, concentrations in influent (river water) were lower than in corresponding ponds for Kj-N, TAN, NO_2-N, TP, alkalinity, TC, TOC, Chlorophyll-a, COD and BOD_5. The same was observed in US ponds for TAN, TP, alkalinity, TC, Chlorophyll-a, COD and BOD_5 ($P<0.05$). Concentrations in DS and US pond influent (river water) were similar to concentrations observed in DS and US-ponds for NO_3-N, PO_4-P and CO_2, respectively.

Table 2. 4: Water quality in pond influent (from river) and in downstream and upstream ponds, averaged over a full production cycle and location. Values are mean ± S.D.

Parameter	Unit	Influent Mean ±SD	Min-Max	Downstream pond Mean ±SD	Min-Max	Influent Mean ±SD	Min-Max	Upstream pond Mean ±SD	Min-Max	p-value
Temperature										
- morning	^{o}C	-	-	30.4^{a}±1.1	28.0-33.0	-	-	29.5^{b}±1.6	27.0-33.0	0.001
- afternoon	^{o}C	-	-	31.6^{a}±1.3	29.0-35.0	-	-	30.9^{b}±1.2	28.0-33.0	0.001
pH										
- morning		6.4^{ab}±0.2	6-7	6.3^{c}±0.3	6.0-7.0	6.4^{a}±0.2	5.7-7.0	6.2^{c}±0.4	5.6-7.2	0.012
- afternoon				6.6^{b}±0.4	6.0-7.9			6.7^{a}±0.6	5.7-7.0	0.001
Oxygen morning										
- 1m depth	mg l^{-1}			1.5^{b}±0.7	0.7-3.8			1.9^{a}±0.6	0.6-3.6	0.001
- 2m depth	mg l^{-1}	4.9^{a}±0.9	2-6.8	1.2^{d}±0.6	0.5-3.4	4.7^{b}±1	2.0-6.8	1.6^{c}±0.5	0.6-3.5	0.001
- 3m depth	mg l^{-1}			0.8^{b}±0.4	0.2-2.7			1.4^{a}±0.4	0.5-3.4	0.001
- 4m depth	mg l^{-1}			0.5^{b}±0.1	0.3-0.7			1.1^{a}±0.4	0.5-3.2	0.001
afternoon										
- 1m depth	mg l^{-1}			1.9^{b}±0.1	1.6-10.6			2.2^{a}±0.1	1.9 -9.0	0.001
- 2m depth	mg l^{-1}			1.5^{b}±0.1	1.0-8.7			1.8^{a}±0.1	1.2-8.7	0.008
- 3m depth	mg l^{-1}			1.0^{b}±0.1	0.8-7.2			1.3^{a}±0.1	1.2-18.6	0.001
- 4m depth	mg l^{-1}			-	-			0.8±0.1	0.5-3.0	-
Transparency										
- morning	cm			24.6^{b}±6.8	14.0-47.0			30.2^{a}±4.4	20.0-50.0	0.001
- afternoon	cm			24.2^{b}±5.0	15.0-48.0			28.2^{a}±4.5	20.0-42.0	0.001
Kj-N	mg l^{-1}	3.2^{bc}±1.8	0.5-7.3	9.9^{a}±8.9	3.0-45.7	6.2^{ab}±3.9	2.0-16.7	7.1^{ab}±3.7	1.6-22.8	0.001
TAN	mg l^{-1}	0.19^{cd}±1.8	0.01-0.7	1.4^{a}±1.3	0.3-5.6	0.2^{c}±0.4	0.01-1.9	1.2^{ab}±1.1	0.03-5.5	0.001
NH_3-N	mg l^{-1}	0.0^{bd}	0.0-0.001	0.01^{b}±0.0	0.0-0.02	0.01^{abc}±0.02	0.0-0.7	0.02^{a}±0.0	0.0-0.1	0.001
NO_2-N	mg l^{-1}	0.02^{bd}±0.02	0-0.7	0.3^{a}±0.3	0.0-1.4	0.03^{bf}±0.06	0.0-0.2	0.1^{b}±0.1	0.0-1.04	0.001
NO_3-N	mg l^{-1}	0.5^{a}±0.3	0.1-1	0.4^{a}±0.3	0.3-1.0	0.3^{a}±0.2	0.1-1.0	0.4^{a}±0.3	0.02-0.5	0.300

Table 2.4 (continued- 1): Water quality in pond influent (from river) and in downstream and upstream ponds, averaged over a full production cycle and location. Values are mean ± S.D.

Parameter		Influent		Downstream pond		Influent		Upstream pond		
	Unit	Mean ±SD	Min-Max	Mean ±SD	Min-Max	Mean ±SD	Min-Max	Mean ±SD	Min-Max	p-value
H_2S	mg l^{-1}	-	-	0.2^{a}±0.1	0.01-0.5	-	-	0.1^{a}±0.2	0.0-0.8	0.615
PO_4-P	mg l^{-1}	0.04^{a}±0.02	0.01-0.1	0.4^{a}±0.5	0.0-1.7	0.4^{a}±1.7	0.0-7.5	0.4^{a}±0.5	0.01-1.5	0.400
TP	mg l^{-1}	0.5^{cd}±0.3	0.2-1.0	2.4^{a}±1.2	0.4-5.9	0.7^{c}±0.3	0.2-1.6	1.5^{b}±1.0	0.2-5.1	0.001
Alkalinity	mg l^{-1}	50.7^{bc}±8.6	40.3-65.3	61.6^{a}±9.8	48.9-83.2	43.0^{cd}±9.2	28.9-60.3	51.1^{b}±8.7	40.2-67.3	0.001
CO_2	mg l^{-1}	34.3^{a}±12.8	17.8-65.7	42.4^{a}±28.1	7.4-106.9	28.2^{a}±14.2	4.4-59.5	30.9^{a}±19.3	1.2-68.3	0.057
TC	mg l^{-1}	13.7^{d}±5.5	5.6-27.3	28.9^{b}±16.3	10.2-103.2	14.8^{cd}±4.6	5.6-27.3	30.4^{ab}±24.1	7.0-129.4	0.001
TOC	mg l^{-1}	2.3^{bd}±2.2	0.5-9.4	8.4^{a}±11.5	1.03-64.9	3.2^{abc}±2.3	0.8-9.4	6.3^{ab}±5.4	1.3-29.9	0.015
TSS	mg l^{-1}	182.9^{ab}±47.7	139.7-335.1	80.8^{c}±33.6	31.8-155.8	221.3^{a}±51.2	122.4-306.8	49.3^{d}±21.5	17.5-122	0.001
Chlorophyll-a	µg l^{-1}	6.0^{c}±3.2	2.1-11.6	114.3^{a}±73.5	23.3-267.8	5.7^{cd}±2.3	1.9-10.2	100.0^{ab}±45.9	27.9-189.8	0.001
COD	mg l^{-1}	6.0^{c}±1.5	3.5-9.7	19.4^{a}±5.7	11.5-29.4	6.9^{cd}±1.4	4.3-8.8	19.4^{ab}±5.1	7.9-29.0	0.001
BOD_5	mg l^{-1}	5.4^{c}±0.9	4.2-7.2	15.4^{a}±5.0	9.1-23.7	4.9^{cd}±1.1	3.2-6.8	15.0^{ab}±4.2	7.0-24.0	0.615
Salinity	ppt	0.4^{a}±0.3	0.1-1.2	0.4^{a}±0.3	0.1-1.2	0.06^{b}±0.02	0.04-0.08	0.06^{b}±0.02	0.04-0.08	0.002

2.3.2. Growth performance

Table 2. 5: Fish performance and feeding in striped catfish ponds. Values are mean ± S.D; n = 2.

Parameter		DS pond			US pond			
	Unit	Mean	±	SD	Mean	±	SD	p-value
Area	ha	1.11	±	0.1	1.08	±	0.1	0.767
Water depth	m	3.40	±	0.1	4.20	±	0.1	0.015
Culture period	day	234.00	±	6.0	277.00	±	3.0	0.013
Initial BW	g ind^{-1}	46.00	±	0.7	31.00	±	0.0	0.001
Final BW	g ind^{-1}	791.00	±	14.1	875.00	±	63.6	0.210
Initial number fish ($x10^3$)	#	600.00	±	183.8	553.50	±	1.4	0.755
Final number fish ($x10^3$)*	#	446.70	±	62.6	282.30	±	3.9	0.066
Initial biomass*	MT	27.40	±	7.9	15.70	±	0.4	0.171
Final biomass	MT	352.80	±	43.1	247.10	±	21.4	0.090
Yield	kg fish m^{-2}	31.80	±	3.9	22.90	±	2.0	0.117
	kg fish m^{-3}	9.30	±	0.4	5.50	±	1.2	0.054
Total feed	MT	486.40	±	15.0	381.00	±	28.8	0.044
Survival *	%	76.40	±	13.0	51.00	±	0.6	0.110
Geometric mean BW	g	190.70	±	3.2	164.60	±	6.0	0.032
Specific growth rate (SGR)	% bw d^{-1}	1.24	±	0.0	1.21	±	0.4	0.746
-SGR in period 1	% bw d^{-1}	0.82	±	0.2	0.95	±	0.2	0.575
-SGR in period 2	% bw d^{-1}	1.19	±	0.6	1.34	±	0.2	0.764
-SGR in period 3	% bw d^{-1}	1.45	±	0.1	1.24	±	0.1	0.191
Feed conversion ratio (FCR)	-	1.50	±	0.1	1.65	±	0.0	0.211
Metabolic feeding rate (MFR)	g $kg^{-0.8}$ d^{-1}	17.70	±	2.2	20.60	±	0.9	0.223
Metabolic growth rate (MGR)	g $kg^{-0.8}$ d^{-1}	12.30	±	0.4	12.90	±	0.7	0.270
FCR (metabolic)*	'	1.47	±	0.1	1.60	±	0.0	0.316
Fillet percentage	%	35.50	±	1.2	35.20	±	1.7	0.465
Fillet colour grade	1-3	1.47	±	0.6	1.20	±	0.5	0.071

Parameters with a significant ($P < 0.05$) co-variable effect of individual weight at stocking are indicated by '*'. Period 1: day 1 -59, Period 2: day 60 -122, Period 3: day 123 – harvest.

Fish behaviour during feeding was monitored and feeding was stopped when fish showed low appetite. In DS ponds, this resulted in realized feeding rates of 1.0±0.4, 2.3±0.5 and 2.5±0.2

percent body weight d^{-1}, respectively, during culture days 1 through 59, 60 through 122, and 123 until harvest. In US ponds, realized feeding rates were 1.1±0.2, 0.8±0.1 and 1.7±0.8 percent body weight d^{-1}, respectively, during culture days 1 through 59, 60 through 122, and 123 until harvest.

In all ponds, fish were harvested when above 700 g average individual weight, which is considered the minimum market size. The total harvested biomass was 352.8±43.1 MT in DS ponds and 247.1±21.4 MT in US ponds. Total feed load was higher in DS ponds when compared with US ponds ($P < 0.05$). Growth was similar between DS and US ponds ($P > 0.05$). Also survival, FCR, fillet dress out percentage and fillet coloration were similar between US and DS ponds ($P > 0.05$) (Table 2.5).

2.3.3. Nutrient mass balances in striped catfish ponds

Dry matter mass balance. Feed DM input was 434 MT in DS and 341 MT in US-ponds. Of this, 28–30% was retained in fish biomass in DS and US ponds ($P > 0.05$). Assuming all feed was consumed, the feed not retained in fish biomass was metabolic waste. At pond level, DM input was the sum of DM in metabolic waste and in influent water (Table 2.6). Mekong water provided 33% of the DM input in DS ponds and 56% in US ponds, which was significantly different ($P < 0.05$). The largest fraction of DM input was removed by pumping out sludge, which was similar between DS and US ponds (46% in DS ponds, 58% in US ponds, $P > 0.05$). Unaccounted DM was also similar between DS and US ponds (37% in DS and 29% in US ponds, $P > 0.05$).

Table 2. 6: Dry matter (DM) mass balance in downstream and upstream striped catfish ponds per production cycle. Values in 10^3 kg are mean ± S.D.; n = 2.

Mass balance component	DS pond				US pond				
	Mean	±	SD	%	Mean	±	SD	%	p-value
At fish level									
DM in feed	434.0[a]	±	13.4	100.0	340.6[a]	±	25.8	100.0	0.045
-DM in retained in fish	123.7[a]	±	14.0	28.5	88.1	±	8.6	25.9	0.092
-DM in fish mortality	4.7[a]	±	2.1	1.1	6.2[a]	±	0.9	1.8	0.415
-DM metabolic waste	305.6[a]	±	2.8	70.4	246.3[b]	±	17.1	72.3	0.040
At pond level									
DM input	453.9[b]	±	10.4	100.0	557.9[a]	±	28.2	100.0	0.039
-DM metabolic waste	305.6[a]		2.8	67.3	246.3[b]		17.1	44.1	0.040
-DM in influent	148.3[b]	±	13.1	32.7	311.6[a]	±	11.1	55.9	0.006
DM Output									
-DM in effluent	74.5[a]	±	5.9	16.4	68.7[a]	±	0.5	12.3	0.295
-DM in sludge	209.5[a]	±	35.7	46.2	325.6[a]	±	22.6	58.4	0.060
-DM unaccounted	169.9[a]	±	31.3	37.4	163.6[a]	±	5.1	29.3	0.806

Phosphorous mass balance. More P was administrated with the feed in DS ponds than in US ponds ($P < 0.05$). On average, 18% of feed-P was retained in fish biomass including dead fish in both DS and US ponds. The remaining 82% became metabolic waste (Table 2.7). Of the combined P input through metabolic waste and river influent water, 34% in DS ponds and 38% in US ponds was discharged with effluent water. In the P mass balance, 0.8 and 2.0% remained unaccounted in DS and US ponds, respectively, at the end of the production cycle. The largest fraction of P was removed with sludge pumped out of the pond (65% of metabolic waste in DS and 59% in US ponds, $P > 0.05$).

Table 2. 7: Phosphorous (P) mass balance in downstream and upstream striped catfish ponds per production cycle. Values in 10^3 kg are mean ± S.D.; n = 2.

Mass balance component	DS pond				US pond				
	Mean	±	SD	%	Mean	±	SD	%	p-value
Fish level									
P in feed	6.73[a]	±	0.20	100.0	5.11[b]	±	0.38	100.0	0.034
-*P retained in fish*	1.09[a]	±	0.11	16.2	0.77[a]	±	0.88	15.1	0.082
-*P in fish mortality*	0.10[a]	±	0.03	1.5	0.13[a]	±	0.06	2.5	0.261
-*P metabolic waste*	5.54[a]	±	0.07	82.5	4.20[b]	±	0.30	82.2	0.025
Pond level									
P input	6.00[a]	±	0.07	100.0	5.13[a]	±	0.46	100.0	0.119
-*P metabolic waste*	5.54[a]	±	0.07	92.3	4.20[b]	±	0.30	81.9	0.025
-*P in influent*	0.46[a]	±	0.01	7.7	0.93[a]	±	0.16	18.1	0.055
P output									
-*P in effluent*	2.05[a]	±	0.27	34.2	1.96[a]	±	0.20	38.2	0.746
-*P in sludge*	3.90[a]	±	0.18	65.0	3.05[a]	±	0.30	59.2	0.075
-*P unaccounted*	0.05[a]	±	0.01	0.8	0.11[a]	±	0.04	2.1	0.152

Nitrogen mass balance. More N was administrated with the feed in DS ponds than in US ponds ($P < 0.05$) (Table 2.8). The percentage of feed-N retained in fish including dead fish was similar in DS and US ponds, fluctuating between 39 to 44% ($P > 0.05$). The remaining 56 to 60% of feed-N became metabolic waste. Of the combined N input through metabolic waste and influent water, 57–59% was discharged with effluent water, 9–11% pumped out with sludge, and 30–34% remained unaccounted.

These results were similar for DS-ponds and US-ponds ($P > 0.05$) (Table 2.8). Assuming the N-unaccounted volatilized, then in DS ponds 44% of the metabolic waste volatilized. In US ponds 53% volatilized. In consequence, 56 and 47% of the metabolic waste was discharged to the Mekong River from DS and US ponds, respectively.

Table 2. 8: Nitrogen (N) mass balance in downstream and upstream striped catfish ponds per production cycle. Values in 10^3 kg are mean ± S.D; n = 2.

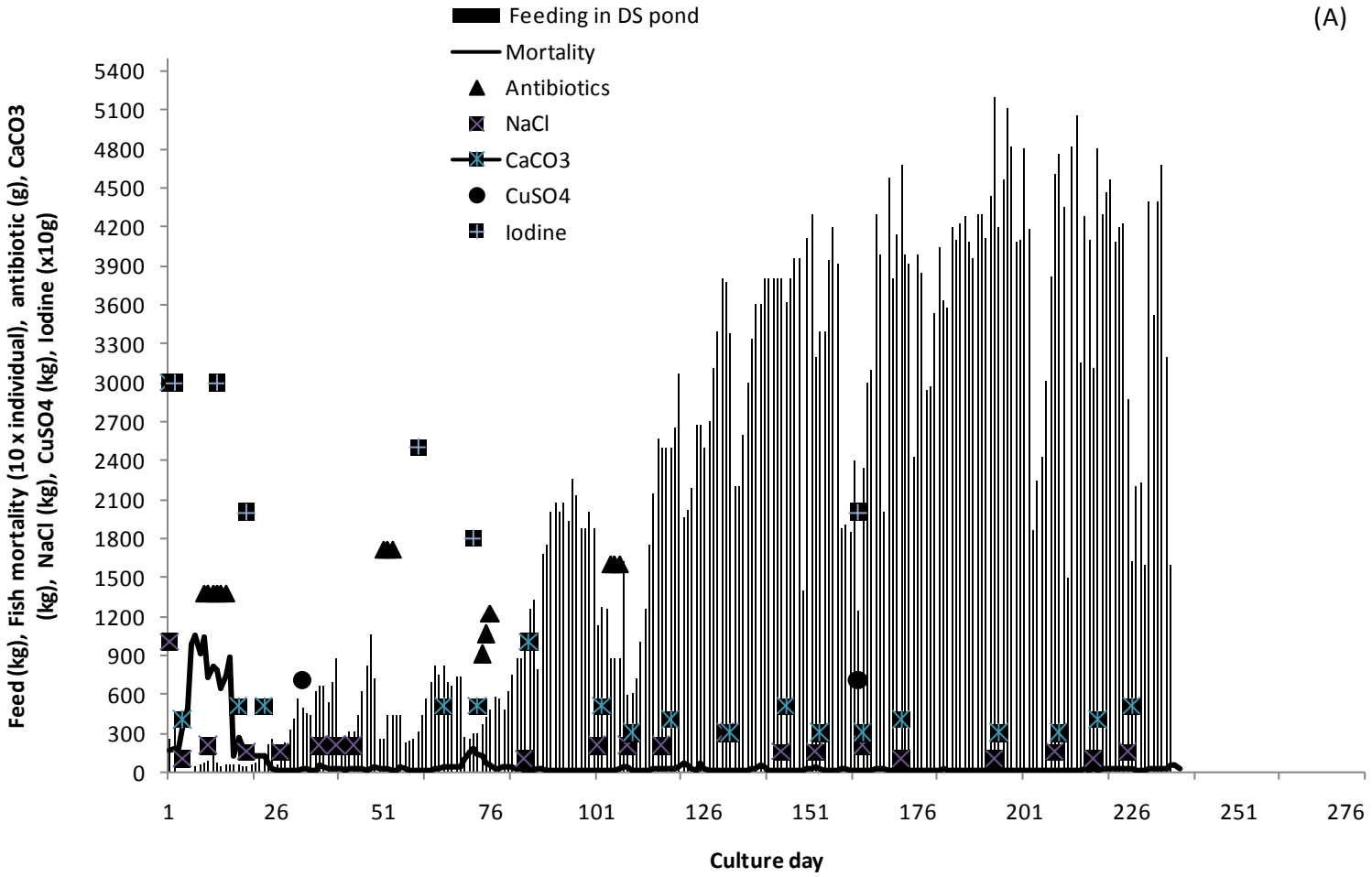
Mass balance component	DS pond				US pond				
	Mean	±	SD	%	Mean	±	SD	%	p-value
Fish level									
N in feed	20.78[a]	±	0.69	100.0	16.13[b]	±	1.22	100.0	0.043
-N in fish retained	8.22[a]	±	0.84	39.6	5.88[a]	±	0.58	36.5	0.082
-N in fish mortality	0.96[a]	±	0.67	4.6	0.50[a]	±	0.02	3.1	0.441
-N in metabolic waste	11.60[a]	±	0.52	55.8	9.76[a]	±	0.67	60.4	0.092
Pond level									
N input	15.07[a]	±	0.87	100.0	17.03[a]	±	0.12	100.0	0.088
-N in metabolic waste	11.60[a]	±	0.52	77.0	9.76[a]	±	0.67	57.3	0.092
-N in influent	3.47[b]	±	0.35	23.0	7.27[a]	±	0.55	42.7	0.014
N output									
-N in effluent	8.51[a]	±	1.17	56.5	10.0[a]	±	0.15	58.7	0.215
-N in sludge	1.42[a]	±	0.91	9.4	1.89[a]	±	0.22	11.1	0.557
-N unaccounted	5.139[a]	±	1.12	34.1	5.15[a]	±	0.21	30.2	0.991

2.3.4. Pond management

Pond management indicators are presented in Table 2.9. Use of water, electricity, labour, lime, salt and antibiotics per kg fish produced was respectively 61, 75, 45, 66, 49, and 60% lower in DS ponds than in US ponds ($P < 0.05$). However diesel oil use and sludge removal frequency per kg fish produced were respectively 113% and 200% higher in DS ponds than in US ponds ($P < 0.05$). No differences between DS and US ponds were observed for $CuSO_4$ and iodine use ($P > 0.05$). The period of no antibiotics use before harvesting was 133 days in DS ponds and 126 days in US ponds (Figure 2.3).

Table 2. 9: Pond management indicators expressed per kg feed (ww) consumed and per kg fish produced for downstream and upstream striped catfish ponds. Values are mean ± S.D.; n = 2.

Parameter		DS pond				US pond				
	Unit	Mean	±	SD	Min-Max	Mean	±	SD	Min-Max	p-value
Resource use										
Fingerlings use	# kg^{-1}fish	1.70	±	0.30	1.50-1.90	2.20	±	0.20	2.10-2.40	0.161
Water use	m^3 kg^{-1}feed	1.90	±	0.10	1.80-1.90	4.30	±	0.10	4.30-4.40	0.001
	m^3 kg^{-1}fish	2.80	±	0.10	2.70-2.90	7.10	±	0.0	7.12-7.14	0.001
Diesel oil use	ml kg^{-1}feed	1.10	±	0.10	1.10-1.20	0.50	±	0.01	0.40-0.60	0.013
	ml kg^{-1}fish	1.60	±	0.30	1.40-1.80	0.75	±	0.03	0.70-0.80	0.048
Electricity use	kWh kg^{-1}feed	0.02	±	0.01	0.02-0.03	0.09	±	0.01	0.08-1.00	0.007
	kWh kg^{-1}fish	0.04	±	0.01	0.03-0.04	0.14	±	0.01	0.10-0.15	0.004
Labour use	hr kg^{-1}feed	0.05	±	0.00	0.046-0.05	0.07	±	0.01	0.06-0.07	0.030
	hr kg^{-1}fish	0.06	±	0.00	0.050-0.07	0.11	±	0.01	0.10-0.12	0.035
Chemical use										
Lime ($CaCO_3$)	g kg^{-1}feed	8.50	±	0.30	8.30-8.70	25.40	±	2.70	23.50-27.30	0.013
	g kg^{-1}fish	14.40	±	3.60	11.80-16.90	41.90	±	5.10	38.30-45.50	0.025
Salt (NaCl)	g kg^{-1}feed	7.10	±	0.60	6.60-7.40	12.00	±	0.90	11.40-12.60	0.023
	g kg^{-1}fish	10.60	±	1.70	9.40-11.80	20.90	±	0.30	20.70-21.10	0.014
$CuSO_4$	g kg^{-1}feed	0.03	±	0.02	0.01-0.04	0.04	±	0.01	0.03-0.06	0.235
	g kg^{-1}fish	0.04	±	0.02	0.02-0.05	0.09	±	0.01	0.06-0.11	0.226
Iodine	g kg^{-1}feed	0.16	±	0.06	0.12-0.20	0.20	±	0.12	0.20-0.21	0.173
	g kg^{-1}fish	0.30	±	0.08	0.17-0.33	0.40	±	0.01	0.04-0.43	0.165
Antibiotic use	g kg^{-1}feed	0.04	±	0.01	0.03-0.05	0.09	±	0.01	0.08-0.97	0.020
	g kg^{-1}fish	0.06	±	0.01	0.05-0.07	0.15	±	0.01	0.14-0.16	0.020
Nutrient use efficiency										
DM retained	%	29.5	±	2.8	27.5-31.5	27.7	±	0.5	27.3-28.0	0.451
N retained	%	44.2	±	0.7	43.7-44.6	39.5	±	0.4	39.2-39.8	0.014
P retained	%	17.7	±	1.4	16.7-18.7	17.6	±	0.4	17.3-17.9	0.973
Nutrient discharge										
DM discharge	g kg^{-1}fish	414.7	±	42.0	384.6-444.9	356.5	±	19.3	342.8-370.2	0.221
	g kg^{-1}feed	278.2	±	49.9	242.9-313.6	216.5	±	14.9	205.9-227.1	0.236
N discharge	g kg^{-1}fish	19.8	±	2.0	17.2-22.9	20.1	±	4.0	18.4-21.2	0.943
	g kg^{-1}feed	13.3	±	1.7	12.1-14.5	12.0	±	1.4	11.1-13.0	0.493
P discharge	g kg^{-1}fish	17.0	±	1.6	15.8-18.1	17.7	±	0.2	17.6-17.8	0.579
	g kg^{-1}feed	11.3	±	0.2	11.2-11.4	10.7	±	0.1	10.7-10.8	0.054
Sludge removal frequency	# $cycle^{-1}$	3			-	1				-


(A)
Feeding in DS pond
Mortality
Antibiotics
NaCl
CaCO3
CuSO4
Iodine
Feed (kg), Fish mortality (10 x individual), antibiotic (g), CaCO3 (kg), NaCl (kg), CuSO4 (kg), Iodine (x10g)
5400
5100
4800
4500
4200
3900
3600
3300
3000
2700
2400
2100
1800
1500
1200
900
600
300
0
1
26
51
76
101
126
151
176
201
226
251
276
Culture day

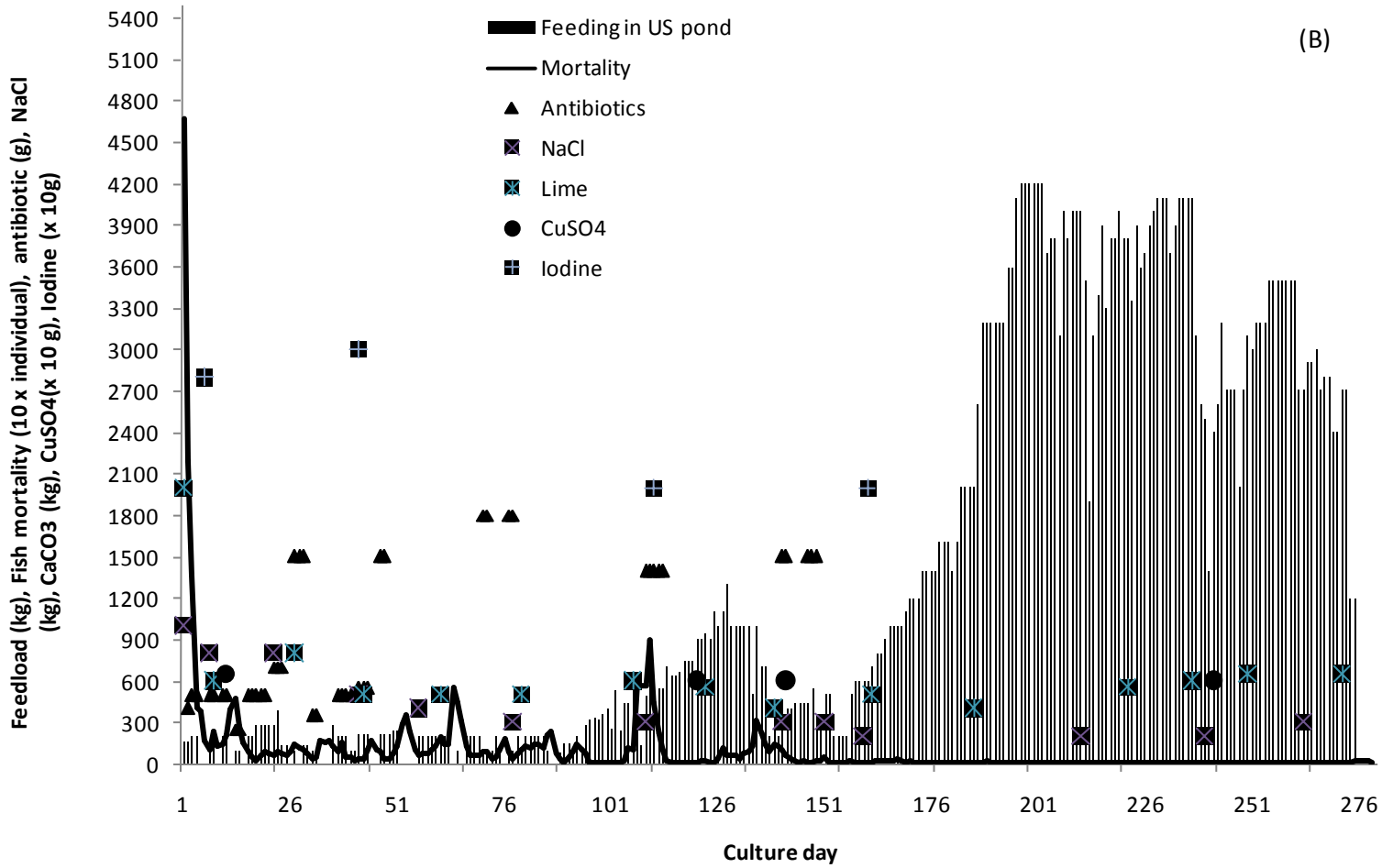


Figure 2. 3: Daily feed load, fish mortality, antibiotics, and chemicals use during the culture period in DS ponds (A) and US ponds (B).

2.4. Discussion

First the main outcomes of the sampling program were compared to reported values, followed by an analysis of observed differences between DS and US ponds. Secondly, the sustainability indicators for striped catfish pond culture were compared to reported indicators for other species, and options for improvement were explored.

2.4.1. Pond performance

None of the measured water quality parameters in the DS and US pond influent exceeded the limits set by the Vietnamese Ministry of Agriculture and Rural Development for striped catfish pond farming (MARD, 2003). Fish grew in 234–277 days from 31–46 g to 791–875g. The FCR over the full production cycle was on average 1.5 in DS ponds and 1.7 in US ponds, in agreement with earlier reported FCRs for striped catfish farming in the Mekong delta (Bosma et al., 2009; Phan et al., 2009). Observed differences in survival between DS and US ponds were possibly due to differences in management and water regime in the two locations. In all ponds, the fraction of input-P remaining unexplained over the complete production cycle remained below 2%, which is lower than reported by Adhikari et al. (2014) and Thakur and Lin (2003). A higher fraction of DM and N remained unexplained in the mass balance, because, contrary to P, not all possible sinks were measured. It is assumed that the unaccounted fractions of DM and N in the mass balances were mainly volatilized as CO_2 and N_2, respectively (Adhikari et al., 2014; Boyd, 1985; Funge-Smith and Briggs, 1998; Gross et al., 2000; Lin et al., 1997), and that other fractions in the mass balances were quantified with the same accuracy as for phosphorous.

2.4.2. Water exchange and water quality

Water exchange was one of the main factors influencing pond water quality. Water use in US ponds was 7.1 m^3 kg^{-1} fish, which was 2.5 times higher than the 2.8 m^3 kg^{-1}fishexchanged in DS ponds. Higher daily water exchange with the Mekong River in US ponds concurred with significantly lower pond concentrations for Kj-N, NO_2-N, total P, alkalinity, CO_2 and chlorophyll-a compared to US ponds. The observed difference in Kj-N concentration of 3.2 mg l^{-1} in influent in DS ponds compared to 6.2 mg l^{-1} in US ponds resulted in 16% additional protein load per kg feed in US ponds. Considering Kj-N concentrations in influent water, an

estimated 58g additional protein (13%) per kg feed entered DS ponds and 161g (58%) per kg feed entered US ponds. This might – depending on settling properties, in-pond water retention time and degradability – impact oxygen availability for fish and microbial respiration. If completely decomposed, 1 g protein consumes 1.77 g oxygen (Dalsgaard and Pedersen, 2011), which indicates an additional oxygen demand of 102 and 285 g O_2 kg $feed^{-1}$ in DS and US ponds, respectively. If only 10% of this protein would be degraded in the pond it means 35% and 59% more oxygen in DS and US ponds, respectively, will be required than the amount supplied by the influent water. Hence, the exchange water significantly increased pond oxygen demand.

The low difference in oxygen concentration of 4.9 mg l^{-1} in influent water to DS ponds versus 4.7 mg l^{-1} to US ponds, had a negligible effect on striped catfish growth performance. During the production cycle, growth performance remained favourable, even with DO dropping below 2 mg O_2 l^{-1} at 1m depth within 2 months after stocking. This is possible because striped catfish is an facultative air-breather (Lefevre et al., 2011c) for which dissolved oxygen in the water is less important for maintaining growth. To indicate this, we assumed an conservative oxygen consumption of 200 g O_2 per kg feed intake by striped catfish, similar to the air breathing African catfish (*Clarias gariepinus*) (Eding and Weerd, 1999). This concurs with a feed based oxygen consumption by striped catfish of 37.4 g O_2 m^{-2} d^{-1}. For the pond culture conditions in the current study the oxygen supply was estimated to be 0.96 g O_2 m^2 day^{-1} through water exchange, 21.5 g O_2 m^2 day^{-1} through primary production and 3 g O_2 m^2 day^{-1} through the water surface oxygen exchange. Oxygen consumption in the water column was estimated at 10.47 g O_2 m^2 day^{-1} and 12.42 g O_2 m^2 day^{-1} in the sediment. This leads to an oxygen deficit of 34.82 g O_2 m^2 day^{-1}, equalling 93% (34.82/37.4 * 100%) of the oxygen requirement of striped catfish in the pond (Table 2.10). This in part explains why the fish remained close to the surface, also in deep ponds (Lefevre et al., 2011b). Striped catfish prefers under non-fed conditions to consume oxygen from the water column provided there is sufficient oxygen (Lefevre et al., 2011c). However, under fed conditions, results show that striped catfish in the Mekong delta is produced under dissolved oxygen deficient conditions. This observation means that striped catfish can be produced under low water exchange conditions, because only 7% of the oxygen demand per kg feed (=14 g O_2 kg $^{-1}$feed) was provided. Assuming surface aeration is negligible then at 30 ^oC a flow rate of 7 to 8 m^3 kg^-

^{1}feed day^{-1} should be sufficient to cover the water oxygen demand of striped catfish (influent concentration 7.2 mg O_2 l^{-1} (95% saturated), effluent 4.5 mg O_2 l^{-1}). A higher flow rate of 12 m^3 kg^{-1} feed was reported for African catfish by Eding and Kamstra (2002), to control oxygen and ammonia and assuming the fish consumed 300 g O_2 kg feed^{-1}.

The average H_2S concentration was 0.1–0.2 mg l^{-1}, and similar in DS and US ponds, with peak concentrations reaching 0.5–0.8 mg l^{-1} (Table 2.4). Considering the NO_3-Nconcentrations in the ponds were low (0.3–0.5 mg l^{-1}), diffusion of NO_3-N into the flocculent bottom layer was too low to prevent H_2S formation under oxygen depleted conditions. The large water volume and daily exchange in the ponds helped in keeping H_2S concentrations low. The sludge removal frequency was sufficient in maintaining a favourable water quality as in our study no correlation was found between fish growth or fish mortality and H_2S concentration ($P > 0.05$). Nevertheless, the H_2S concentration increased (H_2S (mg l^{-1}) = 0.0016*culture day - 0.0192; $r^2 = 0.5649$; $P < 0.05$) during culture and was similar between DS and US locations ($P > 0.05$). Furthermore, the observed H_2S concentrations always remained below threshold concentrations reported to affect fish growth and fillet meat quality (Linh, 2011). H_2S concentrations were measured biweekly, and occasionally reached a peak value of 0.8 mg l^{-1}. Linh (2011) reported an LC_{10} for striped catfish of 0.83 mg l^{-1} over a 60 day observation period. Daily monitoring of H_2S concentrations and investigating possible chronical effects of low H_2S on striped catfish survival over the full production cycle requires further research.

The NO_2-N concentration was never higher than 1 mg l^{-1}, which is much lower than reported safe levels for striped catfish (Huong et al., 2011). The higher salinity of influent water to DS ponds (0.4 ppt; min–max: 0.1–1.2) compared to US ponds (0.06 ppt; min-max: 0.04–0.08) might have been beneficial. The latter increased survival in chinook salmon smolts significantly after a stress test; resulted in lower cortisol stress response and faster recovery from stress; reduced NO_2 toxicity (DS pond water: 0.3±0.3 mg l^{-1} NO_2-N; US pond water: 0.1±0.1 mg l^{-1}); improved the release from the blood of ammonia and other pollutants; cleared the gills from excess mucus; improved the release of ammonia from the blood; prevented the loss of body salt and aided body salt recovery; and increased the production of mucus, a higher mucus turnover helping to eliminate parasites (Clifford et al., 1977; Tomasso et al.,

1979). The tendency to observe better survival (P = 0.11) and yield (P=0.12) in DS ponds compared to US ponds might have been related to higher pond salinity (P < 0.05).

Table 2. 10: Estimated oxygen mass balance of an average downstream pond, based on average water quality, feed input and fish biomass considering the complete production cycle.

Parameter	g O_2 m^2 day^{-1}		% of fish respiration		Reference
	Input	Sink	Input	Sink	
Fish respiration (200 g O_2 kg $feed^{-1}$; average feed load 187 g feed m^2 day^{-1})		37.40		100.0	Eding and Weerd (1999)
Water exchange (4.9 – 1.2 = 2.7 g O_2 m^{-3}, 0.36 m^3 m^{-2})	0.96		2.60		Present study
Water column respiration (based on BOD_5 measurement)		10.47		28.0	Present study
Gross photosynthesis (assuming 3% chl-a in algae DM; 3.47 g O_2 g C $fixed^{-1}$)	21.51		57.05		Desortová (1981); Drapcho and Brune (2000)
Surface gas exchange (2 m s^{-1} wind speed at 30^0C)	3.00		8.00		Boyd & Tucker (2012)
Sediment oxygen demand (based on BOD_5 measurement)		12.42		33.20	Present study
DO deficit	34.82		93.10		Present study

2.4.3. Water quality maintenance for fish growth

Fish grew in 234–277 days from 31–46 g to 791–875g. The FCR over the full production cycle was on average 1.5 in DS ponds and 1.7 in US ponds, in agreement with earlier reported FCRs for striped catfish farming in the Mekong delta (Bosma et al., 2009; Phan et al., 2009). The fact that the specific growth rate (SGR) increased in DS ponds during the production cycle (Table 2.5) is contradictory to normal culture conditions for other fish species, including striped catfish. Temperature remained close to optimal during the culture period, and cannot explain the increase in SGR during culture. Fish mortality due to disease was high during period 1 (Table 2.2) and when disease occurred, feeding was lowered or even suspended (Phan et al., 2009). Ponds were not fed 15 and 17% of culture days in DS and US ponds,

respectively (Figure 2.3A & 2.3B). The larger feed input deficiency in US ponds concurred with a culture period of 277 days compared to 234 days in DS ponds. A longer culture period concurs with a higher labour and energy use, and delays income from fish sales. In this study, the longer culture period concurred with reduced feeding during disease treatment, either by application of antibiotics or $CuSO_4$ (Fig 3A & 3B). Improving biosecurity, stocking disease free fingerlings, controlling pathogens (incl. parasites) in the exchange water (Phan et al., 2009) and reducing transmission by possible hosts (Bondad-Reantaso et al., 2005), would contribute to better growth and feed utilization. Disease control in striped catfish pond culture remains a challenge, considering it is an open system connected with the Mekong River.

Striped catfish farmers prefer 1-ha 2–6 m deep ponds located along channels and rivers accessible by ships to supply feed and to transport harvested fish to the processing plant (Phan et al., 2009). The culture cycle starts with 2–3 m water depth during the first month, subsequently raising the depth to the maximum pond depth and maintaining this depth until the end of the production cycle. In deep ponds, water movement above the bottom is small, resulting in negligible resuspension of settled sludge. Fish driven resuspension is also negligible as the fish stays close to the surface. The farmer checks the height of the sludge layer above the sediment, and removes it when it becomes too high. Water exchange pipes are situated at ± 2 m depth, allowing to exchange large volumes of surface water, with minimum disturbance of the deep-water layers in the pond, especially the flocculent layer. By exchanging the surface water and disturbing the bottom water as little as possible, in-pond resuspension is minimal so that gill damage due to a high suspended solids water load is avoided. The choice to culture striped catfish in 4–6 m deep ponds developed as an adaptation to the semi-tidal conditions in the Mekong delta with two 3-hour tidal water exchange periods daily, allowing to use the first period to discharge and the second period to take in water. In consequence, between tides, the pond water volume is smaller than when the pond is full. By using deep ponds the risks of resuspension and a high suspended solid concentration in the water column is reduced thereby reducing risks of gill damage during the period of low water level. The sludge is removed when the conditions in the flocculent layer switch from a situation favoring denitrification to anaerobic fermentation. Through denitrification, a large fraction of the waste is volatilized in situ, keeping pollution per kg fish produced low (Phu and Tinh, 2012). Assuming that 2.86 g of COD is used to remove 1 g NO_3-N by

denitrification and that oxidation of 1 g OM (organic matter) requires 1.42 g oxygen (Henze et al., 1997), 14698 and 14818 kg of OM COD (30–39g COD kg feed^{-1} or 21–27g OM kg feed^{-1}) was on average removed from the DS and US ponds, respectively (Table 2.8; unaccounted N). Averaging for the whole pond area, 1.72 and 1.98 g NO_3-N m^{-2} day^{-1} was removed daily in US and DS ponds, respectively. This concurs with 4.95 and 5.66 g COD removal m^{-2} day^{-1}, and 0.11 and 0.13 equivalents of alkalinity production m^{-2} day^{-1} (1 mole NO_3-N removal equals 0.91 alkalinity equivalents, Henze et al, 1997) in US and DS ponds, respectively. The alkalinity production compensated a daily application of 9.1 and 10.8 g $NaHCO_3$ m^{-2} day^{-1} (1 alkalinity equivalent equals 83 g $NaHCO_3$) in US and DS ponds, respectively.

2.4.4. Sustainability indicators

Fingerling use. The mortality (calculated as the difference between the number of fingerlings stocked and fish harvested) of striped catfish in this study was higher than reported for channel catfish production in ponds, trout in raceways and tilapia in recirculation systems (Table 2.11). As discussed in section 4.3, reducing disease related mortality is important. Because the mortality occurred mainly during the first two months of the production cycle and fingerlings are cheap, farmers do not consider lowering mortality a priority. However, when taking into account the reduction in feeding days, the high variation in feed input (Figure 2.3) and the longer culture period, then the lack of disease free fingerlings and the higher feed conversion ratio than necessary turns out to be costly to the industry. Developing culture methods to produce fingerlings in closed recirculation systems could be instrumental in producing disease free and high quality fingerlings. This needs to coincide with avoiding contamination during transport to the grow-out ponds.

Water use. Water use per kg production in aquaculture varies over a broad range from 0.15 to more than 100 m^3 per kg fish produced (Verdegem and Bosma, 2009). This was 20–77 times smaller than for trout production in raceways and 3–14 times smaller than for intensive shrimp ponds (Table 2.11). Water use in semi-intensive channel catfish ponds was similar with striped catfish production in the Mekong delta. The water use is further reduced in recirculating aquaculture systems (RAS), where it was 12 -30 times lower than for striped catfish pond culture (Table 2.11) (Verreth and Oberdieck, 2009).

Fossil energy use. On-farm (fossil) energy use to produce 1 kg of striped catfish was 6–113 times lower than the other cultures listed in Table 2.11. This shows that culturing an air breeding species in ponds with tidal water exchange required considerable less on-farm energy input than any other type of fish culture (Boyd and Gross, 2000; Boyd and Tucker, 1998 ; d'Orbcastel et al., 2009a; Verreth and Oberdieck, 2009).

N-utilization efficiency. The N utilization efficiency of the feed was higher in striped catfish compared to channel catfish, shrimp, tilapia and trout culture (Table 2.11). Better N utilization efficiencies were obtained by integrating cultures or by switching to RAS using high quality feed (Table 2.11). N-discharge per kg fish produced from striped catfish ponds was 1.6–6.8 times lower than for other mono-cultures listed in Table 2.11. These differences in discharge when compared other species are much higher than for N utilization efficiency. A unique feature of the 4–6 m deep striped catfish ponds is the occurrence of denitrification at the bottom, which significantly reduced N-discharge. The present practice of the industry is to discharge directly to the river. If however, the sludge would be treated before discharge, then striped catfish farming would actually remove OM from the river. This would not be the case for N and P, because the majority of these nutrients are discharged with the effluent. In a lab scale experiment Anh and Mai (2009) reduced the TSS load in the effluent close to 20% by passing it through a stabilization pond operated with a retention time of 1 hour.

P utilization efficiency. The P utilization efficiency of the feed in striped catfish ponds was better than for shrimp or trout. However, the opposite was true for channel catfish and tilapia in RAS. Possibly, the use of plant ingredients in striped catfish feeds, lowered the P availability for fish growth (Cao et al., 2008; Gatlin et al., 2007; Hung et al., 2015; Kumar et al., 2012). Nevertheless, P discharge per kg striped catfish produced was similar to intensive culture of trout, channel catfish and shrimp, and higher than in polyculture ponds (Table 2.11). In semi-extensive polyculture ponds, a larger fraction of P accumulated in the sediment (Nhan et al., 2006; Nhan et al., 2008). In the deep striped catfish ponds 58 to 60% of the metabolic waste of P accumulated in the system or was taken out during sludge removal. If this P could be trapped in sedimentation ponds (Anh et al., 2010), transformation into fertilizer would become an option (Da et al., 2015; Phung et al., 2009). In addition, reducing the P content in the feed and improving P availability, as was done in trout feeds (Ketola and Richmond, 1994) could also contribute to a further reduction of P discharge.

Table 2. 11: Some key sustainability indicators of different species culture.

Species	Culture system	Mortality (%)	Water use m^3 kg^{-1}fish	Energy use kWh kg^{-1} fish	Labour use hr kg^{-1}fish	N retained % of input	P retained % of input	N discharge g kg^{-1}fish	P discharge g kg^{-1}fish
Striped catfish	Pond	23.6-49[(1)]	2.8-7.1[(1)]	0.04-0.14[(1)]	0.06-0.11[(1)]	43.7- 44.3[(1)]	17.6 - 17.7[(1)]	19.8-20.1[(1)]	17.0-17.7[(1)]
Channel catfish	Pond	6[(12)]	3.7[(2)]	0.9[(3)]	-	18.3-34.8[(4)]	20.4[(4)]	29.1[(5)]	10.6-15.4[(5)]
Tilapia	RAS	0.5[(6)]	0.24[(6)]	1.8[(6)]	0.013[(6)]	32[(6)]	43[(6)]	45.9[(6)]	8.3[(6)]
Trout	FT	0.1[(7)]	148-215[(8)]	1-1.7[(7)]	-	18.9[(8)]	13.2[(8)]	73.3-124.2[(8,13)]	11.0-25.6[(8,13)]
Intensive shrimp	Pond	22-50[(10)]	20-40[(9)]	4.5[(11)]	-	22.8-30.7[(10)]	10.5-12.8[(10)]	36.5-102[(10)]	11.6-18[(10)]
Salmon	Cages	-	-	-	-	28.2-30.0[(14)]	17.7-19.6[(14)]	80.0-84.7[(14)]	16.7-18.9[(14)]

(1): This study, (2): Boyd (2005), (3):Boyd et al. (2000), (4):Gross et al. (2000), (5):Gross et al. (1998), (6):Verreth and Oberdieck (2009), (7):d'Orbcastel et al. (2009b),(8):Foy and Rosell (1991),(9):Yoo and C.E.Boyd. (1994), (10): Thakur and Lin (2003), (11): Boyd and Tucker (1998), (12): Gross et al. (1998), (13):Warrer-Hansen (1982),Sumari (1982) and Solbe (1982):Hall et al. (1992) with commercial feed,(14) .

2.5. Conclusions and recommendations

Considering the sustainability indicators energy use, labour use and N and P retention and discharge, striped catfish production in deep ponds in the Mekong delta performed well in comparison to other aquaculture species and systems (Table 2.11). By developing the 2–6 m deep pond culture systems, striped catfish farmers made maximum use of the unique topography and water regime in the Mekong delta, allowing them to keep on-farm energy and labour inputs low, while realizing nutrient efficiencies that compare well to other major aquaculture species. This in part explains the success of striped catfish farming. Nevertheless, further improvements remain possible. The intensity of striped catfish farming makes it an excellent candidate to develop RAS technology that includes full control of waste streams resulting from culture (d'Orbcastel et al., 2009a). An additional advantage of culturing in RAS would be the reduction in water exchange with the Mekong River, which will minimize horizontal transmission of pollutants, parasites or diseases originating from other farms along the Mekong River.

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CHAPTER 3

Nutrient mass balances, water quality and water use of striped catfish (*Pangasianodon hypophthalmus*, Sauvage, *1878*) in flow-through and recirculation systems

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Abstract

This study compared sustainability indicators on animal and production performance, and calculated nitrogen (N), dry matter (DM), chemical oxygen demand (COD) and phosphorous (P) mass balances in flow-through (FT) and recirculation aquaculture systems (RAS) for striped catfish (*Pangasianodon hypophthalmus, Sauvage, 1878)*. Fish were cultured indoors in 0.85 m^3 tanks that were part of RAS and FT systems, using a standard commercial grow-out feed during one full production cycle (207 days).

All possible sources and sinks of nutrients were measured, except gas exchange. A similar feed conversion ratio (FCR 1.25-1.27), survival (93-96%) and product quality (e.g. fillet dress out percentage and color grade) were realized in RAS and FT. However, RAS performed significantly better than FT in sustainability indicators, using less water, showing higher nutrient utilization efficiencies and discharging less. Respectively 52, 57 and 74% of the N, DM and COD input in RAS could not be accounted in the mass balances. It was concluded that striped catfish culture in RAS is more sustainable than present pond and FT farming practices, and therefore merits further testing and gradual upscaling by the industry in the Mekong Delta.

Key words**:** *recirculating aquaculture system, pangasius, striped catfish, nutrient budget, water quality, flow-through system*

3.1. Introduction

Striped catfish (*Pangasianodon hypophthalmus, Sauvage, 1878)* is one of the key aquaculture products of Vietnam. Today, striped catfish is produced in 2-6 m deep earthen ponds in the Mekong Delta of Vietnam. In 2014, the total catfish production reached 1.1 million metric tons, using 5,500 ha of ponds. Processed striped catfish products have been exported to over 150 countries (MARD, 2014a). Pond production of striped catfish relies on 2 to 7 m^3 of water, exchanged with the Mekong river system, per kg fish produced (Nhut et al., Submitted-a).

The latter exchange is a potential source of pollutants, parasites and diseases and hence a threat to biosecurity. Disease related mortality is common and farmers rely on antibiotics and chemicals to minimize disease related mortality (Bosma et al., 2009; Nhut et al., Submitted-a). Although striped catfish production in ponds is effective, retaining on average 38% of nitrogen (N) and 14% of phosphorous (P) supplied with feed in fish biomass, and converting 46 – 51% of dietary N into N_2 gas through denitrification, the farms still discharge considerable amounts through water exchange and sludge removal (De Silva et al., 2010; Nhut et al., Submitted-a). Sludge removed from striped catfish ponds has a high mineral content and is diluted which limits options to re-use it as a fertilizer (Phu and Tinh, 2012).

Considering sustainability indicators, including water and fossil energy use, human labor, discharge of dry matter (DM), N, P, and use of $CuSO_4$, NaCl, iodine and antibiotics per kg fish produced, striped catfish farming in the Mekong delta performs equally well or better than other important culture species like tilapia, shrimp or trout (Nhut et al., Submitted-a). Striped catfish farming has the advantage of being intensive, producing on average > 200 MT per ha per year. Considering a feed conversion ratio in striped catfish ponds of 1.6 (Bosma et al., 2009), on average 320 MT of feed is applied per ha per year. In general, a 26% protein feed, containing 1.4% P by weight is used, resulting annually in 13,300 kg N and 4,480 kg P input per ha. Hence, the waste amount per unit surface area is very high, making striped catfish a good candidate to be grown in recirculating aquaculture systems (RAS). RAS technology has been adopted to minimize environmental impact from waste discharge, improve sustainability and enhance production (d'Orbcastel et al., 2009a; Verreth and

Oberdieck, 2009). In addition, biosecurity will be improved due to reduced or fully eliminated water exchange with the river.

The aim of this study was to estimate production, sustainability indicators and DM, N, COD and P mass balances for striped catfish grown in flow-through tanks (FT) and RAS covering a full production cycle from fingerling to market size.

3.2. Materials and methods

3.2.1. Experimental design

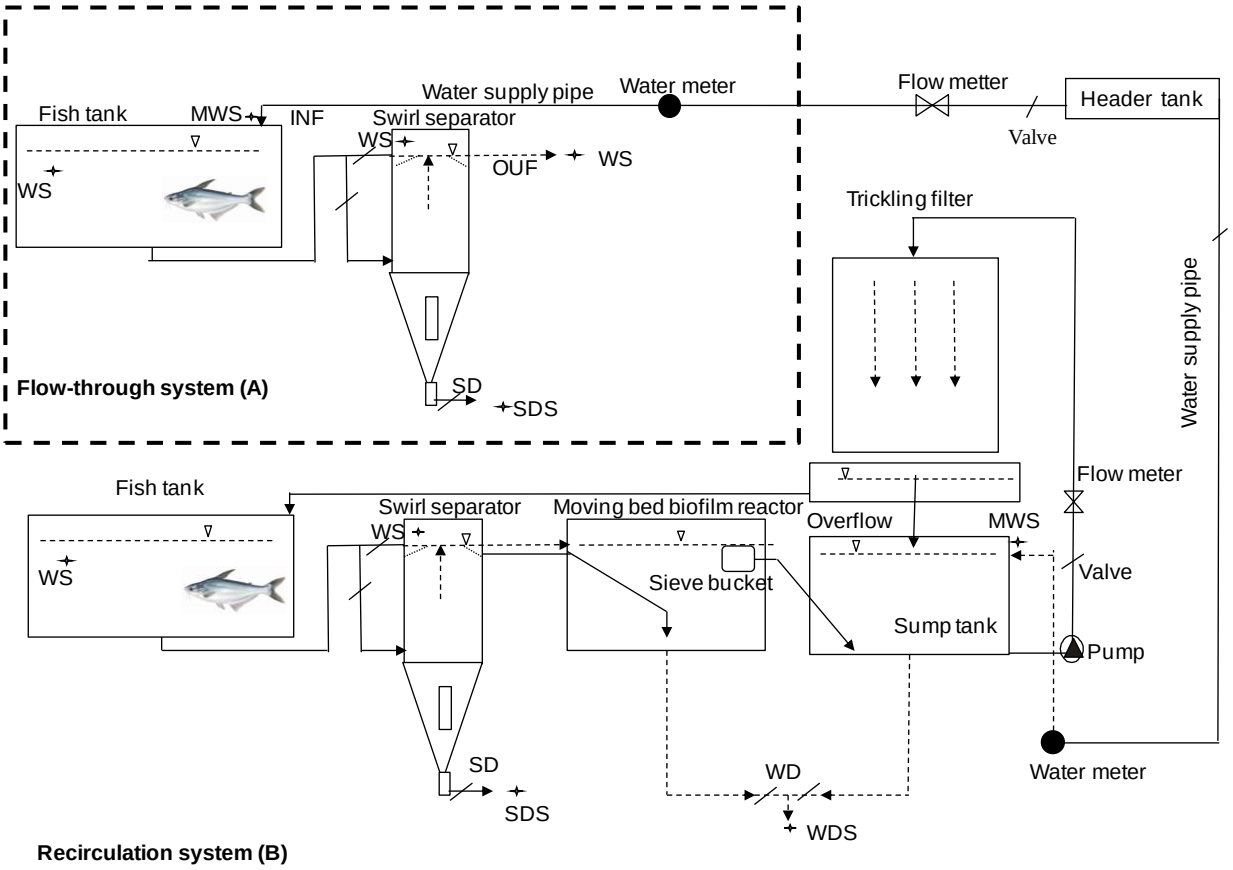


Figure 3. 1: A schematic of flow-through tanks (FT; A) and recirculating aquaculture systems (RAS; B) (not to scale). Sampling point (+); WS: water sampling; MWS: makeup water sampling; INF: inflow; OUF: outflow; SD: sludge discharge; SDS: sludge discharge sampling; WD: water discharge; WDS: water discharge sampling.

Table 3. 1:Components of flow-through tanks (FT) and recirculating aquaculture systems (RAS). n=3.

System component	Type	Unit	FT	RAS
Fish tank water volume	Round fiberglass tank: Ø 1.2m, height 0.75m, with central drain	m^3	0.85	0.85
Water flow through fish tank		m^3hr^{-1}	0.46±0.01	3.8±0.0
Swirl separator volume	Stainless steel: inner diameter (0.85m), inner surface area ($0.57m^2$)	m^3	0.26	0.26
Moving bed reactor	Round fiberglass tank with central drain	m^3		0.53
-Bio-media	*Bio-media type Helix 12mm PN10: SSA ($834m^2m^{-3}$) Fleuren&Nooijen, the Netherlands.*	*m^3*		*0.20*
-Specific surface area bio-media		*m^2m^{-3}*		*834*
-HSL moving bed reactor*		*md^{-1}*		*53.6*
Trickling filter reactor				
-Bio-media	*Bio-blok®-200 (EXPO-NET Danmark)*	*m^3*		*1.40*
-Specific surface		*m^2m^{-3}*		*200*
-HSL trickling filter*		*md^{-1}*		*200*
Sump tank	Round composite	m^3		0.58
Overflow tank	Stainless steel: 100cm x 100cm x 20cm (W x L x H)	m^3		0.20
Pipe volume	PVC pipe, Ø 90 mm	L	12.7	38.1
Total system volume		m^3	1.12	2.23
HRT*		hr	2.4±0.01	0.6±0.0
Flow meter	Type Z-4004, Ningbo KIO, China	unit	1	1
Water meter	ASAHI WVM 1/2" - Thailand	unit	1	1
Pump	250W/50Hz, EBARA ITALY - Submerged pump	unit		1

* HSL = hydraulic surface load; Ø: diameter. HRT: Hydraulic retention time in system.

Three replicate RAS and 3 FT tank systems, each holding an 850-L fish tank were purposely build for this experiment. Each RAS contained a fish tank, a swirl separator, a moving bed reactor, a trickling filter with overflow tank, and a sump with one pump, whereas each FT contained the same type of fish tank and swirl separator as the RAS. Water from a reservoir pond was passed first through a settling tank and subsequently through a 1200 m^2 sand bed filter pond, before being pumped into a 200 m^3 concrete overhead reservoir tank, from where it flowed by gravity to the RAS and FT tanks (Figure 3.1). One 1.5-kWh air-blower provided aeration to all fish tanks and moving bed reactors. Lay-out and connections between components in RAS and FT are shown in Figure 3.1, Table 3.1 and Table 3.5. All experimental units were operated indoors and exposed to natural day light through large windows at National Breeding Centre for Southern Freshwater Aquaculture of the Research Institute for Aquaculture No2 (RIA2) in Vietnam. The photoperiod was extended to 16 hours light using artificial light in the early morning and evening.

3.2.2. Experimental operation

In total, 1560 striped catfish fingerlings were obtained from RIA2. To remove possible ectoparasites, the fingerling were upon arrival stocked in 10 ‰ saline water in a concrete quarantine tank and treated with 37% formalin applied at 30 mg l^{-1} each 24hours during 7 consecutive days.

Two weeks before stocking fingerling in the RAS, the development of nitrifying bacteria in trickling filters and moving bed reactors was stimulated by adding daily ammonium chloride and sodium bicarbonate. Prior to stocking, the amount of total ammonia nitrogen (TAN) removed daily in each RAS was checked if sufficient to remove the amount of TAN released in each system due to the feed load applied on day one of the growth cycle. This was the case, so the fingerlings were stocked. To make water quality uniform between systems at the moment of stocking, the water volume in all systems was replaced with water from the overhead tank. In RAS, on days when fish showed low appetite, 3 - 10 % of the systemvolume was exchanged. In FT tanks water was exchanged continuously (Figure 3.2). The culture period was 207 days, starting on 17 November, 2011. In all systems, 260

fingerlings were stocked. For calculations in FT, a total system volume of 1.12 m^3 was used, representing the combined water volume in the fish tank, swirl separator and connection pipes.

Table 3. 2: Proximate composition of feed for striped catfish (in percentage of dry weight).

Feed[1] composition	Unit	Value
Pellet diameter[2]		
-period 1- 60 days	mm	2.0 - 2.5
-period 61-207days	mm	4.0 - 4.5
Dry matter	%	90.0
Crude protein	%	26.8
Crude lipid	%	7.0
Carbohydrate	%	49.0
Ash	%	7.2
AIA*	%	1.8
Gross energy	KJ g^{-1}DM	8.8

* Acid Insoluble Ash; DM: dry matter. [1]Feed is provided by Vinhhoan Corporation, National Road 30, Ward 11, Cao Lanh City, Dong Thap Province, Vietnam. [2]The same composition of feed was applied during the full culture period. Pellet size during the first 60 days had a 2.0-2.5 mm diameter, and from day 61 onwards a 4.0-4.5mm diameter.

In RAS, the combined water volume of all components totaled 2.23 m^3 (Table 3.1). Fish density and average body weight at stocking in FT and RAS are given in Table 3.5. Each day, fish were fed ad-libitum by hand at 9a.m. and 1, 5 and 9p.m. The same standard commercial striped catfish pond diet was used in RAS and FT (Table 3.2) during the whole culture period. Feeding behaviour was observed during feeding and feeding was stopped when fish showed low appetite to avoid sinking uneaten feed pellets to accumulate in the swirl separator.

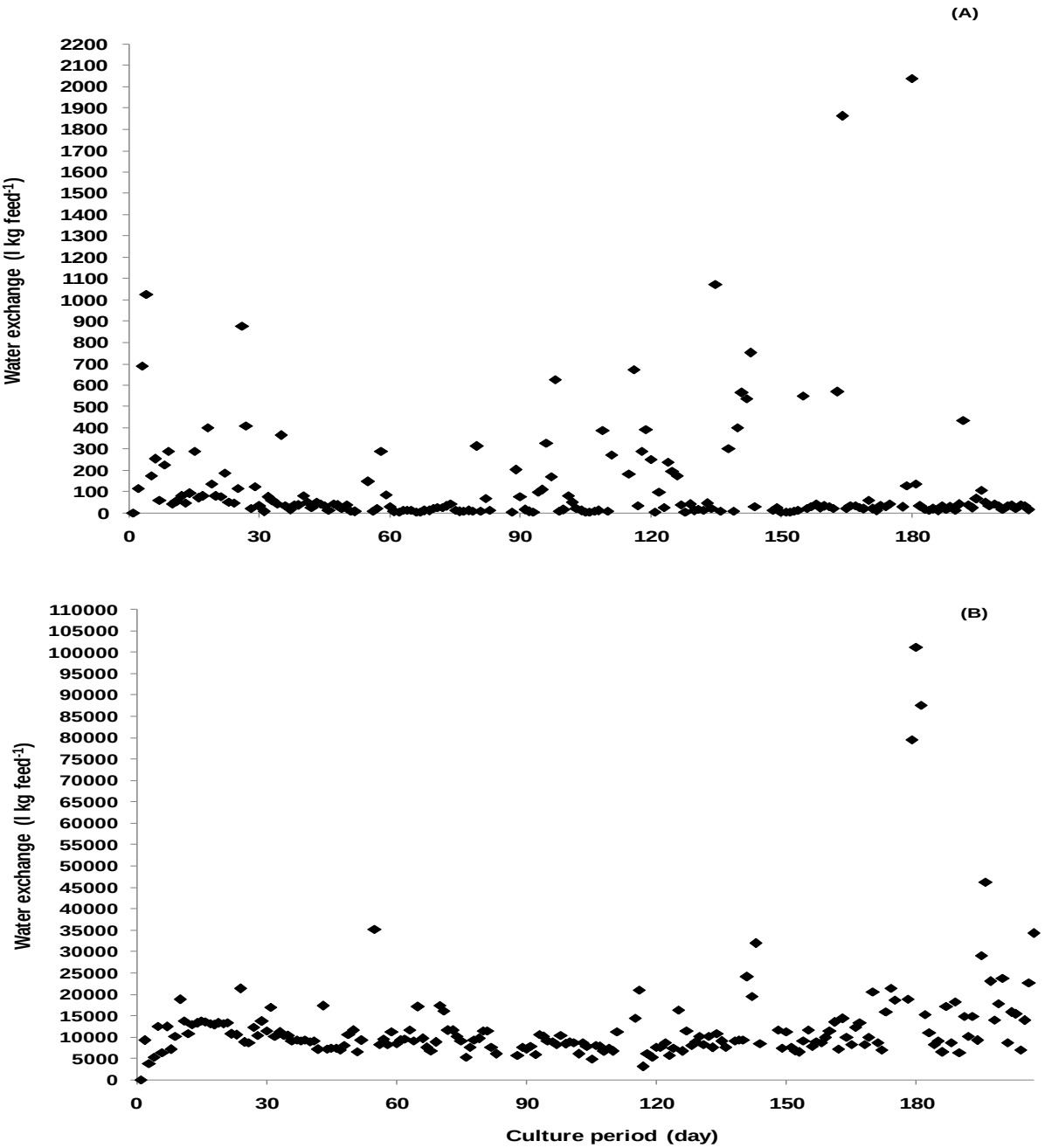


Figure 3. 2: Amount of water exchanged per kg feed per day in flow-through tanks (FT; B) and recirculating aquaculture systems (RAS; A). Values are mean; n=3.

Sampling

Starting the day of stocking, a semi-monthly sampling scheme was adopted. Fish in the tanks were constantly moving and touching the tank wall which could be felt laying hands on the outer tank surface. Any noise or movement around the systems made the fish move faster,

indicating a high sensitivity to disturbance. Therefore, to minimize disturbance, no samples was taking directly from the fish tank. The sampling points in FT and RAS are shown in Figure 3.1. In both FT systems and RAS, samples were collected of outflow water from the fish tank when entering the swirl separator and at the bottom outlet of the swirl separator. In FT, samples of water coming from the reservoir tank just before entering the fish tank were also collected.

In RAS, samples were collected from an outlet connecting the bottom outlet of the moving bed reactor and of the sump tank, and of the make-up water coming from the reservoir tank before entering the sump tank. On each bi-monthly sampling day, samples were collected at all sampling points in all systems before the first feeding of the day, except for bottom samples from the swirl separator (explained below). In addition, when 3 – 10 % of RAS water was exchanged, the discharged water was sampled and the volume recorded.

On semi-monthly sampling days, 15 fish were collected randomly from each RAS and FT, anaesthetized (MS22-Sigma, 50 mg l^{-1}tricaine methanesulfonate (98%)) and batch weighed to estimate the average body weight. After measuring, 12 fish were returned to the fish tank and three fish were culled and pooled to determine the proximate composition (crude protein (CP), total phosphorus (TP), crude lipid (CL), carbohydrate, dry matter (DM) and ash). These data were used to document proximate body composition of striped catfish in function of individual fish biomass.

At harvest, all fish per system were counted and batch weighed to determine the average individual body weight. In addition, 15 fish were anaesthetized and processed to record fillet yield, fillet coloration and off-flavour.

Sludge was collected from the swirl separators in RAS and FT every 4 hours to quantify the daily sludge production. In addition, on semi-monthly sampling days, the 4-hr samples were kept at 4^{o}C, pooled at the end of the day and homogenized to analyze DM, organic matter (OM), ash, chemical oxygen demand (COD), total organic carbon (TOC), total carbon (TC), N and TP. During the culture period, feed was collected on day 1, 61 and 180 to measure DM, TP, CP, CL, gross energy, carbohydrate, acid insoluble ash (AIA) and ash.

3.2.3. Measurements and analyses

Water

Dissolved oxygen (mg l^{-1}) (DO), pH and water temperature (^{o}C) were measured daily at 8 a.m. in swirl separators and make-up water, using a multi-parameter meter HI9828, Hanna Instruments, Rhode Islands, USA.

Semi-monthly collected water samples from swirl separators and make-up water were analyzed following APHA (1999) for COD (dichromate reflux), 5-day biological oxygen demand (BOD_5), salinity (Salinity Refractometer Model 2493 Master-S/Mill M – Atago-Japan), TOC (high temperature combustion method on acidifying sample to inorganic carbon), TC (high temperature combustion method), carbon dioxide (CO_2,free CO_2 reacts with sodium hydroxide to form sodium bicarbonate), total alkalinity (TA, titration with sulfuric acid and methyl orange indicator), Kjeldahl N (TKN; Kjeldahl method), TAN (colorimetric method), NO_2-N (colorimetric method with diazotized sulfanilamide), NO_3-N (cadmium reduction to nitrite and measurement of nitrite), hydrogen sulfide (H_2S, photometric method), total suspended solid (TSS, dried to constant weight at 103 - 105 ^{o}C), chlorophyll-a (spectrophotometer method), orthophosphate (PO_4-P,ammonium molyndate and potassium antimonyl tartrate method) and TP (photometric method).

Fish, feed and sludge

Whole individual fish was analyzed for DM, CP, CL, TP and ash. Before analysis, feed rests in stomach and intestine were removed, and then each fish was minced and homogenized. Observed dead fish were collected, weighted and counted, and the associated nutrient content was calculated based on the semi-monthly measured proximate composition. The fifteen fish collected per system at the end of the culture period were filleted by hand by an employee of Vinh Hoan company[1], and fillet yield and color grade were determined according to Sang et al. (2012). The grade of fillet coloration was defined as white (score 1), pink (score 2) and yellow (score 3). Off-flavor of fillet was determined by an employee of a processing company, and was defined as good-flavor (score- 1) and off-flavor (score 2). Feed also minced and homogenized for proximate composition (DM, TP, CP, CL, carbohydrate, acid

[1]VinhHoan Corporation, National Road 30, Ward 11, Cao Lanh City, Dong Thap Province, Vietnam.

insoluble ash (AIA) and ash). Sludge was homogenized to analyze DM, OM, TP, TC, TOC, Kjeldahl N and ash.

The DM was calculated by gravimetric analysis after drying at 105 ^{o}C for 24 hours (Foy and Rosell, 1991). The ash in whole fish, feed and sludge were analyzed according to APHA (1999). The OM in sludge was calculated as the weight difference between DM and ash content (after burning at 550^{o}C) according to APHA (1999). The TKN was analyzed by the Kjeldahl method (Foy and Rosell, 1991). The CP in feed was calculated by 6.25 x TKN. COD in sludge was analyzed according to APHA (1999).

The TP in whole fish, feed and sludge was analyzed spectrophotometrically following Kitson and Mellon (1944). The CL in whole fish and feed were analyzed by acid-hydrolysis Soxhlet method (AOAC, 2000). For sludge, the TC was determined by high temperature combustion method, the TOC by high temperature combustion method on acidified samples according to APHA (1999).

The carbohydrate in feed was determined as the difference in DM content minus CP, ash and fat. The AIA in feed was analyzed according to AOAC (2000).

3.2.4. Calculations and statistics

Calculations

Details of calculations related to water quantity and quality, chemical application, energy consumption, fish growth and production, nutrient utilization and nutrients mass balances in FT and RAS are given in Table 3.3.

Table 3. 3: Units and formulas.

Parameter	Unit	Formulas
Resource utilisation		
Water use (WU)	l kg^{-1}fish	$WU = V_{tot\ inflow} / (W_{totfinal} - W_{totinitial})$
	l kg^{-1}feed	$WU = V_{tot\ inflow} / FC$
Chemical use (CU)	l kg^{-1}fish	$CU = M_{tot\ chemical} / (W_{totfinal} - W_{totinitial})$
	l kg^{-1}feed	$CU = M_{tot\ chemical} / FC$
Energy use (EU)	kWhkg^{-1}fish	$EU = E_{tot\ electricity} / (W_{totfinal} - W_{totinitial})$
	kWhkg^{-1}feed	$EU = E_{totelectricity} / FC$
Labour use (LU)	hrkg^{-1} fish	LU = total labour hours / $(W_{tot\ final} - W_{tot\ initial})$
	hrkg^{-1}feed	LU = total labour hours / FC
Fingerlings use (FU)	#kg^{-1}fish	$FU = N_{tot\ initial} / (W_{totfinal} - W_{totinitial})$
Nutrient utilisation		
N, TP, DM and total COD in fish biomass		
- Initial fish ($Nu_{initial\ fish}$)	g	$Nu_{initial\ fish} = 1000 * C_{Nu\ initial\ fish} /100 * W_{totinitial}$
- Final fish ($Nu_{final\ fish}$)	g	$Nu_{final\ fish} = 1000 * C_{Nu\ final\ fish} /100 * W_{totfinal}$
- Mortality ($Nu_{mortality}$)	g	$Nu_{mortality} = 1000 * C_{Nu\ fish\ mortality}/100 * W_{tot\ mortality}$
Nutrient utilisation efficiency (NuUE) of N, P, DM and COD	%	$NuUE = [\{(Nu_{final\ fish} - Nu_{initial\ fish})/1000\} / \{C_{Nu\ in\ feed} * FC / 100\}] * 100$
Waste discharge		
Nutrient discharge in FT (NuDF)of N, P, DM and COD	gkg^{-1}fish	$NuDF = [[(C_{Nufeed} /100 * FC*1000)*\{1-(NuUE / 100)\}] - Nu_{mortality}] / (W_{totfinal} - W_{totinitial})$
Nutrient discharge in RAS (NuDR)of N, P, DM and COD	gkg^{-1}fish	$NuDR = [(C_{Nuoutflow} * V_{totoutflow} / 1000) + (C_{Nu\ dry\ sludge} * M_{tot\ sludge}) - (C_{Nuinflow} * V_{totinflow} / 1000)] / (W_{totfinal} - W_{tot\ initial})$
Fish growth performance		
Total initial fish biomass ($W_{tot\ initial}$)	kg	$W_{tot\ initial} = N_{tot\ initial} * W_{I\ initial}$
Total final fish biomass ($W_{tot\ final}$)	kg	$W_{totfinal} = N_{tot\ final} * W_{I\ final}$
Initial density (ID)	kgm^{-3}	$ID = W_{tot\ initial} / V_{tot\ fish\ tank}$
Final density (FD)	kgm^{-3}	$FD = W_{tot\ final} / V_{tot\ fish\ tank}$
Total dead fish biomass ($W_{tot\ mortality}$)	kg	$W_{tot\ mortality}$ = cumulative weight of dead fish in kg
Survival (SR)	%	$SR = 100 * N_{tot\ final} / N_{tot\ initial}$
Mortality (M)	%	M = 100 – SR
Geometric mean body weight (Wg)	g	$W_g = e^{[(\ln(W_{Ifinal} * 1000) + \ln(W_{Iinitial} * 1000))/2]}$
Specific growth rate (SGR)	%bwd^{-1}	$SGR = 100 * (\ln W_{I\ final} - \ln W_{I\ initial}) / D$

Table 3.3 (continued- 1): Units and formulas.

Parameter	Unit	Formulas
Feed conversion ratio (FCR)	-	$FCR = FC/(W_{totfinal} - W_{totinitial})$
Metabolic feeding rate (MFR)	$g\ kg^{-0.8}d^{-1}$	$[1000*FC/\ \{(N_{tot.initial}+N_{tot\cdot final})/2\}] * \{(W_g/1000)^{-0.8}\}\ /D$
Metabolic growth rate (MGR)	$g\ kg^{-0.8}d^{-1}$	$(W_{Ifinal} - W_{Iinitial}) * \{(W_g/1000)^{-0.8}\}\ /D$
Harvested fish quality		
Fillet yield (FY)	%	$FY = 100*(FW/W_{I\ final} * 1000)$
Fillet colouration (FIC)	#	$FIC = \{(1*n_{white})+ (2*n_{pink})+(3*n_{yellow})\}\ /n$
Off-flavor (OFL)	#	$OFL = \{(1*n_{good\text{-}flavor}) + (2*n_{off\text{-}flavor})\}\ /n$
tCOD in feed and fish		
COD crude protein[a] (COD_{CP})	$g\ O_2g^{-1}CP$	$COD_{CP} = 1.66* CP$
COD crude fat[a] (COD_{CF})	$g\ O_2g^{-1}CF$	$COD_{CF} = 2.78* CF$
COD carbohydrate [b] (COD_{St})	$g\ O_2g^{-1}Carbo$	$COD_{St} = 1.19*Carbo$
tCODfeed (fish)	$g\ O_2g^{-1}feed(fish)$	$tCODfeed(fish) = COD_{CP} + COD_{CF} + COD_{carbo}$
Nutrients mass balance		
<u>At fish level</u>		
Nutrient in feed (NU_{feed})	$gkg^{-1}feed$	$NU_{feed} = [(C_{Nufeed}\ /100) * FC * 1000]/FC$
Nutrient retained in fish ($NU_{fish\ retained}$)	$gkg^{-1}feed$	$NU_{fish\ retained} = [\{(C_{Nufinal\ fish}\ /100)* W_{totfinal}\} - \{(C_{Nuinitial\ fish}/100)* W_{totinitial}\} * 1000]/FC$
Nutrient in dead fish ($NU_{fish\ mortality}$)	$gkg^{-1}feed$	$NU_{fishmortality} = (C_{Nu\ mortality}\ /100\ )* W_{tot\ mortality} * 1000\ /FC$
<u>At system level</u>		
Input (NU_{input})	$gkg^{-1}feed$	$NU_{input} = NU_{MW} + NU_{influent}$
Nutrient not retained in fish (NU_{MW})	$gkg^{-1}feed$	$NU_{MW} = NU_{feed} - (NU_{fish\ retained} + NU_{fishmortality})$
Nutrient in influent ($NU_{\ influent}$)	$gkg^{-1}feed$	$NU_{influent} = C_{Nuinflow} *V_{totinflow}\ /1000\ /FC$
Output (NU_{output})	$gkg^{-1}feed$	$NU_{output} = NU_{effluent} + NU_{sludge} + NU_{unaccounted}$
Nutrient in effluent in RAS ($NU_{effluent\ RAS}$)	$gkg^{-1}feed$	$NU_{effluentRAS} = C_{Nuoutflow} * V_{totoutflow} / 1000 / FC$
Nutrient not measured in FT ($NU_{effluent\ FT}$)	$gkg^{-1}feed$	$NU_{effluent\ FT} = NU_{MW} + NU_{influent} - NU_{sludge}$
Nutrient in sludge (NU_{sludge})	$gkg^{-1}feed$	$NU_{sludge} = (C_{Nu\ dry\ sludge}\ /100)* M_{tot\ sludge}\ /FC$
Nutrient not measured in RAS ($NU_{\ no\ measurement}$)	$gkg^{-1}feed$	$NU_{\ no\ measurement} = NU_{input} - NU_{effluent} - NU_{sludge}$

[a]Based on Henken et al. (1986), [b]stoichiometric oxygen demand calculated according to Tchobanoglous et al. (2004). **tCODfeed(fish):** total COD in feed consumption and whole fish (gO_2g^{-1} feed(fish)); **CF**: crude fat (g); $\mathbf{C_{Nu\ dry\ sludge}}$: nutrients percentage of N,TP, DM and COD in sludge (% of dry sludge); $\mathbf{C_{Nu\ final\ fish}}$: nutrient percentage of N, TP, DM and COD in fish harvested (% of wet body weight); $\mathbf{C_{Nu\ in\ feed}}$: nutrient percentage of N, TP, DM and COD in feed (% of dry feed); $\mathbf{C_{Nuinflow}}$: nutrient concentration in N,TP, DM and COD in influent water (gm^{-3}); $\mathbf{C_{Nu\ initial\ fish}}$: nutrient percentage of N, TP, DM and COD in fish stocked (% of wet body weight); $\mathbf{C_{Nu\ mortality}}$: nutrient percentage of N, TP, DM and COD in dead fish (% of wet body weight); $\mathbf{C_{Nuoutflow}}$: nutrient concentration in N,TP, DM and COD in discharge water ($g\ m^{-3}$); **CP**: crude protein (g); **D**: days of culture period (d); $\mathbf{E_{tot\ electricity}}$: total electricity consumption including light, air-blower, water pumping and other activities during full production cycle per experimental unit (kWh); **FC:** cumulative feed input (kg); **FW**: average weight of complete skinless fillet from one individual after removing fat and red muscle following standard process

for export market (g); $\mathbf{M_{tot\ chemical}}$: total amount of chemicals applied including sodium bicarbonate and sodium chloride (g); $\mathbf{M_{tot\ sludge}}$: total weight of dry sludge (g); **n**: number of fish in sample (#); $\mathbf{n_{good\text{-}flavor}}$: number of fish in sample with no off-flavor (#); $\mathbf{n_{off\text{-}flavor}}$: number of fish with off-flavor (#); $\mathbf{n_{pink}}$: number of fish with pink fillet (#); $\mathbf{N_{tot\ final}}$: total number of fish harvested (#); $\mathbf{N_{tot\ initial}}$: total number of fish stocked (#); $\mathbf{n_{white}}$: number of fish with white fillet (#); $\mathbf{n_{yellow}}$: number of fish with yellow fillet (#); $\mathbf{Nu_{dead\ fish}}$: amount of N, P, DM or COD in fish biomass collected during production cycle (g); $\mathbf{Nu_{final\ fish}}$: amount of N, P, DM or COD in fish biomass harvested (g); $\mathbf{Nu_{initial\ fish}}$: amount of N, P, DM or COD in fish biomass stocked (g); **St:** starch (g); **Carbo**: Carbohydrate (g); **total labour hours**: accumulative hours of human labour per experimental unit during full production cycle (hr); $\mathbf{V_{tot\ fish\ tank}}$: water volume fish tank (m^3); $\mathbf{V_{tot\ inflow}}$:total water volume during culture period (l); $\mathbf{V_{totoutflow}}$:total volume of water discharge (l); $\mathbf{W_{Iinitial}}$: individual initial body weight (kg); $\mathbf{W_{Ifinal}}$: individual final body weight (kg).

Statistics

Water quality parameters were averaged over the complete culture period. The daily or semi-monthly sludge production and waste discharge were summed over the complete culture period, as were water, chemical and energy use. Fish growth parameters and mass balances were calculated by experimental unit. The amount of sludge collected by feeding load and the body composition (CP, CL, DM, TP and Ash) by whole fish wet body weight were analysed by regression. Results for FT and RAS were compared by one-way ANOVA ($P < 0.05$) with the SPSS version 11.

3.3. Results

3.3.1. Water quality in flow-through tanks (FT) and recirculating aquaculture systems (RAS)

Table 3. 4: Water quality in flow-through tanks (FT) and recirculating aquaculture systems (RAS). n=3.

Parameter	Unit	FT			RAS			p-value
		Mean	±	SD	Mean	±	SD	
Daily water quality (n= 207):								
pH		7.5^{b}	±	0.3	7.7^{a}	±	0.3	0.001
Temperature	^{o}C	31.7^{a}	±	1.1	28.7^{b}	±	1.1	0.001
Dissolved oxygen	mgl^{-1}	3.2^{b}	±	1.3	5.4^{a}	±	0.9	0.001
Semi-monthly water quality (n=30):								
Alkalinity	mg $CaCO_3$ l^{-1}	246.7^{a}	±	1.4	134.8^{b}	±	20.0	0.001
Carbon dioxide (CO_2)	mgl^{-1}	14.3^{a}	±	0.7	17.5^{a}	±	1.9	0.081
Total nitrogen (TN)	mgl^{-1}	4.3^{b}	±	0.4	58.8^{a}	±	4.0	0.001
Total ammonia nitrogen (TAN)	mgl^{-1}	1.5^{a}	±	0.1	1.0^{b}	±	0.1	0.001
Nitrite nitrogen (NO_2-N)	mgl^{-1}	0.2^{b}	±	0.1	0.7^{a}	±	0.1	0.001
Nitrate nitrogen (NO_3-N)	mgl^{-1}	3.2^{b}	±	0.7	52.8^{a}	±	2.4	0.001
Total phosphorus (TP)	mgl^{-1}	1.4^{b}	±	0.8	24.1^{a}	±	5.4	0.003
Orthophosphate (PO_4-P)	mgl^{-1}	0.5^{b}	±	0.1	20.7^{a}	±	5.3	0.002
Total suspended solids (TSS)	mgl^{-1}	6.6^{b}	±	0.8	31.9^{a}	±	0.3	0.001
Chemical oxygen demand (COD)	mgl^{-1}	35.1^{a}	±	0.4	32.2^{a}	±	1.4	0.061
Total organic carbon (TOC)	mgl^{-1}	9.3^{b}	±	0.2	16.7^{a}	±	1.8	0.001
Total carbon (TC)	mgl^{-1}	78.2^{a}	±	1.1	58.8^{b}	±	5.0	0.002
Biological oxygen demand (BOD_5)	mgl^{-1}	5.0^{b}	±	0.1	16.6^{a}	±	0.3	0.001
Hydrogen sulfide (H_2S)	mgl^{-1}	0.01^{b}	±	0.0	0.11^{a}	±	0.0	0.001
Salinity	‰	0.0^{b}	±	0.0	2.6^{a}	±	0.1	0.001

SD = standard deviation. Means with different superscript within each row are significantly different ($P < 0.05$).

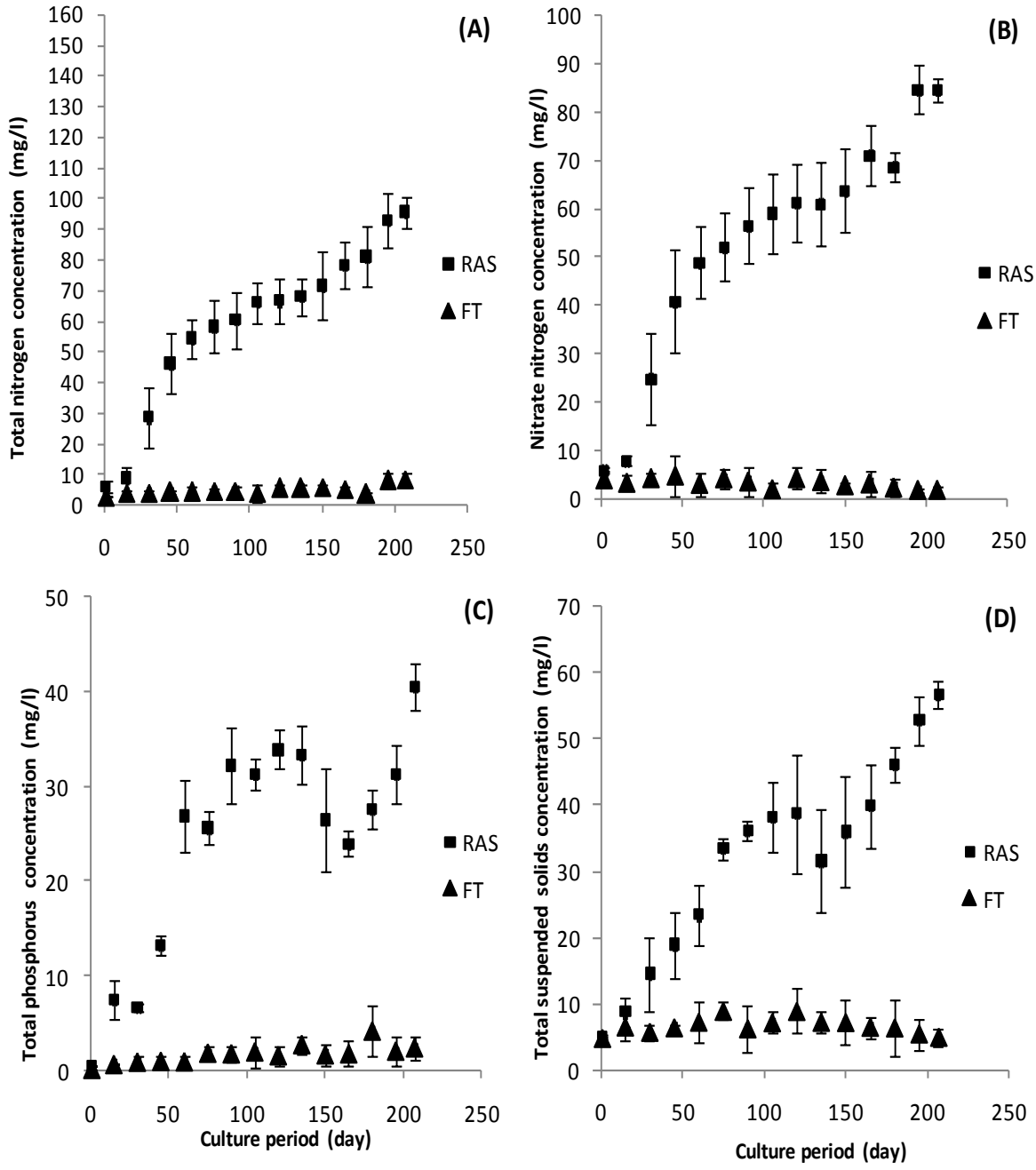


Figure 3. 3: Accumulation of TN (A), NO_3-N (B), TP (C) and TSS (D) in recirculation systems (RAS) and flow-through (FT). Values are mean ± SD; n=3.

Water quality parameters were different between FT and RAS ($P < 0.05$), except for CO_2 ($P > 0.05$) and chemical oxygen demand (COD) ($P > 0.05$) (Table 3.4). The temperature, alkalinity, TAN and TC were lower in RAS than in FT ($P < 0.05$). In contrast, pH, DO, TN, NO_2-N, NO_3-N, P, TSS, BOD_5, H_2S and salinity were higher in RAS than in FT ($P < 0.05$).

In RAS, TN, NO_3-N, TP and TSS accumulated during the culture period (Figure 3.3). In FT, these nutrients did not accumulate.

3.3.2. Fish performance

3.3.2.1. Fish growth

Striped catfish grew slower and ate less in RAS than in FT ($P < 0.05$). Survival and feed conversion ratio (FCR) were similar in FT and RAS ($P > 0.05$). Fish quality parameters were also similar between systems ($P > 0.05$) (Table 3.5).

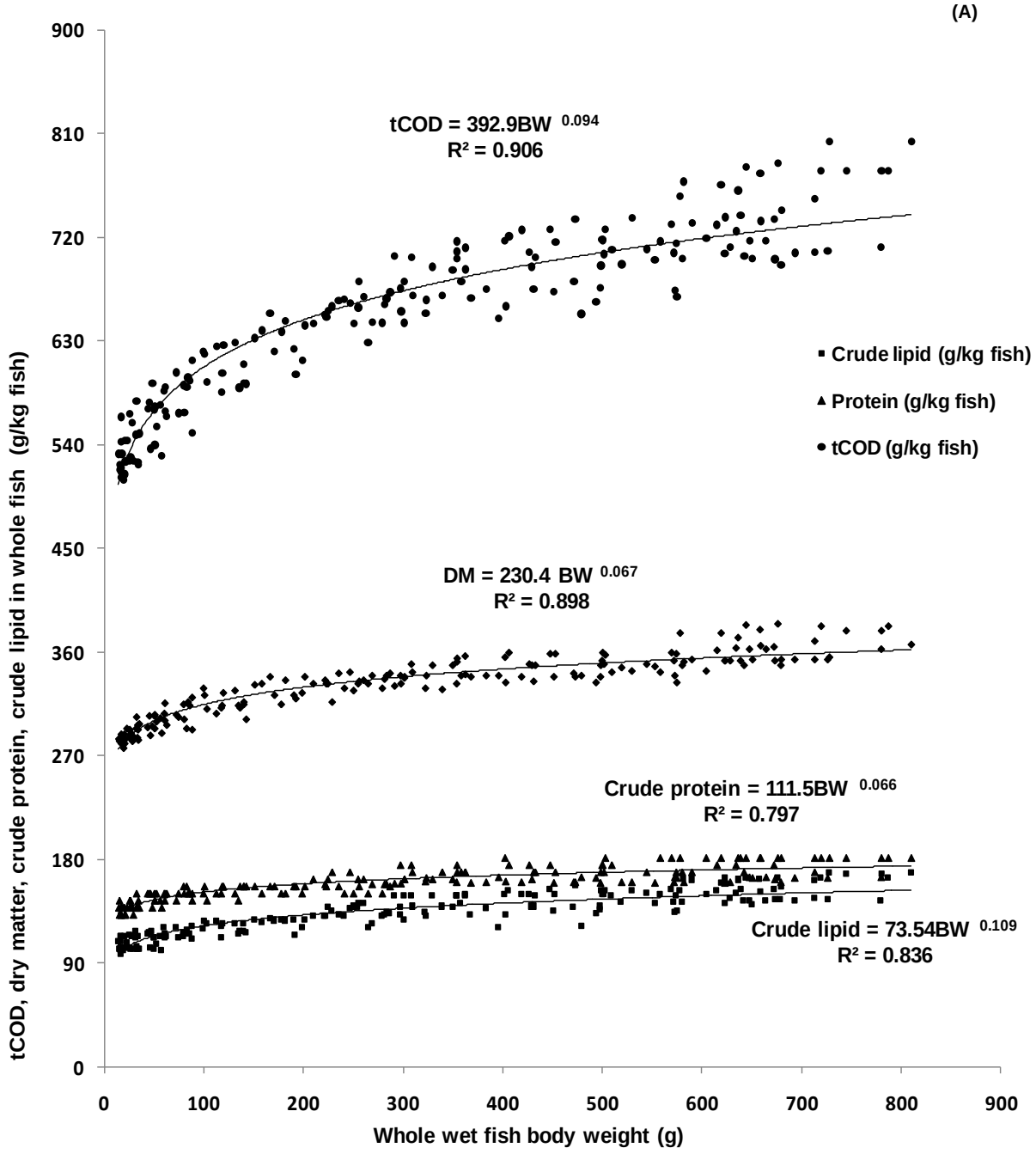
Table 3. 5: Striped catfish performance parameters in flow-through tanks (FT) and recirculating aquaculture systems (RAS). n=3.

Parameter	Unit	FT			RAS			p-value
		Mean	±	SD	Mean	±	SD	
Fish growth:								
Average body weight	g ind^{-1}	15.6	±	1.0	18.0	±	2.6	
Stocking density	kg m^{-3}	4.8	±	0.3	5.5	±	0.8	0.196
Fish per experimental unit	# $system^{-1}$	260			260			
Final individual weight	g ind^{-1}	738.60[a]	±	274.90	659.40[b]	±	297.60	0.040
Fish density at harvest	kg m^{-3}	196.60[a]	±	9.90	171.40[a]	±	12.60	0.053
Survival	%	95.76[a]	±	1.76	93.84[a]	±	2.69	0.359
Specific growth rate	% bw d^{-1}	1.86[a]	±	0.04	1.74[b]	±	0.06	0.040
Feed conversion ratio	-	1.27[a]	±	0.10	1.25[a]	±	0.10	0.716
Feeding rate	g $kg^{-0.8}d^{-1}$	25.62[a]	±	0.38	21.80[b]	±	1.08	0.005
Growth rate	g $kg^{-0.8}d^{-1}$	20.87[a]	±	0.94	18.27[b]	±	0.92	0.027
Fish quality:								
Fillet percentage	%	37.80[a]	±	1.40	37.20[a]	±	2.00	0.502
Fillet coloration*	-	1.00		-	1.00		-	-
Off-flavor*	-	1.00		-	1.00		-	-

* Off-flavor of fillet was defined as good-flavor (score 1) and off-flavor (score 2). Fillet coloration was defined as white (score 1), pink (score 2) and yellow (score 3). S.D. = standard deviation. Means with different superscript within each row are significantly different ($P < 0.05$).

3.3.2.2. Fish body composition

The fish body composition in RAS and FT was similar ($P > 0.05$) and changed with fish size (Figure 3. 4). Regression explained at least 83% of the variation in the data set.



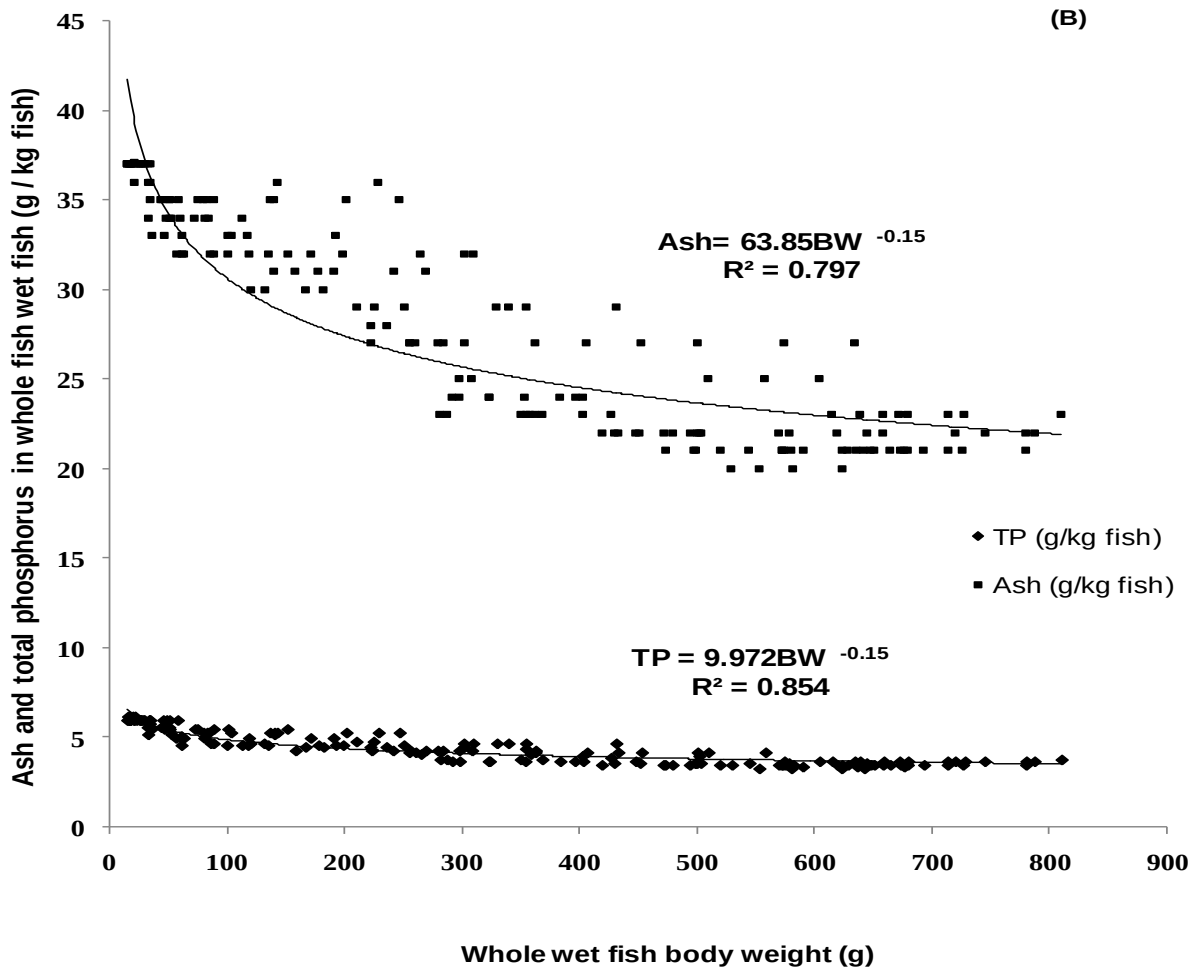
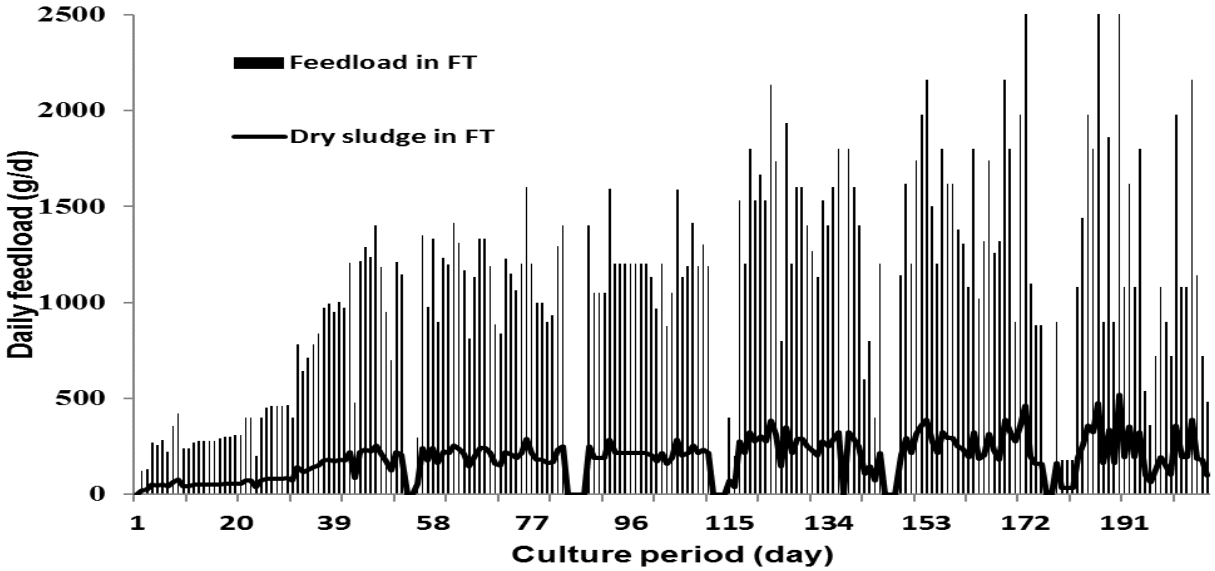
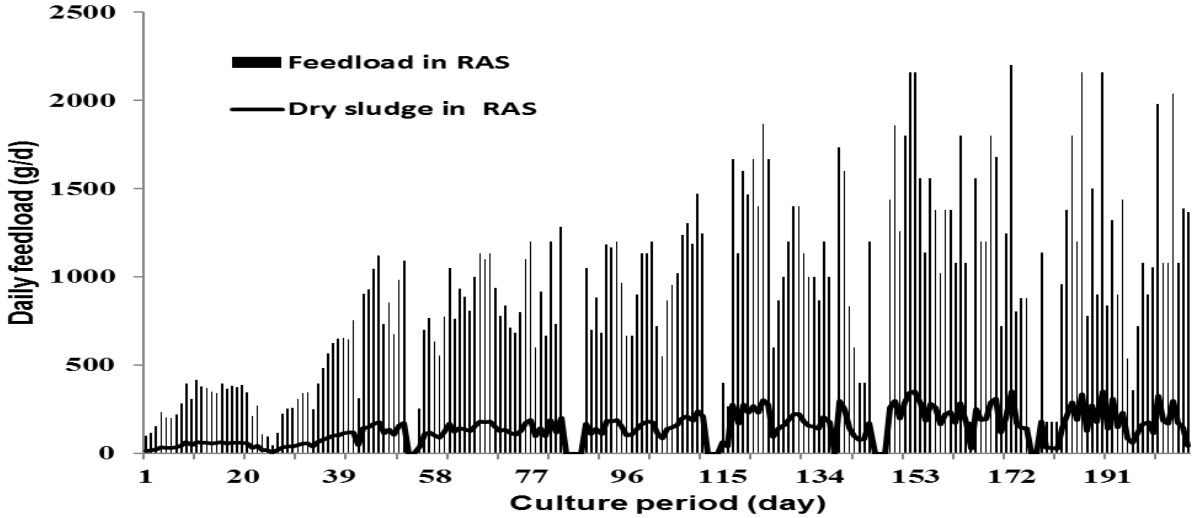


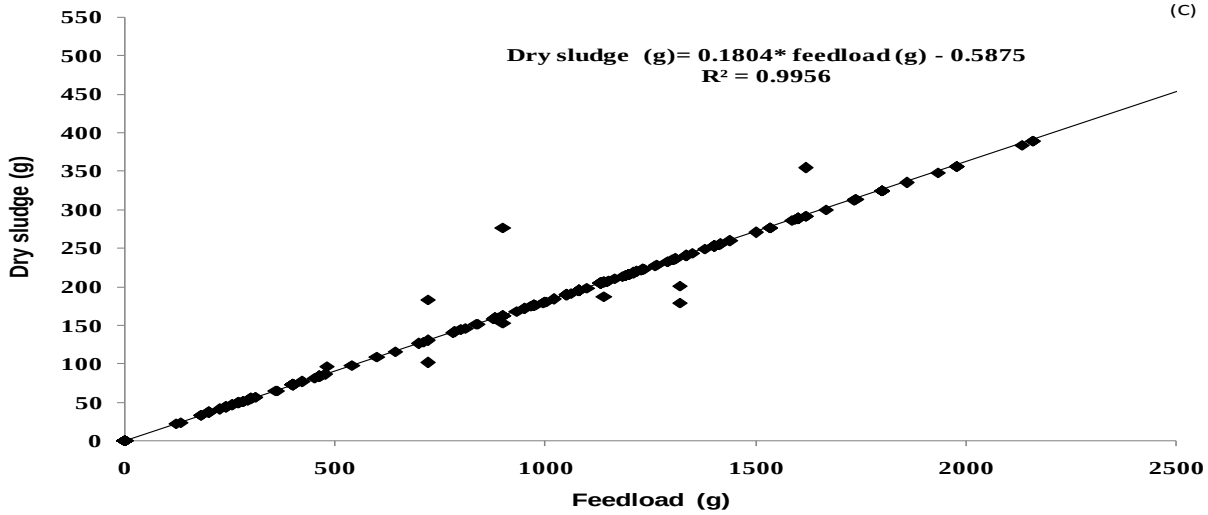
Figure 3. 4: Compositions of whole striped catfish body in flow-through tanks (FT) and recirculation aquaculture systems (RAS). The line of dry matter, crude protein, crude lipid (A) and ash, total phosphorus (TP) (B), bw: whole striped catfish body (g) (on wet weight basis).

The amounts of sludge (dry weight) collected from the swirl separator and feed (wet weight) given each day in FT and RAS are shown in Figure 3.5. On average, 161.0 ±7.2 g sludge was collected per kg feed in RAS and 180.4 ± 0.3 g in FT ($P < 0.05$).The sludge collected in RAS had a lower organic and higher ash content than FT sludge ($P < 0.05$). The concentrations of N and P in sludge were similar between FT and RAS ($P > 0.05$) (Table 3.6).


Feedload in FT
Dry sludge in FT
Daily feedload (g/d)
Culture period (day)


Feedload in RAS
Dry sludge in RAS
Daily feedload (g/d)
Culture period (day)

(C)


Dry sludge (g)= 0.1804* feedload (g) - 0.5875
R² = 0.9956
Dry sludge (g)
Feedload (g)

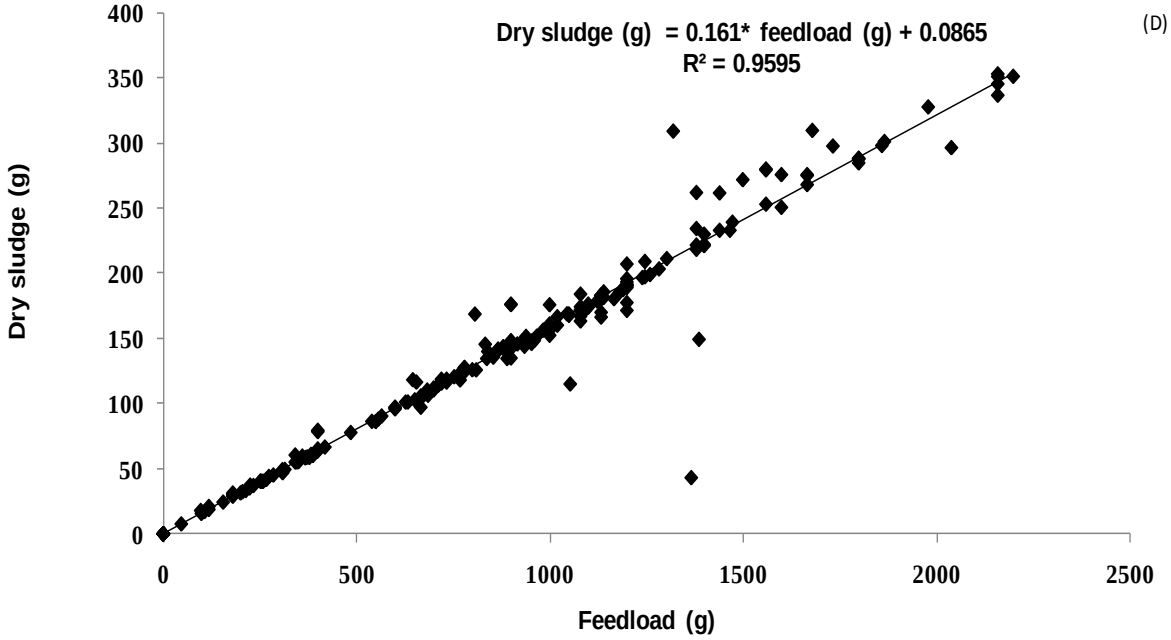


Figure 3. 5: Relationship of amount of dry sludge collected and accumulative feed load in flow-through (FT) (C) and in recirculating aquaculture systems (RAS) (D). Daily amount of dry sludge collected and cumulative feed load in FT (A) and in RAS (B).

Table 3. 6: Sludge composition (in g^{-1}kg dry sludge) in flow-through tanks (FT) and recirculating aquaculture systems (RAS). n=3.

Parameter	FT			RAS			p-value
	Mean	±	SD	Mean	±	SD	
Organic matter	610.4[a]	±	57.8	578.6[b]	±	58.7	0.011
Total organic carbon	335.3[a]	±	34.0	313.1[b]	±	33.6	0.002
Total carbon	344.8[a]	±	34.0	328.1[b]	±	31.7	0.018
Total COD	867.2[a]	±	81.5	821.7[b]	±	83.4	0.010
Ash	389.5[b]	±	57.8	421.4[a]	±	58.7	0.011
Total nitrogen	30.3[a]	±	6.5	28.4[a]	±	7.6	0.219
Total phosphorus	25.2[a]	±	4.3	25.7[a]	±	5.3	0.605

SD = standard deviation. Means with different superscript within each row are significantly different ($P < 0.05$).

3.3.3. Sustainability indicators in flow-through and recirculating aquaculture systems

The total water use in RAS was 120 times smaller than in FT. In FT, 99.8% of the water used was discharged, while in RAS this was 56.4% (Table 3.7). Of the remaining water consumption in RAS, 30% was lost through evaporation and 12% was discharged during harvest at the end of the production cycle. About 45l evaporated per kg feed applied in RAS (Table 3.7).

Table 3. 7: Water budgets (l) in flow-through (FT) tanks and recirculating aquaculture systems (RAS) averaged over the full production cycle. n=3.

	FT			RAS			p-value
Parameter	Mean	± SD	%	Mean	± SD	%	
Water filling	1 120	± 0	0.05	2 193	± 0	11.51	
Water intake	2 286 001[a]	± 61 702	99.95	16 868[b]	± 5 985	88.49	0.001
Total	2 287 121[a]	± 61 702	100.0	19 061[b]	± 5 985	100.0	0.001
Water output							
Harvest drainage	1 120	± 0	0.05	2 193	± 0	11.51	
Water discharge	2 282 456[a]	± 61 702	99.80	10 757[b]	± 5 945	56.43	0.001
Water evaporation	3 185[b]	± 0	0.14	5 812[a]	± 0	30.49	0.001
Water sampling	28	± 0	0.00	28	± 0	0.14	
Water unaccounted	333[a]	± 32	0.01	271[a]	± 113	1.42	0.398
Total	2 287 121	± 5 984	100.0	19 061	± 6 171	100.0	0.001

SD = standard deviation. Means with different superscript within each row are significantly different ($P < 0.05$).

Water use per kg fish produced or kg feed consumed in RAS was 100 times lower than in FT ($P < 0.05$) (Table 3.8). In contrast, energy, NaCl, $NaHCO_3$ and labour use in RAS was higher than in FT ($P < 0.05$). Similar amounts of N, P and DM per kg fish produced or per kg feed applied were retained in fish in FT and RAS ($P > 0.05$). Nevertheless, compared to RAS, twice as much N, P and DM were discharged per kg fish produced in FT ($P < 0.05$). For COD, the discharge in FT was even 4 times higher than in RAS ($P < 0.05$).

Table 3. 8: Sustainability indicators in flow-through tanks (FT) and recirculating aquaculture systems (RAS). n=3.

Parameter	Unit	FT			RAS			p-value
		Mean	±	SD	Mean	±	SD	
Water use	l kg^{-1}fish	14749.6[a]	±	743.0	146.3[b]	±	59.2	0.001
	l kg^{-1}feed	11033.3[a]	±	171.4	109.8[b]	±	35.5	0.001
Energy use	kWh kg^{-1}fish	6.7[b]	±	0.3	13.6[a]	±	0.2	0.001
	kWh kg^{-1}feed	5.0[b]	±	0.1	10.5[a]	±	0.5	0.001
Nutrient retention in fish								
-Nitrogen	g kg^{-1}fish	28.3[a]	±	0.9	27.4[a]	±	0.5	0.206
	g kg^{-1}feed	21.2[a]	±	1.3	20.7[a]	±	1.1	0.588
-Phosphorus	g kg^{-1}fish	3.7[a]	±	0.1	3.7[a]	±	0.1	0.832
	g kg^{-1}feed	2.7[a]	±	0.10	2.8[a]	±	0.20	0.625
-DM	g kg^{-1}fish	381.2[a]	±	9.3	374.0[a]	±	5.4	0.311
	g kg^{-1}feed	285.9[a]	±	21.3	282.2[a]	±	16.8	0.821
-COD	g kg^{-1}fish	758.4[a]	±	17.3	765.0[a]	±	28.9	0.752
	g kg^{-1}feed	559.2[a]	±	22.4	576.6[a]	±	26.8	0.437
Waste discharge								
-Nitrogen	g kg^{-1}fish	29.0[a]	±	1.5	13.7[b]	±	3.5	0.020
	g kg^{-1}feed	21.0[a]	±	1.2	10.3[b]	±	1.9	0.001
-Phosphorus	g kg^{-1}fish	14.5[a]	±	0.5	11.9[b]	±	1.5	0.040
	g kg^{-1}feed	10.7[a]	±	0.1	8.9[b]	±	0.5	0.003
-DM	g kg^{-1}fish	826.2[a]	±	41.4	348.7[b]	±	40.9	0.001
	g kg^{-1}feed	603.7[a]	±	21.7	261.6[b]	±	23.4	0.001
-COD	g kg^{-1}fish	826.1[a]	±	50.0	199.5[b]	±	19	0.001
	g kg^{-1}feed	608.4[a]	±	21.0	149.9[b]	±	4.5	0.001
NaCl	g kg^{-1}fish	12.0[b]	±	0.6	15.2[a]	±	1.2	0.014
	g kg^{-1}feed	9.7[b]	±	0.3	12.5[a]	±	0.3	0.001
$NaHCO_3$	g kg^{-1}fish	0.0[b]		-	43.6[a]	±	1.3	0.001
	g kg^{-1}feed	0.0[b]		-	36.0[a]	±	2.4	0.001
Antibiotic use		No			No			
Labor use	hr kg^{-1}fish	1.35[a]	±	0.07	1.56[a]	±	0.13	0.073
	hr kg^{-1}feed	1.00[b]	±	0.01	1.17[a]	±	0.04	0.001

SD = standard deviation. Means with different superscript within each row are significantly different ($P < 0.05$).

3.3.4. Nutrients mass balance

3.3.4.1. Nitrogen mass balance

Per kg feed, similar amounts of N were retained in fish biomass (incl. dead fish) in FT and RAS ($P > 0.05$) (Table 3.9). However, N input in FT was 2.6 times higher than in RAS ($P < 0.05$), due to supply of N with the exchange water. In FT, about 8% of N input was N unaccounted, while in RAS, 52% of the N input remained unaccounted. Less N was discharged in RAS with effluent and with sludge than in FT ($P < 0.05$).

Table 3. 9: Nitrogen mass balance (in g kg^{-1} feed) in flow-through (FT) tanks and recirculation aquaculture systems (RAS). n=3.

Parameter	FT			RAS			p-value
	Mean ± SD	%		Mean ± SD	%		
Fish level							
N in Feed	43.0	100		43.0	100.0		-
-N in fish retained	21.2^{a} ± 1.3	49.3		20.7^{a} ± 1.1	48.1		0.588
-N in fish mortality	0.8^{a} ± 0.4	1.9		0.9^{a} ± 0.5	2.1		0.726
-N not retained in fish	21.0^{a} ± 0.6	48.8		21.4^{a} ± 1.1	49.8		0.857
System level							
N input	57.2^{a} ± 6.0	100		21.7^{b} ± 0.9	100.0		0.001
-N not retained in fish	21.0^{a} ± 0.6	37.6		21.4^{a} ± 1.1	98.6		0.857
-N in influent	36.2^{a} ± 5.6	63.3		0.3^{b} ± 0.1	01.4		0.001
N output							
-N in sludge	5.2^{a} ± 0.5	9.1		4.5^{a} ± 0.5	20.7		0.155
-N in effluent	48.2^{a} ± 2.1	83.0		6.0^{b} ± 2.2	27.6		0.001
-N not measured	4.5^{b} ± 0.8	7.9		11.2^{a} ± 1.5	51.6		0.012

Values of N not retained in fish and N not measured was calculated. Values of remaining all parameters were measured.SD = standard deviation. Mean with different superscript within each row are significantly different ($p < 0.05$).

3.3.4.2. Dry matter mass balance

Per kg feed, similar amounts of DM were retained in fish biomass (incl. dead fish) in FT and RAS ($P > 0.05$) (Table 3.10). In FT, the influent water accounted for 5.8% of the DM input, while in RAS this was negligible ($P < 0.05$). A higher fraction of the DM input was present as sludge (incl.sludge removed from swirl separator and sludge accumulated in bio-filters, sump and overflow tank) in RAS than in FT ($P < 0.05$). However, by weight, more sludge was collected per kg feed from the in swirl separator in FT than in RAS ($P < 0.05$). About 57% of the DM input in RAS remained unaccounted, most likely due to in situ digestion, while 12% of the DM input was unaccounted in FT.

Table 3. 10: Dry matter mass balance (in g kg^{-1}feed) in flow-through tanks (FT) and recirculating aquaculture systems (RAS). n=3.

Parameter	FT			RAS			p-value
	Mean ± SD	%		Mean ± SD	%		
Fish level							
DM in feed	900	100.0		900	100.0		-
-DM in fish retained	285.9^{a} ± 21.3	31.8		282.2^{a} ± 16.8	31.4		0.821
-DM in fish mortality	10.4^{a} ± 5.5	1.2		12.1^{a} ± 6.2	1.3		0.735
-DM not retained in fish	603.7^{a} ± 6.3	67.1		605.7^{a} ± 16.8	67.3		0.357
System level							
DM input	641.0^{a} ± 3.9	100.0		606^{b} ± 16.9	100.0		0.019
-DM not retained in fish	603.7^{a} ± 6.3	94.2		605.7^{a} ± 16.8	99.9		0.357
-DM in influent	37.3^{a} ± 2.7	5.8		0.3^{b} ± 0.1	0.1		0.001
DM output							
-DM in sludge	182.7^{b} ± 5.2	28.5		259.0^{a} ± 23.0	42.7		0.013
-DM in effluent	380.4 ± 4.8	59.3		2.9 ± 0.6	0.6		0.015
-DM not measured	77.9 ± 4.7	12.2		344.0 ± 11.7	56.7		0.001

Values of DM not retained in fish and DM not measured was calculated. Values of remaining all parameters were measured. SD = standard deviation. Mean with different superscript within each row are significantly different ($p < 0.05$).

3.3.4.3. Phosphorus mass balance

In FT and RAS alike (P > 0.05), about 21% of P supplied with the feed was retained in life and dead fish biomass. The remaining 79 % of P fed became waste (Table 3.11). However, due to P loading through influent water, the total P input in FT was higher than in RAS (P < 0.05). On average, per kg of feed consumed,10.7 and 9.3g P were discharged from FT and RAS, respectively. In RAS, 15% of the P input could not be traced back and is reported as P unaccounted (Table 3.11).

Table 3. 11: Phosphorus mass balance (in g kg^{-1}feed) in flow-through and recirculating aquaculture systems (RAS). n=3.

Parameter	FT			RAS			p-value
	Mean	± SD	%	Mean	± SD	%	
Fish level							
P in Feed	13.50		100	13.50		100	-
-P in fish retained	2.70[a]	± 0.10	20.0	2.80[a]	± 0.20	20.7	0.625
-P in fish mortality	0.10[a]	± 0.06	0.7	0.13[a]	± 0.07	1.0	0.609
-P not retained in fish	10.70[a]	± 0.10	79.3	10.57[a]	± 0.20	78.3	0.625
System level							
P input	19.80[a]	± 0.4	100	10.68[b]	± 0.27	100	0.047
-P not retained in fish	10.70[a]	± 0.10	54.0	10.57[a]	± 0.20	99.0	0.625
-P in influent	9.10[a]	± 0.50	46.0	0.11[b]	± 0.0	1.0	0.001
P output							
-P in sludge	4.45[a]	± 0.13	22.5	4.05[b]	± 0.16	37.9	0.031
-P in effluent	14.94[a]	± 1.3	75.4	4.98[b]	± 0.65	46.6	0.017
-P not measured	0.41[b]	± 0.16	2.1	1.65[a]	± 0.35	15.4	0.031

Values of P not retained in fish and P not measured was calculated. Values of remaining all parameters were measured. SD = standard deviation. Mean with different superscript within each row are significantly different (p < 0.05).

3.3.4.4.COD mass balance

In both FT and RAS, about half of the COD supplied with the feed was retained in fish biomass, and was similar in both systems (P > 0.05). Nevertheless, the amount of COD discharged per kg feed consumed in RAS was 24 times smaller than in FT (P < 0.05). In

RAS, nearly 75% of the COD input which was not retained in fish biomass could not be traced back and is reported as not measured.

Table 3. 12: COD mass balance (in g kg^{-1}feed) in flow-through (FT) and recirculation aquaculture systems (RAS). n=3.

Parameter	FT			RAS			p-value
	Mean	± SD	%	Mean	± SD	%	
Fish level							
COD in Feed	1188.9		100.0	1188.9		100.0	-
-COD in fish retained	559.2[a]	± 22.4	47.0	576.6[a]	± 26.8	48.5	0.437
-COD in fish mortality	21.3[a]	± 9.0	1.8	24.4[a]	± 13.0	2.1	0.753
-COD not retained in fish	608.4[a]	± 22.4	51.2	587.9[a]	± 26.7	49.4	0.437
System level							
COD input	649.2[a]	± 23.0	100.0	588.3[b]	± 26.9	100.0	0.023
-COD not retained in fish	608.4[a]	± 22.4	93.7	587.9[a]	± 26.7	99.9	0.437
-COD in influent	40.8[a]	± 0.7	6.3	0.4[b]	± 0.1	0.1	0.001
COD output							
-COD in sludge	155.7[a]	± 3.7	24.0	130.9[b]	± 6.2	22.3	0.004
-COD in effluent	386.3	± 24.3	59.5	19.4	± 10.2	3.3	-
-COD not measured	107.2[b]	± 11.7	16.5	438.0[a]	± 21.2	74.4	0.032

Values of COD not retained in fish and COD not measured was calculated. Values of remaining all parameters were measured. SD = standard deviation. Mean with different superscript within each row are significantly different ($p < 0.05$).

3.4. Discussion

3.4.1. Water quality in FT and RAS

Temperature, pH and dissolved oxygen, H_2S, TAN, nitrite, nitrate and TSS concentrations remained favourable for striped catfish during the full culture period in both FT and RAS. Temperature in RAS was always slightly above 27^oC, which is optimal for striped catfish (Phuc et al., 2015). In FT, the temperature was on average 3^oC higher than in RAS ($P < 0.5$), because in the latter the trickling filter acted as cooling tower. In ponds, where temperature

fluctuates seasonally, temperature can drop below 26^{o}C, resulting in reduced appetite (Nhut et al., Submitted-a).

The dissolved oxygen concentration was 2.2 mg l^{-1} higher in RAS than in FT ($P < 0.05$) and 3.7 mg l^{-1} higher than in ponds (Nhut et al., Submitted-a). According to Lefevre et al. (2011c), striped catfish benefits from higher oxygen availability. However, comparing RAS and FT, higher oxygen availability in RAS did not result in better growth, production or feed conversion ($P > 0.05$). Although a pond feed was fed in our RAS and FT, striped catfish grew slower in ponds (Nhut et al., submitted). This could have been caused by the higher oxygen concentration in RAS and FT than in ponds (Lefevre et al., 2011a), but it could also be that more feed was lost in ponds. More research is needed on oxygen consumption by striped catfish in culture systems.

Higher H_2S concentrations were observed in RAS than in FT during the production cycle, although the observed levels in RAS (Table 3. 4) were still much lower than the 0.96 mg H_2S l^{-1} reported by Linh (2011). Four hours for sludge retention in the swirl separator and sludge accumulated in components of RAS (bottom of the moving bed biomedia reactor (MBBR), sump tank, overflow tank and pipe) can produced H_2S through passive denitrification, while water in FT was one-way flow. According to Linh (2011) reported that maintaining 0.96 mg H_2S l^{-1} reduced 40% striped catfish growth and survival and reduced fillet quality. The concentrations of TAN and nitrite were also below reported threshold levels for striped catfish (Huong et al., 2011; Nguyen et al., 2014) and on average lower than observed in RAS for African catfish and tilapia culture (Akinwole and Faturoti, 2007; Bovendeur et al., 1987; Shnel et al., 2002). A possible explanation is the accumulation of sludge under the moving bed reactor in our RAS, which facilitated denitrification.

Under the low water exchange conditions in RAS, on average 4.5 g N was discharged with the exchange water while 11.2 g N was not accounted, the bulk of which was converted into N_2 gas by denitrification (Bovendeur et al., 1987). In consequence, accumulation of NO_3-N was low, reaching maximum 89 mg NO_3-N l^{-1} during the production cycle (Figure 3.3B). Nitrate nitrogen tolerance levels for striped catfish have not been reported yet in literature, but numerous other species were investigated including rainbow trout (Davidson et al., 2011),

sturgeon (Hamlin, 2006), chinook salmon and rainbow trout fingerlings (Westin, 1974), juvenile turbot (Van Bussel et al., 2012a), eel (Kamstra and Heul., 1998) and tiger shrimp larvae (Muir et al., 1991). Reported chronic, acute and sub-lethal levels of NO_3-N vary with development stage and species (Camargo et al., 2005). The 89 mg NO_3-N l^{-1} reached in our study was below recommended safe levels in RAS for African catfish (Bovendeur et al., 1987; Schram et al., 2014) and eel (Eding et al., 2006).

The concentration of total suspended solids in RAS (Table 3.4) was lower and different in composition than in ponds (Nhut et al., Submitted-a). The TSS ash content in ponds was above 90% compared to less than 50% in RAS. The higher TSS ash content in ponds concurred with a 1.6 times lower COD in the rearing water than in RAS. In ponds, suspended solids enter with the exchange water. During the rainy season concentrations > 200 mg TSS l^{-1} in intake water to ponds were observed (Nhut et al., Submitted-a).

The 31 mg TSS l^{-1} average concentration in RAS with striped catfish was higher than for African catfish (Akinwole and Faturoti, 2007), Arctic char and rainbow trout (Davidson and Summerfelt, 2005) in RAS. Solid removal efficiencies in RAS depend on the method(s) used, and faeces consistency. Faeces of striped catfish are low in solid matter, diffuse easily in the water column and contain mainly non-settable particles. Both in FT and RAS, only 17% of the dry matter in feed was removed in the swirl separators, which is low compared to other fish species (Couturier et al., 2009; Davidson and Summerfelt, 2005; Piedrahita, 2003; Summerfelt and Penne, 2005). More research on the physical properties of striped catfish faeces is required to improve solids removal of striped catfish in RAS.

3.4.2. Fish performance and quality

Striped catfish grew slower in RAS than in FT which could be due to the higher NO_3-N concentration and lower temperature in RAS. Continuous exposure to a high nitrate concentration for example reduced growth in African catfish (Schram et al., 2014) and turbot (Van Bussel et al., 2012a). Growth of warm water fish improves with increasing temperature up to a few degrees below the upper lethal temperature (Corey et al., 1983; Heap and Thorpe, 1987; Talbot, 1993). For instance, channel catfish grew faster when the temperature was 3^0C

above average (Buentello et al., 2000), while striped catfish kept at 6‰ salinity grew 2.1 times better at 35^{o}C than at 30^{o}C (Phuc et al., 2015).

Striped catfish survival was 30% higher in RAS (this study) than in ponds (Nhut et al., Submitted-a). Factors that might contribute to the lower performance in ponds include fingerling quality, dependence on large volumes of high quality exchange water and limitations on water quality control, including diurnal or seasonal temperature or dissolved oxygen fluctuations (Bosma et al., 2011; Dung et al., 2008; Nguyen et al., 2007; Phan et al., 2009). In RAS and FT, mortality was highest during the first months of the production cycle, a fact commonly observed in the striped catfish industry (Phan et al., 2009). In Vietnam, obtaining healthy pathogen free fingerlings is difficult, with about 15 different diseases occurring frequently (Nguyen et al., 2007; Phan et al., 2009). When disease occurs, farmers reduce or even suspend feeding to avoid feed wastage and maintain water quality, thus reducing production output. Stocking infected fingerling also affected production in our FT and RAS. However, the stocking density was higher than in ponds in our FT and RAS units, making it easier and more cost effective to apply treatment. Disease related mortality occurred during the first weeks of culture but was effectively controlled, observing no disease related mortality during the rest of the culture period in both FT and RAS. However, considering 100 times less water was exchanged per kg fish produced in RAS (0.14 m^3) compared to FT (14 m^3) (Table 3.8) the risk of importing new infections is much less and bio-security measures are easier to apply.

Striped catfish produced in our FT and RAS was of export quality (Sang et al., 2012; Sang et al., 2009): fillets were 100% white with no trace of off-flavour and a fillet yield of 37-38% (Table 3.5). In contrast, striped catfish harvested from traditional ponds yielded 53 - 93% white fillets and off-flavour was regularly reported (Phu et al., 2014). Nitrifying biofilters in RAS are potential production sites of geosmin and 2-methylisoborneol (MIB), substances that cause off-flavour (Guttman and van Rijn, 2008). Occurrence of “earthy’’ or ‘’musty’’ off-flavour due to geosmin and MIB, respectively, is commonly reported in RAS (Bai et al., 2013; Burr et al., 2012; Guttman and van Rijn, 2008, 2009; Schrader et al., 2010; Tucker, 2000; Tucker and van der Ploeg, 1999). So, the expectation was that off-flavour would develop during the production cycle in our RAS. Possibly, the high frequency of sludge removal through 4-hr interval in swirl separator, every 10-day in the MBBR and sump tank

kept the concentration of decomposing wastes in the system as source of off-flavours compounds sufficient low, not only in FT but also in RAS. In addition, denitrification occurred in our RAS (Table 3.9), which could have reduced significantly geosmin and MIB concentrations (Guttman and van Rijn, 2009). Besides sludge removal, high water exchange rates in FT also reduced potential exposure of striped catfish to geosmin or MIB. Brown and Boyd (1982) reported off-flavour in channel catfish in ponds with a high concentration of algae and COD and not in ponds with few algae and low COD. This concurs with our exchange water in FT where chlorophyll*a* concentration was very low (data not reported).

3.4.3. Sustainability indicators

Table 3. 13: Key sustainability indicators in striped catfish culture systems.

Indicator	Unit	RAS	FT	Conventional pond
Resource utilisation efficiency				
Mortality	%	6.2(1)	4.2(1)	36.3(2)
Fingerlings	#kg^{-1}fish	1.8(1)	1.6(1)	1.9 (2)
Feed	kg feed kg^{-1}fish	1.25(1)	1.27(1)	1.6(2);1.69(6) 1.86(3)
Water use	l kg^{-1}fish	146.3(1)	14,749.6(1)	2,800-7,100(2); 2,500(3); 4,02(4);9,13(5)
Energy use	kWh kg^{-1}fish	13.6(1)	6.7(1)	0.09(2); 0.043(3)
Antibiotic	gkg^{-1}fish	No use	No use	0.15(3)
Labour	hrkg^{-1}fish	1.56(1)	1.35(1)	0.06-0.11(2)
Nutrient utilisation efficiency				
Nitrogen	%	48.1(1)	49.3(1)	38.3(2)
Phosphorus	%	20.7(1)	20.0(1)	14.3(2)
Dry matter	%	31.4(1)	31.8(1)	28.5(2)
COD	%	48.5(1)	47.0(1)	-
Waste discharge				
Nitrogen	gkg^{-1}fish	13.7(1)	29.0(1)	18.5(2);46(6)
Phosphorus	g kg^{-1}fish	11.9(1)	14.5(1)	16.7(2);14.4(6)
Dry matter	gkg^{-1}fish	348.7(1)	826.2(1)	359.6(2)
COD	g kg^{-1}fish	199.5(1)	826.1(1)	-

(1) This study, (2) pond study (Nhut et al., Submitted-a), (3) Bosma et al. (2011), (4) Phan et al. (2009),(5) Anh et al. (2010), (6) De Silva et al. (2010).

In contrast to ponds (Nhut et al., Submitted-a), a small amount of chemicals and no antibiotics were used in our RAS. About 36 g sodium bicarbonate was applied per kg feed to maintain pH which is less than normally applied in RAS (Timmons, 2002). By contrast, in ponds, 8 disinfectants, 4 parasiticides and 20 antibiotics are commonly applied in Vietnam to striped catfish ponds to fight disease (Rico et al., 2013). On average, 0.15 g antibiotics are

applied per kg striped catfish produced in ponds (Bosma et al., 2011). Farmers can eliminate residual antibiotics from fish tissue within 2-5 days after application, minimizing risks to consumers (Danyi et al., 2011). Nevertheless, the discharge and accumulation of antibiotic residues remains a risk factor for the development of bacterial resistance (Dung et al., 2008; Nguyen et al., 2007; Sarter et al., 2007a) and remains a major concern for the striped catfish industry.

Water use per kg striped catfish produced in our RAS was 17 – 62 times lower than water use in traditional ponds (Anh et al., 2010; Bosma et al., 2009; Phan et al., 2009). The low water use per kg production in RAS also reduced the volume of discharge water. Small volumes can be treated in smaller facilities, allowing better control on effluent quality, while more attention can be given to water quality and minimizing contamination risks. RAS technology thus allows to target future improvement of the present ASC standard on water use in striped catfish culture (ASC, 2012) .

The recirculating flow in RAS was 45 – 91 m^3 per kg feed per day which was 6 – 8 times higher than in FT. The fact that fish production was similar in FT and RAS suggests that water flow and associated pumping costs can be reduced. The experimental RAS was overdesigned causing energy use to be higher than reported in commercial RAS for tilapia, African catfish and European eel (Eding and Kamstra, 2002; Verreth and Oberdieck, 2009). Daily monitoring and water sample and sludge collection every 4 hours also made labour use in this experiment much higher than in commercial RAS farms. In the Netherlands one person can operate a tilapia RAS with an annual production capacity of 100 MT (Verreth and Oberdieck, 2009) or an 250 MT African catfish RAS (Eding, personal communication). With striped catfish, reaching labour efficiencies as for African catfish could be set as a long-term target for the industry.

Overall, RAS production improves water quality and minimizes environmental impacts from aquaculture, as shown for rainbow trout in Europe (d'Orbcastel et al., 2009a; d'Orbcastel et al., 2009b). The same was observed for striped catfish production in experimental scale RAS. Nutrient retention efficiency and discharge are important sustainability indicators (Verreth and Oberdieck, 2009). High N, P, COD and DM retention efficiencies were realized in

experimental FT and RAS, compared to traditional ponds. The combination of better survival, fast growth and low feed conversion ratio in RAS partially explains the high nutrient retention efficiencies realized in our FT and RAS. It would be interesting to explore if these retention efficiencies can be further improved by applying improved diets (Eding and Kamstra, 2001; Eding and Kamstra, 2002; Eding et al., 2009). Future research should focus on improvement of nutrient retention efficiencies and shortening of the production cycle, as for instance was done for production of tilapia in RAS (Eding et al., 2009). A major advantage of RAS was the high reduction in waste discharge (Table 3.13) due to within system mineralization of organic waste. The reported waste discharge from striped catfish ponds was much higher than in our RAS (De Silva et al., 2010).

3.4.4. Nutrient mass balances

Unfortunately, N_2 and CO_2 volatilization were not measured in RAS. More than 50% in input N and DM could not be traced back in the mass balance budgets (Table 3.9 and 3.10) which was most likely due to volatilization. The losses of N and DM were higher than observed in traditional striped catfish ponds (Nhut et al., Submitted-a). In contrast, in FT the amount of unaccounted waste was much less, because time for volatilization was negligible while discharge through sludge and exchange water was monitored in detail. The latter losses accounted for 54% of N, 45% of DM and COD and 17% of P of the input nutrients not retained in fish. Specially, in FT and RAS, 183 – 259 g DM was collected in sludge per kg feed, containing 10 – 12 % of N and 30 – 33% of P applied with the feed. The sludge is relatively easy to collect and ways to recuperate or reuse these nutrients could be explored. The N, P and OM concentrations in sludge collected from FT and RAS were higher than reported for traditional striped catfish ponds (Phu and Tinh, 2012). Promising options to explore include reuse as agricultural or aquacultural fertilizer(Adler and Sikora, 2004; Birch et al., 2010; James et al., 1998; Phung et al., 2009) and energy recuperation through methanogenesis (Kugelman and Van Gorder, 1991; Lanari and Franci, 1998; Mirzoyan and Gross, 2013; Mirzoyan et al., 2012; Mirzoyan et al., 2008; Mirzoyan et al., 2010; Mshandete et al., 2004).

3.5. Conclusions and recommendations

Growth and nutrient utilization efficiency was higher in experimental small-scale FT and RAS than reported for striped catfish production in ponds when using a normal 26% protein pond diet and stocking 325 fingerlings per m^3, which is 32 times higher than in ponds. Production of striped catfish in our RAS showed promising improvements on the sustainability indicators water use and discharge of N, P, DM and COD per kg feed or per kg fish produced (Table 3.8). However, fossil energy and human labour input were high, due to over dimensioning of the biofilter capacity (and associated pumping capacity) and the high sampling frequency.

Future research should focus on RAS up scaling to commercial size and lowering energy requirements. In FT, the water turnover rate in the culture tanks was 8 times smaller, realizing better growth and production than in RAS. This suggest that there is room to develop recirculating systems for striped catfish production with a much lower recirculation flow than used in this experiment, thus lowering energy requirements and costs. In addition, research on sludge reuse as fertilizer or for biogas production will help to further improve sustainability.

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CHAPTER 4

Methane production potential and compost composition of sludge from striped catfish (*Pangasianodon hypophthalmus*, Sauvage, *1878*) ponds and recirculating systems

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Abstract

This paper compares the quantity and quality of solid waste obtained from traditional ponds and recirculating aquaculture systems (RAS) for striped catfish, evaluating also methane and compost production. Striped catfish sludge was collected from four commercial ponds along the Mekong river and three indoor RAS. The amount of sludge of dry matter produced per kg fish production in ponds was 6 times higher than in RAS. However, the concentration of nutrients in solid waste from RAS was much higher, leading to better compost quality and higher methane yield than with sludge from ponds. Methane yield of striped catfish solid waste in RAS systems was 201 l CH_4 per kg COD added with 52.7% CH_4 in biogas, compared to 125 l CH_4 per kg COD added in ponds, with 46.8% methane in biogas. The higher methane production from RAS sludge concurred with higher digestibility of COD: 57.5% for RAS and 37.5 for ponds.

Keywords: *striped catfish, sludge, compost, methane, biogas, energy*

4.1. Introduction

In 2014, 1.1 million metric tons of striped catfish was produced in the Mekong delta in 5,500 ha of earthen ponds. Processed pangasius products have been exported to over 150 countries (MARD, 2014). With the goal to improve sustainability, culture in recirculating aquaculture systems (RAS) has been successfully tested in an indoor pilot scale RAS (Nhut et al., Submitted-b).

In grow-out ponds, 22-35% protein diets are used, realizing a feed conversion ratio (FCR) of 1.7 -1.8 (Bosma et al., 2009; Phan et al., 2009). The sludge collected or discharged from ponds contains uneaten feed, faeces and residues of chemicals applied during culture (Phu and Tinh, 2012). Today, 65% of farms discharge effluents, including sludge, directly to the Mekong river and 35% discharge to rice fields or gardens (Phan et al., 2009). For each kg of striped catfish produced, 2.5 to 9.1m^3 water is discharged (Anh et al., 2010; Bosma et al., 2011; Phan et al., 2009). The concentration of wastes in these effluents is low: e.g. Anh et al. (2010) reported 22 mg l^{-1}biological oxygen demand at five days (BOD_5), 27 mg l^{-1} chemical oxygen demand (COD), 61 mg l^{-1} total suspended solid (TSS), 4 mg l^{-1} total nitrogen (TN) and 1 mg l^{-1} total phosphorus (TP) in striped catfish pond effluents. Although these effluents are highly diluted, water volumes discharged are large, with farms discharging 60 to 90% of N (Anh et al., 2010) and 30% of P (Nhut et al., Submitted-a) supplied through feeding. Combined, striped catfish farms in the Mekong delta discharge annually 18,500 tonnes N and 9,300 tonnes P (Nhut et al., Submitted-a).

The sludge accumulating at the bottom of the pond contains 6 and 57% of N and P supplied with the feed, respectively. If pond effluents would be passed through a sedimentation pond, part of the nutrients could be trapped and re-used. Unfortunately, this technology is not widely adopted by the striped catfish industry in the Mekong delta. The direct effluent discharged such as nutrients, transmission of pathogens, residual antibiotics and chemicals from opened aquaculture systems can lead to stress ecological systems of surround environment (Folke and Kautsky, 1992). Particularly, striped catfish ponds locate along the Mekong River. Its effluent can effect on community heath in the Mekong delta through using water with contamination of residual drugs, chemicals and pollution.

The amount of sludge that can be collected is different between fish species or production systems. The sludge production ranges from 0.2 to 0.5 kg dry sludge per kg fish produced (Chen et al., 1997). A part of the nutrients and energy contained in sludge could be re-used,

either through composting or methanogenic fermentation. Composting is done by mixing sludge with rice straw, a resource which is available year round in large quantities in the Mekong delta (Phung et al., 2009). However, sludge collected from ponds contains inorganic soil particles, and this might limit methane production. The latter is less a problem with sludge collected from RAS, where the concentration of volatile solids is sufficiently high to consider on-farm methane production (Gebauer, 2004; Gebauer and Eikebrokk, 2006; Mirzoyan and Gross, 2013; Mirzoyan et al., 2012; Mirzoyan et al., 2008; Mirzoyan et al., 2010).
The aim of this study was to quantify and qualify sludge production in striped catfish ponds and RAS during grow-out, and to determine and compare compost and methane production of the collected pond and RAS sludge.

4.2. Materials and methods

4.2.1. Ponds and RAS for production and sludge collection

Sludge production and composition were determined in four commercial striped catfish ponds and three indoor RAS during a full production cycle. Two ponds were located upstream in Dong- Thap province and two downstream in Vinh Long province. The ponds were 3.5 – 4.5 m deep. Pond design and operation was described in detail by Nhut et al. (Submitted-a). Detailed information on RAS design and operation was presented in Nhut et al. (Submitted-b). Figure 4.1 and Table 4.1 summarize and compare pond and RAS design and operation.

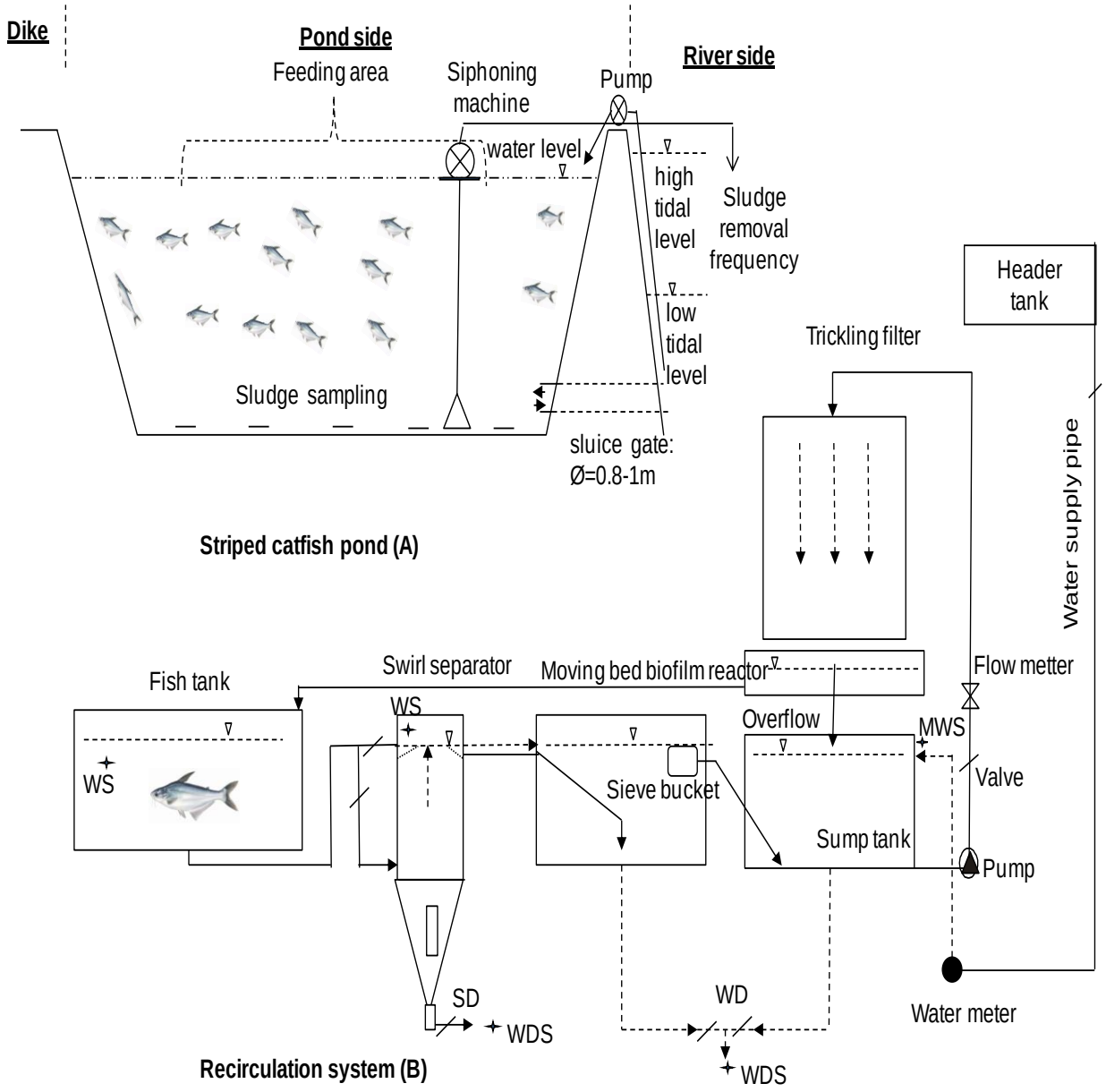

Figure 4. 1:Characterization of traditional striped catfish ponds (A) and recirculating aquaculture systems (B) used for solid waste collection. WS: water sampling; MWS: makeup water sampling; SD: sludge discharge; WDS: water discharge sampling; (+) sampling point.

Table 4. 1: Characterization of the fish ponds (Spond) and recirculating aquaculture systems (SRAS) used for solid waste collection. Values are mean ± standard deviation (S.D.).

Parameter	Unit	Spond			SRAS		
		Mean	±	SD	Mean	±	SD
System information:							
Replicates	#	4			3		
Total culture surface area	m^2	10,946	±	212	0.85	±	0.0
Culture depth	m	3.8	±	0.6	0.8		
Volume	m^3	41,529	±	5,388	0.85	±	0.0
System operation:							
Culture period	days	255			207		
Sludge removal frequency	# $crop^{-1}$	1 or 3*			1,242**		
Stocking density	kg m^{-3}	0.6	±	0.3	4.7	±	0.7
Final density	kg m^{-3}	7.7	±	4.1	171.4	±	12.6
Initial individual weight	g ind^{-1}	38.5	±	18.7	18.0	±	2.6
Final individual weight	g ind^{-1}	833	±	376	658.4	±	30.8
Total fish biomass	kg $system^{-1}$	299,972	±	74,741	145.7	±	10.7
Total feed	kg $system^{-1}$	433,673	±	74,529	176.3	±	5.4
Feed conversion ratio	-	1.53	±	0.14	1.25	±	0.1
Feed composition (in ww)							
Dry matter	%	89.25	±	0.2	90.1	±	0.3
Total nitrogen	%	4.5	±	0.3	4.2	±	0.1
Total carbon	%	43.6	±	1.5	53.2	±	1.5
Carbohydrate	%	47.0	±	1.7	51.4	±	1.9
Total COD	g kg^{-1}feed	1210.4	±	9.3	1,188	±	5.1
Ash	%	7.5	±	0.2	7.2	±	0.2
Total phosphorus	%	1.3	±	0.1	1.4	±	0.2
Total Ca	mg kg^{-1}feed	23,500	±	1,643.2	25,019	±	689.2
Total Mg	mg kg^{-1}feed	3,667	±	516.4	4,030	±	361.4
Total K	mg kg^{-1}feed	2,450	±	821,6	2,011	±	105.6
Influent water							
Make up water use	m^3 kg^{-1}feed	3.1	±	1.7	0.11	±	0.04
	m^3kg^{-1}fish	5.0	±	3.0	0.15	±	0.06
pH	-	6.4	±	0.2	8.1	±	0.1
Salinity	g l^{-1}	0.2	±	0.2	0.0		
TN	mg l^{-1}	4.7	±	2.1	1.3	±	0.7
TC	mg l^{-1}	14.3	±	0.8	55	±	20.0
COD	mg l^{-1}	6.5	±	0.6	3.1	±	1.1
TP	mg l^{-1}	0.4	±	0.1	0.5	±	0.9
TSS	mg l^{-1}	202.1	±	27.2	1.8	±	0.5

Spond: Sludge in traditional striped catfish ponds, SRAS: sludge in striped catfish RAS systems, ww: wet weight. In ponds, feed composition changed with culture phase. In RAS, the same feed was used during the full production cycle. *One time per crop cycle in downstream ponds, three times per crop cycle in upstream ponds. ** Six times of sludge collection per day in RAS.

4.2.2. Sludge sampling from ponds and RAS

At three randomly assigned locations in each pond, a 0.6-m^2 circular sludge trap, and at six randomly assigned locations a 0.4-m^2 ceramic tile, placed horizontally at the sediment surface, was installed. Sludge traps were emptied weekly, but the amount of sludge collected is reported semi-monthly. A weekly sampling frequency was necessary, because the traps could spill over if sampled semi-monthly. Each tile and sludge trap location was marked by a 6-m bamboo pole, with one end fixed in the sediment and the other end visible above the water surface. The sediment accumulating on 3 tiles was left undisturbed until harvest, when the amount of sludge accumulated above the tiles was quantified and analyzed. The sludge accumulating above the other 3 tiles was collected semi-monthly, for sludge composition analysis.

The farmers removed bottom sludge in their ponds each time the sludge bed became higher than 25 cm, using a diesel powered suction pump. When this happened, a sludge sample was collected and the total volume of sludge pumped out was recorded, so that the total amount and composition of the sludge removed could be qualified and quantified. During sludge removal, the locations with ceramic tiles and sediment traps were not disturbed.

The sludge in RAS was collected every four hours from the bottom section of the swirl separator (Figure 4.1). The collection interval was short to prevent the development of floating sludge through gas development and subsequently sludge wash-out from the swirl separator. The amount of sludge collected at 4-hour intervals is also reported semi-monthly.

The sludge collected semi-monthly from above the tiles in each pond was mixed into one composite 1-l sample which was kept cooled (4^oC) conditions for 4 hours during transport to the laboratory where it was analyzed for pH, electric conductivity (EC, mS cm^{-1}), ash, volatile solids (VS), total carbon (TC), total organic carbon (TOC), chemical oxygen demand (COD), total phosphorus (TP) and total nitrogen (TN) (expressed as percentage of dry matter (DM), unless mentioned differently). During the last two months of the production cycle, total calcium (Ca), total magnesium (Mg), and total potassium (K) in sludge were measured semi-monthly (mg kg^{-1} DM). At the end of the culture period, sludge accumulated above the three undisturbed tiles in each pond was collected by a 90-mm inner-diameter circular core and dried at room temperature while in the core. When dry, 5 cm layers were cut starting at the top (0-5 cm, 5-10 cm, 10-15 cm, 15-20 cm and 20-25 cm) and homogenously mixed per horizon per pond to analyze TOC, TC, TN and TP (% DM). Each day, the RAS sludge collected at 4-hr intervals was pooled and analyzed for DM. Semi-monthly, a 1-L sludge samples per RAS was

collected per RAS and analyzed for pH (-), EC (mS cm^{-1}), ash, VS, TC, TOC, COD, TP and TN (expressed as percentage of DM, unless mentioned differently).

4.2.3. Determination of composting potential of sludge

The composition of sludge collected from striped catfish ponds (Spond) and RAS (SRAS) and of rice straw (RS) used to make compost is given in Table 4.2. Pond sludge was collected during the last two months of the production cycle according to Nhut et al. (Submitted-a); Nhut et al. (Submitted-b). Per pond, the same amount of sludge (moisture content 60%) was used for composting. Similarly, sludge collected from each RAS was dried at room temperature until 60% moisture content. Fresh rice straw (RS) was collected in Vinh Long province, and dried at room temperature until 60% moisture content and cut into 2-3cm long pieces.

Table 4. 2: Composition of sludge and rice straw before composting (on dry weight basis, unless stated differently). All sludge and rice samples were standardised to a dry matter content of 40%.

Parameter	Unit	RS			Spond			SRAS		
		Mean	±	SD	Mean	±	SD	Mean	±	SD
pH	-	-			6.5	±	0.0	6.7	±	0.1
EC	$mScm^{-1}$	-			0.8	±	0.2	2.7	±	0.1
DM	% ww	40.3	±	0.4	40.2	±	0.6	40.8	±	0.8
VS	%	84.0	±	0.8	15.6	±	0.3	65.6	±	0.7
Ash	%	16.0	±	0.8	84.4	±	0.3	34.4	±	0.7
TC	%	38.4	±	0.6	5.6	±	0.3	31.1	±	0.9
TN	%	1.36	±	0.02	0.47	±	0.0	2.2	±	0.1
TP	%	0.15	±	0.01	0.28	±	0.0	2.9	±	0.1
C:N ratio	$g\ g^{-1}$	28.2	±	0.6	11.7	±	0.5	13.9	±	0.7
Total Ca	$mg\ kg^{-1}$	2,066	±	306	7,633	±	57	10,966	±	25
Total Mg	$mg\ kg^{-1}$	450.7	±	26	3,833	±	208	3,533	±	58
Total K	$mg\ kg^{-1}$	16,833	±	513	1,566	±	351	353	±	10

RS: rice straw**, Spond**: sludge in striped catfish ponds (n = 4), **SRAS:** sludge in striped catfish RAS (n = 3).
Values are mean ± standard deviation (S.D.),ww: wet weight.

Equal quantities of sludge from each pond were homogeneously pooled, and subsequently divided into 3 equal portions. The same was done with sludge collected from RAS. Then each portion of Spond or SRAS was mixed with rice straw and referred to as Spond+RS and SRAS+RS. The Spond+RS was a homogenous 1:1 (DW basis) mixture of sludge and rice

straw. For the SRAS+RS treatment the ratio sludge: rice straw ratio was 1:2. In this way, the C:N ratio in mixture was raise to 20 or higher (Table 4.6). Per treatment 3 batches of compost were made. Per experimental unit, 3kg Spond+RS or SRAS+RS mixture was put in a polystyrene box (height 40cm x length 40cm x width 40cm) with holes in cover and sides for air ventilation. During composting, moisture was checked every 4 days and adjusted to about 60%. The compost was mixed before closing the box. The composting experiment was terminated after 60 days. Three random samples of final compost in each treatment were collected and kept at 4^0C during transportation to the laboratory for composition analysis.

4.2.4. Determination of methane production potential of sludge

Pond sludge (Spond) was collected during the final 2 months of the production cycle, RAS sludge (SRAS) during the final month of the production cycle. Equal amounts of sludge collected in four ponds were homogeneously pooled into one composite sample. The same was done with sludge collected from three RAS systems. The initial composition of Spond and SRAS is given in Table 4.3.

Table 4. 3: Composition of sludge, inoculum and sludge-inoculum mixture for the biogas reactor (on wet weight basis, unless started differently)

Parameter	Unit	Spond	SRAS	I	Spond+I	SRAS+I
pH		6.9	6.7	7.9	7.5	6.9
EC	$mScm^{-1}$	0.5	1.8	0.7	0.6	1.2
Alkalinity	$mgCaCO_3l^{-1}$	-	-	-	1,807.3	2,321.4
DM	%	6.0	6.0	6.0	6.0	6.0
VS	%	0.3	3.6	3.1	2.0	3.3
Ash	%	5.7	2.4	2.9	4.0	2.7
TKN	mgl^{-1}	342.0	1,698.0	2,765.0	1,795.8	2,338.2
TAN	$mg\ l^{-1}$	58.7	290.3	442.4	305.3	397.5
NO_3-N	$mg\ l^{-1}$	1.1	2.4	3.1	1.8	2.5
COD	$mg\ l^{-1}$	5,488	51,683	27,789	27,427.4	29,915
TP	$mg\ l^{-1}$	203.0	1,500.0	1,342.0	886.4	1,405.2

Spond: sludge collected from traditional striped catfish ponds, SRAS: sludge collected from the swirls separator in striped catfish RAS, Spond +I: sludge in traditional striped catfish ponds with inoculum, SRAS +I: Sludge in striped catfish RAS with inoculum.

Digested sludge from a pig biogas plant in Ho Chi Minh City, Vietnam, was used as inoculum. The digester had been operating under mesophilic conditions for 2 years, prior to the

experiment. The collected inoculum was homogenously mixed and incubated during 7 days at 36^{o}C to deplete residual biodegradable organic matter (OM) and degassed to remove residual methane. After degassing, the inoculum was stored at 4^{o}C until use. The inoculum is further referred to as 'I' and its initial composition is given in Table 4.3.

Different substrates were used to measure methane production:(1) 200ml Spond + 300ml 'I' (Spond+I), (2) 200ml SRAS + 300ml 'I' (SRAS+I) and (3) 200 ml distilled water + 300ml 'I' (Control). All samples were standardized to a dry matter content of 6%. All treatments were executed in triplicate according to Angelidaki et al. (2009). The initial composition of Spond+I and SRAS+I are given in Table 4.3.

Nine 550 ml incubation bottles were used, each filled with 500 ml substrate (either Spond+I, SRAS+I or Control) leaving 50ml headspace. Each bottle was closed with a butyl rubber stopper that was hold in place with an aluminum clamp according to Angelidaki et al. (2009). Pure N_2 gas was flushed 2 minutes through the bottles before and after filling. The bottles were incubated at 36 ± 2^{o}C during 63 days under dark condition. During incubation, the bottles were constantly shaken at 72-75 strokes per minute, except during sampling and biogas volume measurements.

At the end experiment, after 63 days, samples were taken to analyze the composition. The amounts of CH_4 and CO_2 produced were daily measured during the experiment.

4.2.5. Sample analysis

Sludge and compost samples were analyzed for pH, EC, DM, TSS, TS (total solids), VS, COD, total Kjeldahl nitrogen (Kj-N), TP, TC, NH_4-N and NO_3-N. The pH was measured by pH electrode (model Hi99121-HANNA). The EC was measured by conductivity meter (HI98331-Hanna for Soil Test). The total suspended solid (TSS, dried to constant weight at 103 - 105 ^{o}C) was measured according to APHA (1999). The DM or TS was weighed after drying at 105^{o}C for 24 hours (Foy and Rosell, 1991). The VS was calculated as the weight difference between DM and ash content (after burning at 550^{o}C) according to APHA (1999). The chemical oxidation demand (COD) was measured according to APHA (1999). Total Kj-N was analyzed by the Kjeldahl method (Foy and Rosell, 1991). The TP in sludge was analyzed spectrophotometrically following Boyd and Tucker (1998).The TC was determined by high

temperature combustion method, TOC by high temperature combustion method on acidified samples, NO_2-N bydiazotized sulfanilamide method, NO_3-N by cadmium reduction to nitrite method according to APHA (1999). Total Ca, total K and total Mg were measured by atomic absorption spectrophotometer (Hatachi 180-60) after extraction in 1N ammonium acetate (pH7).

Biogas production was determined daily as displaced water volume (in ml) (Figure 4.2). Biogas included CH_4, $CO_2$and other gases. The volume of biogas was measured as displaced water collected daily in the graduated cylinder. Two ml biogas was taken directly by syringe from the headspace and the CH_4 and the CO_2 concentrations were measured by gas chromatography (Shimadzu GC, Japan). The methane production was recalculated according to standard temperature and pressure (X_{STP}) according to Hansen et al. (2004).

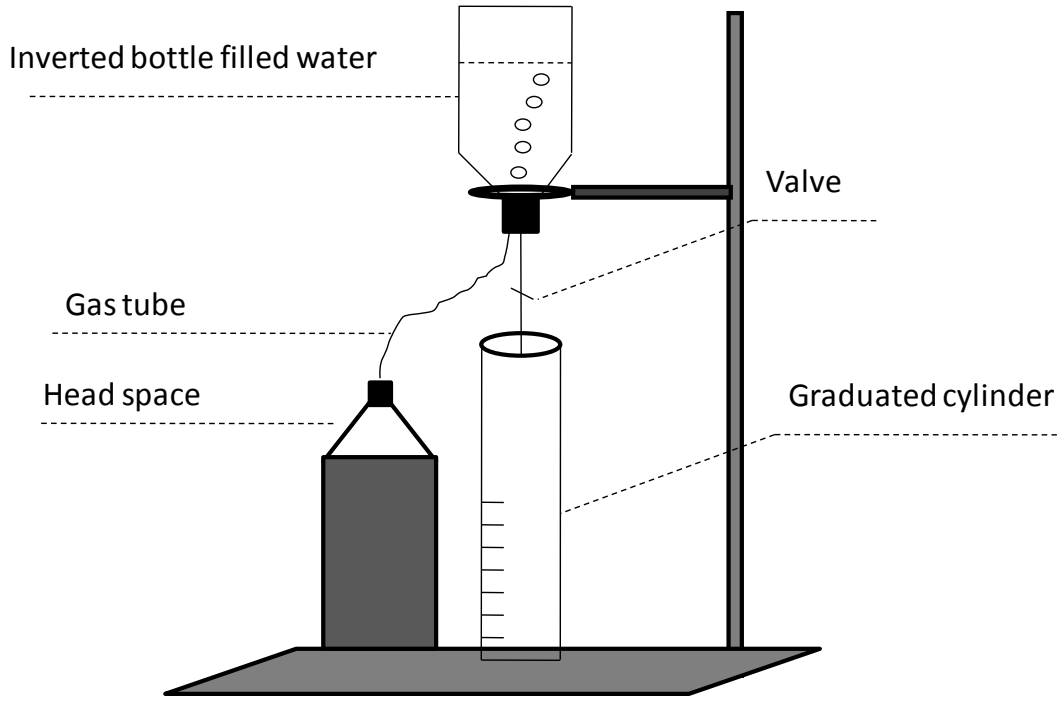

Figure 4. 2: Daily measurement of biogas volume by inverted bottle filled with water.

4.2.6. Formulas and calculations

Details of calculations related to compost, methane potential, energy and nutrients lost during composting are given in Table 4.4.

Table 4. 4: Summary of formulas for calculating in experiments

Parameter	Unit	Formulas
Volatile solids (VS)	g	VS = Dry matter – Ash
Nutrient loss (NL)	%	$NL = 100 * [(NU_{initial} - NU_{final}) / NU_{initial}]$
Mineral loss (ML)	%	$ML = 100 * [(M_{initial} - M_{final}) / M_{initial}]$
Feed conversion ratio (FCR)	-	$FCR = FC / (W_{total\ final\ fish\ bw} - W_{total\ initial\ fish\ bw})$
CH_4substrate production (V_{CH4})	ml d^{-1}	$V_{CH4} = V_{CH4,\ S+I} - V_{CH4,\ I}$
CO_2 substrate production (V_{CO2})	ml d^{-1}	$V_{CO2} = V_{CO2,\ S+I} - V_{CO2,\ I}$
CH_4substrate percentage (% CH_4)	%	$\%\ CH_4 = 100 * V_{CH4} / V_{biogas}$
CO_2substrate percentage (% CO_2)	%	$\%\ CO_2 = 100 * V_{CO2} / V_{biogas}$
Other gas percentage (% other gases)	%	$\%$ Other gases $= 100\% - \%\ CO_2 - \%\ CH_4$
X_{STP}		$X_{STP} = X_m * [T_{standard} * P_m / T_m * P_{standard}]$
CH_4 / VS_{added}	l $CH_4 g^{-1}$VS	$_{CH4}/VS_{added} = [V_{CH4cumulative} / 1000 / VS_{added}]$
CH_4/COD_{added}	l $CH_4 g^{-1}$COD	$_{CH4}/COD_{added} = [V_{CH4cumulative} / 1000 / COD_{added}]$
CH_4/TS_{added}	l $CH_4 g^{-1}$TS	$_{CH4}/TS_{added} = [V_{CH4cumulative} / 1000 / TS_{added}]$
$TS_{removal}$	%	$TS_{removal} = 100 * [V_{S+I} * (C_{initialTS\ inS+I} - C_{finalTS\ inS+I})] / (V_{S+I} * C_{initialTS\ inS+I})$
$VS_{removal}$	%	$VS_{removal} = 100 * [V_{S+I} * (C_{initialVS\ inS+I} - C_{finalVS\ inS+I})] / (V_{S+I} * C_{initialVS\ inS+I})$
$COD_{removal}$	%	$COD_{removal} = 100 * [V_{S+I} * (C_{initialCOD\ inS+I} - C_{finalCOD\ inS+I})] / (V_{S+I} * C_{initialCOD\ inS+I})$
Compost potential		
Per kg fish produced (COF)	kg kg^{-1}fish	$COF = [(S_{dm}\ kg^{-1}\ FB + F * kg^{-1}\ S_{dm}) / (100\% - Mst_{initial})] - Dig * [(S_{dm}\ kg^{-1}\ FB + F * kg^{-1}\ S_{dm}) / (100\% - Mst_{final})]$
Per kg feed consumption (COpF)	kg kg^{-1}feed	COpF = COF/FCR
Methane potential		
Per kg fish produced (MF)	l CH_4 kg^{-1}fish	$MF = CH_4.gVS^{-1} * gVS.kg^{-1}$ fish settled / FB
Per kg feed consumption (MpF)	l CH_4 kg^{-1}feed	$MpF = CH_4.g\ VS^{-1} * gVS.kg^{-1}$ feed settled
Energy potential	kWh	E[(1)]
Per fish produced (EF)	kWh kg^{-1}fish	EF = E*(MF/1000)
Per feed consumption (EpF)	kWh kg^{-1}feed	EpF =E*(EpF/1000)

[(1)] Theoretical energy content of methane gas according to Henze et al. (1997), E = 9.7 kWh/m^3 CH_4, $\mathbf{V_{CH4\ potential}}$: volume of methane production for energy production calculation (m^3), $\mathbf{V_{CH4,\ S+I}}$: volume of methane production from test on substrate including inoculum (ml.d^{-1}), $\mathbf{V_{CH4,\ I}}$: volume of methane production from inoculums reactor (ml.d^{-1}), $\mathbf{V_{CO2,\ S+I}}$: volume of CO_2 measurement in reactor with substrate including inoculum (ml.d^{-1}),$\mathbf{V_{CO2,\ I}}$: volume of CO_2 measurement in reactor with inoculum (ml.d^{-1}), $\mathbf{NU_{initial}}$: amount of VS, TOC, TN or TP in initial sludge in reactor (mg), $\mathbf{NU_{final}}$: amount of VS, TOC, TN or TP in final sludge in reactor (mg), $\mathbf{M_{initial}}$: amount of Ca, K or Mg in initial sludge in reactor (mg), $\mathbf{M_{final}}$: amount of Ca, K or

Mg in sludge in final sludge in reactor (mg), **FC**: cumulative feed input (kg), $\mathbf{W_{total\ final\ fish\ bw}}$: total fish biomass at harvest (kg, wet weight), $\mathbf{W_{total\ initial\ fish\ bw}}$: total fish biomass at stocking (kg, wet weight),$\mathbf{V_{biogas}}$**:** volume of biogas production ($ml.d^{-1}$), $\mathbf{V_{CO2}}$: volume of CO_2 production from substrate ($ml.d^{-1}$), $\mathbf{V_{CH4\ cumulative}}$: accumulative methane production during experimental period 63 days (ml); $\mathbf{VS_{added}}$: total volatile solids in substrate added for test (g), $\mathbf{COD_{added}}$: initial total chemical oxygen demand in substrate added for test (g); $\mathbf{TS_{added}}$: corrected for control reactor (g), $\mathbf{X_{STP}}$: volume gas content at standard temperature (^oC) and pressure (ml), $\mathbf{P_m}$: atmospheric pressure during measurement of gas production during experiment (bar), $\mathbf{X_m}$: gas volume at room temperature (^oC) (ml), $\mathbf{T_{standard}}$: standard temperature, 0^oC (32^oF), $\mathbf{T_m}$: room temperature in (36^oC) 96.8^oF, $\mathbf{P_{standard}}$**:** standard pressure (bar), **COP:** Final compost production (kg in wet weight), **FB:** fish biomass produced (kg), $\mathbf{V_{S+I}}$: total volume of substrate including inoculum in reactor (l), $\mathbf{C_{initialTS\ inS+I}}$: initial total suspended solids concentration of substrate including inoculum in reactor ($mg\ l^{-1}$), $\mathbf{C_{initialTS\ inS+I}}$: final total suspended solids concentration of substrate including inoculum in reactor ($mg\ l^{-1}$), $\mathbf{C_{final\ lVS\ in\ S+I}}$: initial volatile solids concentration of substrate including inoculum in reactor ($mg\ l^{-1}$), $\mathbf{C_{finalVS\ in\ S+I}}$: final volatile solids concentration of substrate including inoculum in reactor ($mg\ l^{-1}$), $\mathbf{C_{initialCOD\ in\ S+I}}$**:** initial chemical oxygen demand concentration of substrate including inoculum in reactor ($mg\ l^{-1}$), $\mathbf{C_{finalCOD\ inS+I}}$: final chemical oxygen demand concentration of substrate including inoculum in reactor ($mg\ l^{-1}$). $\mathbf{S_{dm}}$: sludge of dry matter in pond-sludge or RAS-sludge (kg); **F**: rice straw: sludge ratio on dry weight in initial composting with F=1 for pond-sludge and F=2 for RAS-sludge; $\mathbf{Mst_{initial}}$: moisture content in initial composting for pond-sludge or for RAS-sludge (%); $\mathbf{Mst_{final}}$: moisture content in final compost for pond-sludge or for RAS-sludge (%); **FCR:** feed conversion ratio, 1.53 for ponds and 1.25 for RAS (kg feed consumed/ kg fish produced); **Dig**: percentage of dry matter lost during composting for pond-sludge or for RAS-sludge (%).

Statistics

Treatment effects (pond vs. RAS) on nutrient concentrations in compost, nutrient removal during composting, methane production potential and biogas composition of the bio-methane production test were analyzed by one-way ANOVA, followed by Tukey test in case of significant difference ($P < 0.05$).

4.3. Results

4.3.1. Sludge production

The weight of sludge of dry matter collected per kg fish produced was 6 times higher in ponds than that in RAS ($P < 0.05$). The dry sludge collected per kg fish produced declined with increasing average fish body weight in ponds, whereas this ratio slightly increased with fish body weight in RAS (Figure 4.3A). On average, 1.2 ± 0.50 and 0.2 ± 0.04 kg sludge of dry matter per kg fish produced was collected in ponds and RAS, respectively. The amount of volatile solids collected per kg fish produced in ponds and RAS was similar and increased with fish body weight (Figure 4.3B).

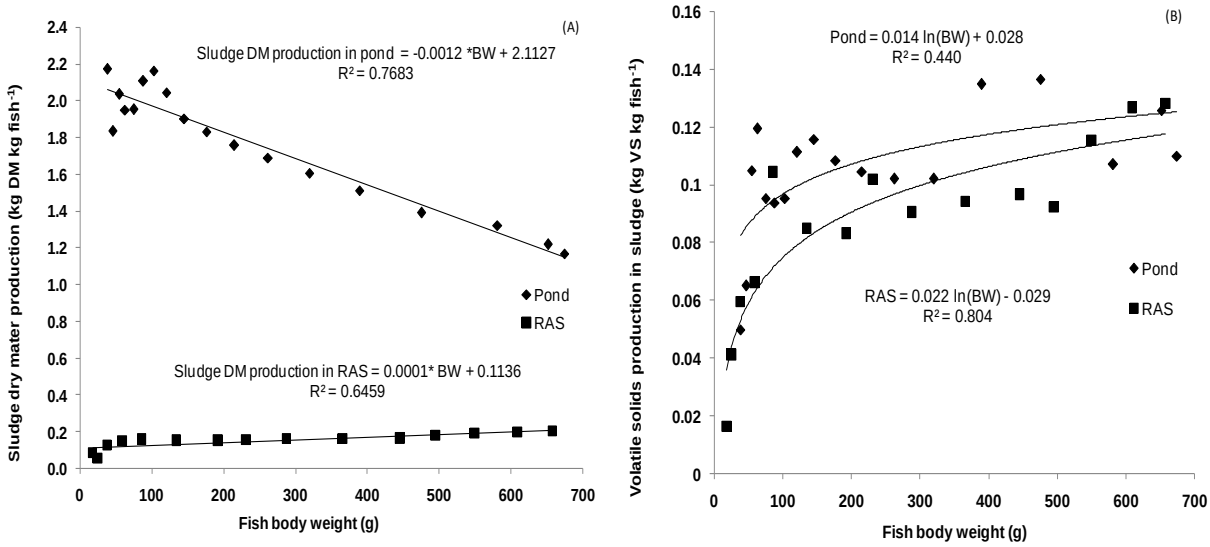

Figure 4. 3: Average sludge dry matter (A) and volatile solids (B) production (on dry weight basis) by average individual fish weight (on wet weight basis) in striped catfish ponds and RAS. Values are mean. n = 3 for RAS and n = 4 for ponds.BW: fish body weight.

4.3.2. Sludge composition in striped catfish ponds and RAS systems

Table 4. 5: Sludge composition (on dry weight basis) in striped catfish ponds (n=4) and RAS (n=3). Values are mean ± standard deviation (S.D.)

Parameter	Unit	Spond			SRAS			p-value
		Mean	±	SD	Mean	±	SD	
pH	-	6.70^{a}	±	0.2	6.43^{b}	±	0.1	0.001
EC	$mScm^{-1}$	0.75^{b}	±	0.5	2.72^{a}	±	0.1	0.001
COD	%	8.70^{b}	±	3.8	82.52^{a}	±	8.7	0.001
Ash	%	93.80^{a}	±	2.8	42.14^{b}	±	5.9	0.001
VS	%	6.20^{b}	±	2.8	57.86^{a}	±	5.9	0.001
TOC	%	3.40^{b}	±	1.5	31.31^{a}	±	3.4	0.001
TC	%	3.50^{b}	±	1.3	32.82^{a}	±	3.2	0.001
TN	%	0.35^{b}	±	0.1	2.84^{a}	±	0.8	0.001
TP	%	0.33^{b}	±	0.2	2.57^{a}	±	0.5	0.001
Total Ca	$mg\ kg^{-1}$	$7,633^{b}$	±	57	$10,966^{a}$	±	25	0.000
Total Mg	$mg\ kg^{-1}$	$3,833^{a}$	±	208	$3,533^{a}$	±	58	0.074
Total K	$mg\ kg^{-1}$	$1,566^{a}$	±	351	353^{b}	±	10	0.004

Mean with different superscript letter within rows are significantly different ($P < 0.05$). **Spond:** sludge in striped catfish ponds, **SRAS:** sludge in RAS.

Sludge collected in ponds was different from sludge collected in RAS ($P < 0.05$). Pond sludge DM contained on average 94% ash compared to 42% for RAS, while RAS sludge contained 9-times more volatile solids ($P < 0.05$). Moreover, the percentage TN, TP and TOC in RAS sludge was close to 10 times higher than in ponds sludge DM ($P < 0.05$) (Table 4.5). In ponds, the concentration of TC and TOC per kg sludge DM in the sludge bed changed significantly with depth. The amount of TOC in sludge in the top 0 – 5 cm was 23g per kg sludge DM and gradually declined with depth reaching 13 g kg^{-1} dry sludge in the 20 – 25 cm depth layer (Figure 4.4). The amount of TN content varied between 2 and 5 g per kg dry sludge showing an irregular pattern with depth, while TP was 1.4 – 1.7 g per kg dry sludge and similar between the 5 cm depth layers.

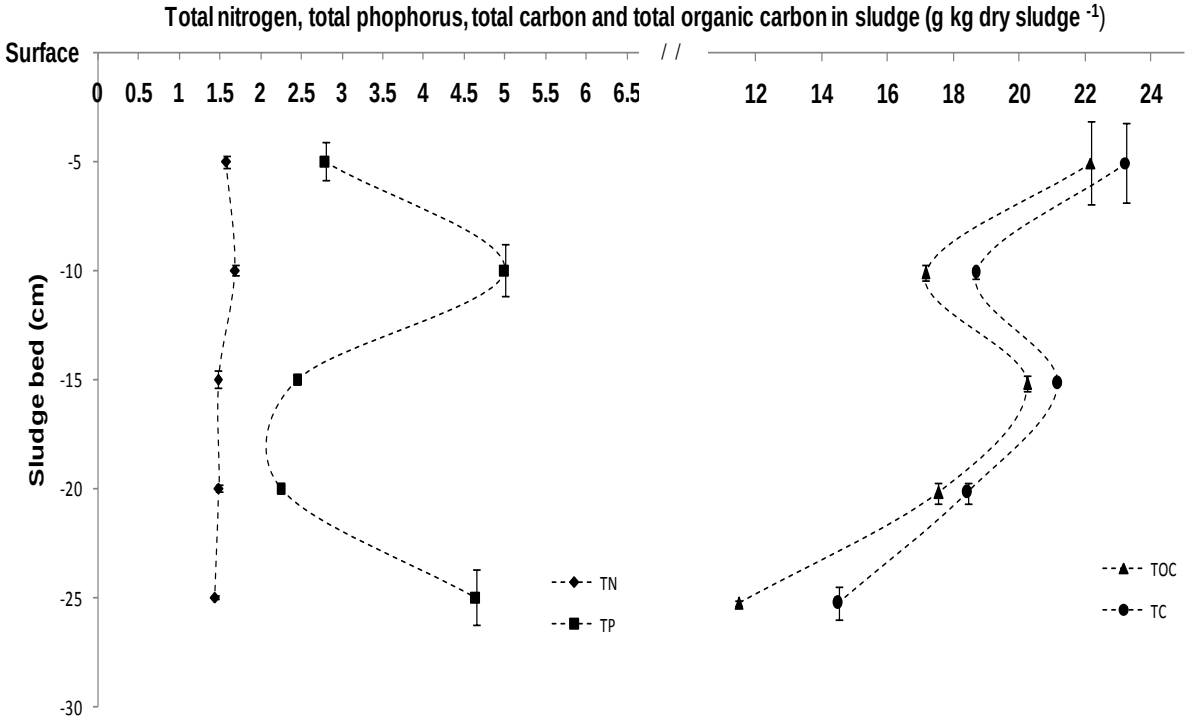

Figure 4. 4: Sludge composition change in accumulative sludge layers in striped catfish ponds. Values are mean, n=4.

4.3.3. Compost composition

Composting decreased the VS content by 36 – 40 % and the TOC with 31 – 53 %. The TN percentage during composting dropped less, showing a 16 – 18 % decline. Nutrient losses were similar for ponds and RAS sludge ($P > 0.05$). Because the TN loss due to composting was smaller than the TOC loss, the C:N ratio dropped from 20 – 23 to 13 – 14 (Table 4.6).

Table 4. 6: Compost composition on dry weight basis, except for moisture (wet weight basis) and RS: sludge ratio. Values are mean ± S.D., n= 3.

Parameter	Unit	Spond+RS			SRAS+RS			p-value
		Mean	±	SD	Mean	±	SD	
Initial composition								
RS: Sludge ratio	g g^{-1}	1			2			-
Moisture	% ww	59.8	±	1.2	59.2	±	2.5	0.589
pH	-	6.9	±	0.1	6.8	±	0.1	0.313
EC	$mScm^{-1}$	2.2^{b}	±	0.1	3.3^{a}	±	0.1	0.001
VS	%	49.8^{b}	±	0.4	77.9^{a}	±	0.7	0.001
Ash	%	50.2^{a}	±	0.4	22.1^{b}	±	0.4	0.001
TOC	%	22.0^{b}	±	0.2	36.0^{a}	±	0.5	0.001
TP	%	0.2^{b}	±	0.02	1.1^{a}	±	0.01	0.001
TN	%	0.9^{b}	±	0.01	1.7^{a}	±	0.01	0.001
C: N ratio	g g^{-1}	20.0^{b}	±	0.1	23.4^{a}	±	0.3	0.001
Total Ca	$mgkg^{-1}$	4,850.0	±	132.3	5,033.3	±	207.4	0.266
Total Mg	$mgkg^{-1}$	$2,142.0^{a}$	±	91.3	$1,478.2^{b}$	±	15.8	0.001
Total K	$mgkg^{-1}$	$9,200.0^{b}$	±	173.2	$11,339.8^{a}$	±	347.0	0.001
Final composition								
Moisture	%	56.3^{a}	±	2.1	58.9^{a}	±	1.7	0.574
pH	-	7.3^{a}	±	0.1	7.6^{b}	±	0.1	0.001
EC	$mScm^{-1}$	2.0^{b}	±	0.1	3.2^{a}	±	0.1	0.001
VS	%	31.6^{b}	±	2.0	46.5^{a}	±	0.3	0.001
Ash	%	68.4^{a}	±	2.0	53.5^{b}	±	0.3	0.001
TOC	%	10.4^{b}	±	1.0	17.5^{a}	±	1.5	0.002
TP	%	0.2^{b}	±	0.01	1.1^{a}	±	0.01	0.001
TN	%	0.8^{b}	±	0.01	1.4^{a}	±	0.01	0.005
C : N ratio	g g^{-1}	13.5^{a}	±	1.0	13.0^{a}	±	1.9	0.728
Total Ca	$mgkg^{-1}$	$4,823.3^{a}$	±	40.4	$4,985.3^{a}$	±	103.7	0.065
Total Mg	mg kg^{-1}	$2,135.7^{a}$	±	29.8	$1,473.7^{b}$	±	118.2	0.001
Total K	mg kg^{-1}	$9,167.0^{b}$	±	152.9	$11,320.3^{a}$	±	5.7	0.001
Nutrient loss*								
DM	%	38.7^{b}	±	0.3	44.4^{a}	±	0.4	0.001
VS	%	36.6^{a}	±	3.9	40.3^{a}	±	0.4	0.171
TOC	%	52.7^{a}	±	4.4	51.4^{a}	±	4.5	0.732
TN	%	15.9^{a}	±	3.8	17.9^{a}	±	10.1	0.764
TP	%	0.2^{a}	±	2.5	0.6^{a}	±	3.0	0.111
Trace mineral loss*								
Total Ca	%	0.5^{a}	±	3.2	0.8^{a}	±	5.2	0.931
Total Mg	%	0.2^{a}	±	3.6	0.2^{a}	±	9.1	0.994
Total K	%	0.4^{a}	±	0.6	0.1^{a}	±	3.1	0.898

Mean with different superscript letter within rows are significantly different ($P < 0.05$). ***** % nutrient and trace mineral loss between initial and final composition divided by the initial composition (expressed as percentage). **Spond+RS :** sludge collected from striped catfish ponds mixed with rice straw**, SRAS+RS:** sludge collected from striped catfish RAS mixed with rice straw .

The TP percentage dropped 0.2 – 0.6 % during composting, while the pH increased by 0.2 and 1.3 units in Spond+RS and SRAS+RS, respectively. Mineral losses were small and similar between Spond+RSand SRAS+RS(P> 0.05). For nearly all parameters, the nutrient content was higher in RAS-compost than in pond-compost (P<0.05), reflecting the quality of the composted sludge.

4.3.4. Methane potential

The CH_4volume obtained after fermentation was 0.26 l per g VS from RAS-sludge, compared to 0.17 l obtained from pond-sludge (P < 0.05). The CH_4 production per g TS from RAS-sludge was 15 times higher than for pond-sludge (P < 0.05) (Table 4.7).

Table 4. 7: Quantity and quality of biogas from digestion of sludge in striped catfish ponds and RAS.

Parameter	Unit	Spond			SRAS			p-value
		Mean	±	SD	Mean	±	SD	
Methane yield								
-per TS added	l CH_4g^{-1}TS	0.010^b	±	0.00	0.157^a	±	0.02	0.001
-per VS added	l CH_4g^{-1}VS	0.165^b	±	0.04	0.264^a	±	0.04	0.019
-per COD added	l CH_4g^{-1}COD	0.125^b	±	0.01	0.201^a	±	0.03	0.017
Biogas quality								
-CH_4	%	46.80^b	±	6.1	52.70^a	±	5.7	0.001
-CO_2	%	49.54^a	±	5.7	43.7^b	±	4.9	0.001
-Other gases	%	3.66^a	±	1.4	3.60^a	±	1.2	0.984

Mean with different superscript letter within rows are significantly different (P < 0.05). **Spond**: sludge in traditional pangasius ponds, **SRAS**: sludge in pangasius RAS systems, n=3.

The daily volume of CH_4, CO_2, and other gases obtained from digestion of RAS-sludge and pond-sludge increased quickly until day 9 – 10 and then declined gradually becoming negligible small after 63 days (Figure 4.5). The percentage of CH_4 in RAS-sludge biogas was 6% higher than pond-sludge biogas (P < 0.05), the latter containing 6% more CO_2 in the biogas mixture (Figure 4.6).

After 63 days of incubation, the amount of nutrients in RAS-sludge with inoculum (SRAS+I) was higher than in pond-sludge with inoculum (Spond+I) (P <0.05), except for TP and NO_3-N

(P > 0.05). The VS and COD digestion efficiencies were1.5 and 1.6 times higher, respectively, for RAS-sludge than for pond-sludge (P < 0.05) (Table 4.8).

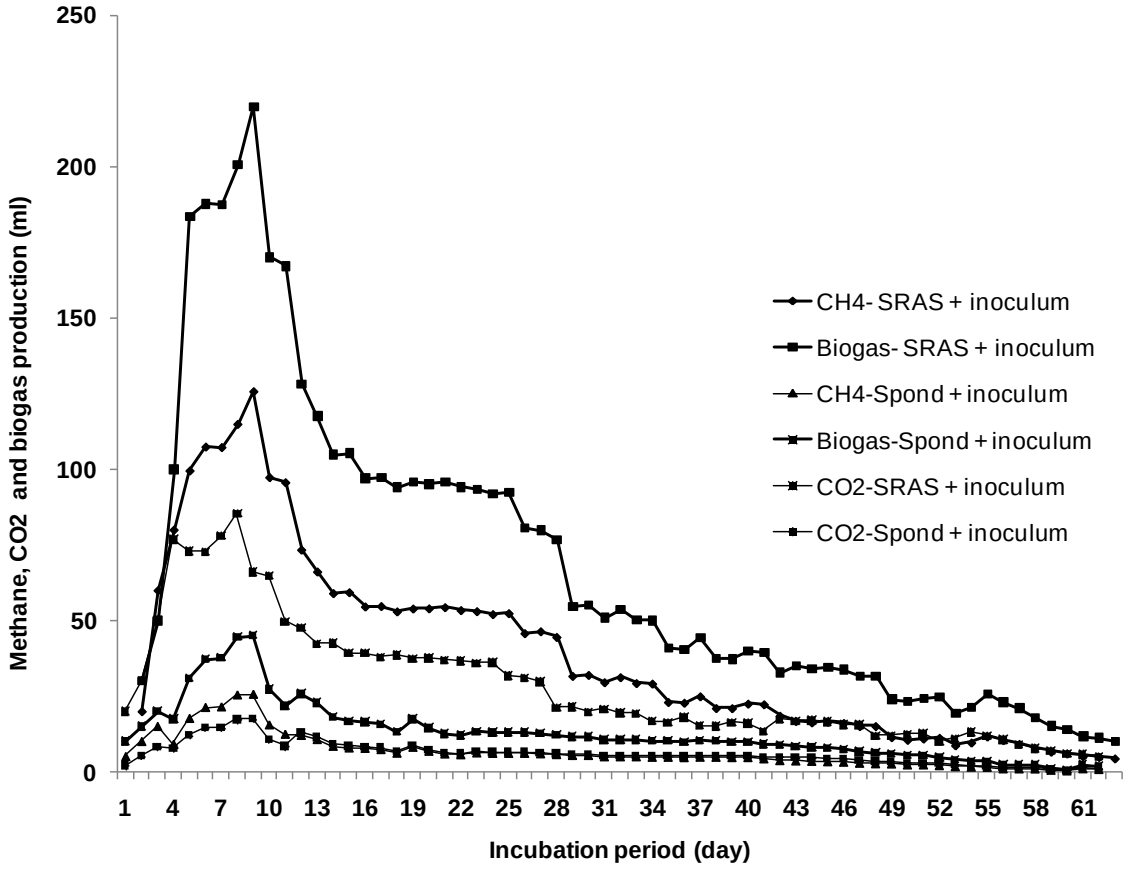

Figure 4. 5: Mean of daily biogas production in 550 ml digestion bottles from striped catfish pond-sludge (Spond) and RAS-sludge (SRAS). n = 3. **CH4-SRAS + inoculum**: volume (ml) of methane gas from RAS-sludge with inoculum, **CH4-Spond + inoculum**: volume (ml) of methane gas from pond-sludge with inoculum, **Biogas-SRAS + inoculum**: volume (ml) of biogas from RAS-sludge with inoculum, **Biogas-Spond + inoculum**: volume (ml) of biogas from pond-sludge with inoculum, **CO2-SRAS + inoculum**: volume (ml) of carbon dioxide from RAS-sludge with inoculum, **CO2-Spond + inoculum**: volume (ml) of carbon dioxide from pond-sludge with inoculum.

The amount of compost obtained per kg fish produced and per kg feed consumed was higher in ponds than in RAS, although not significantly different (P < 0.05), while the volume of methane produced per kg fish produced and per kg feed consumed was higher in RAS than in

ponds ($P < 0.05$). The energy yield from methane with RAS-sludge was more than 2.5 higher than with pond-sludge ($P < 0.05$) (Table 4.9).

Table 4. 8: Change in sludge composition after incubation (wet weight basis).

Parameter	Unit	Spond + I			SRAS + I			p-value
		Mean	±	SD	Mean	±	SD	
Final sludge								
pH		7.7^{b}	±	0.1	8.2^{a}	±	0.1	0.008
EC	$mScm^{-1}$	0.7^{b}	±	0.1	1.4^{a}	±	0.1	0.001
Alkalinity	$mgCaCO_3l^{-1}$	$1,976^{b}$	±	156	$2,416^{a}$	±	30	0.001
DM	%	5.4^{a}	±	0.2	4.0^{b}	±	0.2	0.001
VS	%	1.2^{a}	±	0.1	1.3^{a}	±	0.1	0.101
COD	$mg\ l^{-1}$	$18,799^{b}$	±	73	$20,606^{a}$	±	1,040	0.040
TN	$mg\ l^{-1}$	$1,448^{b}$	±	18.2	$1,874^{a}$	±	15.5	0.001
TAN	$mg\ l^{-1}$	209.6^{b}	±	7.3	282.0^{a}	±	2.5	0.001
NO_3-N	$mg\ l^{-1}$	0.6^{a}	±	0.03	0.5^{a}	±	0.2	0.101
TP	mgl^{-1}	836^{a}	±	2.0	$1,455^{a}$	±	20	0.749
Digestion efficiency								
-VS removal	%	38.3^{b}	±	2.8	59.6^{a}	±	1.7	0.001
-COD removal	%	37.2^{b}	±	0.5	57.5^{a}	±	2.0	0.001

Mean with different superscript within row are significantly different ($P < 0.05$). **Spond + I:** pond-sludge with inoculum, **SRAS + I:** RAS-sludge with inoculums, (n = 3).

Table 4. 9: Compost, methane and energy potential obtained with sludge collected from striped catfish ponds and RAS.

Parameter	Unit	Pond			RAS			p-value
		Mean	±	SD	Mean	±	SD	
Compost potential								
-per fish produced	$kg\ kg^{-1}fish$	3.7^{a}	±	1.6	0.87^{b}	±	0.02	0.034
-per feed consumption	$kgkg^{-1}feed$	2.4^{a}	±	1.1	0.70^{b}	±	0.02	0.043
Methane potential								
-per fish produced	$l\ kg^{-1}fish$	13.9^{b}	±	8.5	33.5^{a}	±	1.8	0.012
-per feed consumption	$l\ kg^{-1}feed$	8.7^{b}	±	5.0	25.3^{a}	±	1.1	0.003
Energy potential								
-per fish produced	$kWhkg^{-1}fish$	0.15^{b}	±	0.1	0.33^{a}	±	0.02	0.012
-per feed consumption	$kWhkg^{-1}feed$	0.08^{b}	±	0.1	0.25^{a}	±	0.01	0.003

Mean with different superscript within each row are significantly different ($P < 0.05$).

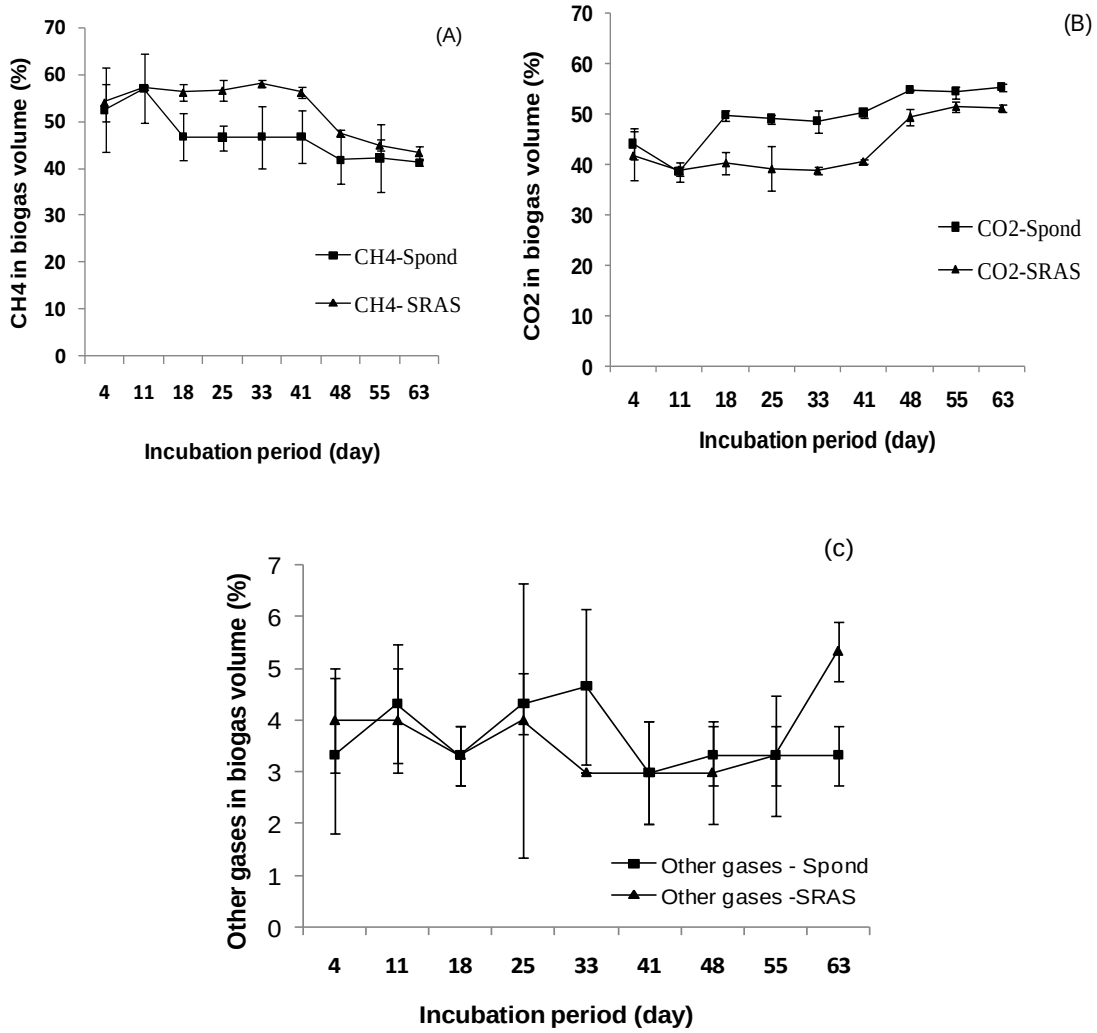

Figure 4. 6: Change in biogas composition (% of total volume) during incubation period (day). **CH4-Spond**: % CH4 in biogas mixture from pond-sludge, **CH4-SRAS**: % CH4 in biogas mixture from RAS-sludge (4.6A). **CO2-Spond**: % CO2 in biogas mixture from pond-sludge, **CO2-SRAS**: % CO2 in biogas mixture from RAS-sludge (4.6B). **Other gases-Spond**: % other gases in biogas mixture from pond-sludge, **Other gases-SRAS**: % other gases in biogas mixture from RAS-sludge (4.6C).

4.4. Discussion

4.4.1. Quantity and quality of sludge in striped catfish culture systems

By weight, 6 times more sludge of dry matter was discharged per kg fish produced from ponds than from RAS. However, similar quantities of volatile solids were discharged from ponds and RAS per kg fish produced. The difference in weight is due to a high soil content in pond-sludge. Farmers exchange pond water with the Mekong River each day, with inflowing water delivering on average 202 mg l^{-1} TSS (Nhut et al., Submitted-a). Per kg fish produced in ponds, 2 – 7 m^3 of water is exchanged with the river. Assuming an average water exchange of 5 m^3 per kg fish produced, then about 1000 g TSS enters the pond, while with out-flowing water only 300 g TSS is discharged. Including feed inputs, about 1,200 g TS accumulates in striped catfish ponds per kg fish produced (Nhut et al., Submitted-a), which is more than usually reported for pond fish culture (Chen et al., 1997).

Striped catfish produces dilute faeces, which are quickly dispersed through the water column and difficult to trap. In RAS, 17% of feed dry matter was collected by a swirl separator placed directly after the fish tank, collecting sludge every 4 hours (Nhut et al., Submitted-b). The quantity of faeces collected daily as sludge from swirl separators in RAS and flow-through tanks fluctuated between 12 and 20 % of the feed input (on wet weight basic) (Nhut et al., Submitted-b) and was difficult to predict. In ponds, numerous factors can affect sludge production, including for example location, season, tidal regime, day of culture cycle, fish density and biomass, water exchange frequency, etc. Nhut et al. (Submitted-a) observed a peak in VS accumulation in ponds after 3 months of culture when the feed load passed 200 – 250 kg ha^{-1} d^{-1} and algae could not compete for nutrients with bacteria, died off and settle, to the pond sediment.

The VS, COD, TOC, TC and TN concentrations in sludge of dry matter change due to decomposition (Adhikari et al., 2014; Boyd, 1985; Boyd, 1995; Chen et al., 1997; Gross et al., 1998; Gross et al., 2000), as shown by differences in concentration at different depths in the undisturbed sludge bed sampled at the end of the culture period. With depth, the TC and TOC concentrations decreased. This was also expected for TN (Boyd, 1995), but this could not be observed in our study. The TP concentrations did not change with depth, which is in line with results of Munsiri et al. (1996). Similar concentrations of VS, TOC, COD, TN and TP for

striped catfish ponds were reported by Phu and Tinh (2012), for channel catfish by Boyd (1995), and for trout in raceways by Stephen et al. (1999).

According to Shrestha et al. (2008), concentrations of Ca, Mg, and K in the Mekong river in Vietnam are low, with 0.73-0.86 mg Ca l^{-1}, 0.38-1.30 mg Mg l^{-1} and 0.04 -0.12 mg K l^{-1}. Soils in the Mekong delta at a depth of 20 - 50 cm contain 1378 mg Ca kg^{-1}, 432 mg Mg kg^{-1} and 164 mg K kg^{-1} (Quang and Guong, 2011). However, in this study, the main supply of Ca, Mg, and K was through feeding (Table 4.1), and the observed concentrations in sludge were higher than previously reported in striped catfish ponds (Phu and Tinh, 2012), freshwater prawn ponds and carp ponds (Wudtisin, 2006). The reason why in our study Ca, Mg and K concentrations were higher is unclear, and merits further study. Possible factors involved are concentrations in the diet, the stage of culture cycle during sampling, the sampling method, the depth and the sample size (Munsiri et al., 1996).

4.4.2. Compost quality, nutrient loss during composting and applicability

The pH, EC, TC, TP and TN concentrations reported by Phung et al. (2009) were similar to the pond-compost (Spond+RS) concentrations in our study, the biggest exception being Ca levels, which were much higher in our study (Table 4.10). The compost produced by Phung et al. (2009) allowed saving 30 kg N fertilizer input per ha in rice cultivation. The nutrient content in RAS-compost (SRAS+RS) was higher than in pond-compost. Both types of compost obtained in our study compared well to compost produced from cattle manure (Eghball et al., 1997), but were less nutrient rich than poultry manure (Abdelhamid et al., 2004), pig manure (Abdelhamid et al., 2004; Roca-Pérez et al., 2009; Tran et al., 2011) and sewage (Roca-Pérez et al., 2009) based composts. The compost based on sludge from striped catfish RAS is a good product due to a favourable macro-nutrients content and the fact that in RAS the use of drugs and chemicals is minimal (James et al., 1998).

During composting, the pH and EC slightly increased. This could be the result of inorganic-N release during composting (Sánchez-Monedero et al., 2001). Slight changes (positive or negative) in pH during composting were also reported by Sánchez-Monedero et al. (2001) and Eghball et al. (1997), depending on the type of compost made. The TOC, VS and TN decreased significantly, while losses of TP, Ca, Mg, and K were negligible. These changes are related to the compost processing methods applied (Eghball et al., 1997; Goyal et al., 2005; Tran et al.,

2011). In our study, composting was done in a partially covered box with holes for air exchange. Most likely, when left uncovered VS and N losses would have been higher. The TN loss during composting pig manure was 25– 30% when compost was covered compared to 63–73 % when not covered (Tran et al., 2011). In general, composting studies with uncovered storage report higher TN losses than obtained in our study (Eghball et al., 1997; Sánchez-Monedero et al., 2001; Sommer, 2001; Tran et al., 2011). The VS loss in this experiment was in line with some previous studies (Eghball et al., 1997; Li et al., 2008; Sommer, 2001). Because TC loss was higher than TN loss during composting the C/N ratio declined. In contrast, negligible amounts of TP minerals were lost during composting (Eghball et al., 1997). As expected, TP did not volatilise during composting.

Considering the compost composition (Table 4.6), compost production per kg fish (Table 4.9) and the total striped catfish production in the Mekong Delta, 4.02 million tonnes compost, containing 36 thousand tonnes of TN and 8 thousand tonnes of TP can be produced from pond-sludge. If producing striped catfish in RAS, then 0.96 million tonnes compost would be produced, containing 16.3 thousand tonnes of TN and 10.6 thousand tonnes of TP, would be obtained. Although less compost would be produced from RAS, the ash content is lower, collection easier and transport costs are smaller than for pond-compost. Per kg fish produced in RAS, 0.87 kg compost with a present market value of US$ 0.02 ($VND 700 kg compost^{-1}) can be produced. This value would be enough to cover 50% of the pumping and aeration costs in RAS for striped catfish in pond (0.8 kWh per kg fish produced equaling US$ 0.06 ($VND 1.300 kg fish^{-1})) (Nhut et al., 2015).The compost can also be applied to other crops than rice (Casado-Vela et al., 2007; Casado-Vela et al., 2006; Phung et al., 2009; Pilar et al., 2005), and would lower nutrient discharge to surface waters by 10%, hence improving sustainability (Verreth and Oberdieck, 2009). Considering that a major fraction of the reused compost nutrients will not volatilize, the greenhouse gas emission potential would also be reduced (Mirzoyan et al., 2010; Møller et al., 2004; Picot et al., 2003; Zhang et al., 2013). The composting RAS-sludge and pond-sludge are feasible and simple method and can be applied for striped catfish farms in the Mekong delta. This compost is considered better than utilisation of fresh sludge to plant because it can control quality and quantity for plants. Currently, some gardens have utilised direct effluent and fresh sludge, but its nutrient quality and quantity have not controlled lead to unpredictable production of gardens.

4.4.3. Methane potential production

The pH, alkalinity and TAN concentration in pond and RAS sludge were below the threshold levels that would inhibit methane production (Angelidaki and Ahring, 1993; Chen et al., 2008; Hansen et al., 1998; Speece, 1996). For free ammonia, anaerobic microbes are inhibited at a concentration above 1,100 mg NH_3l^{-1} (Hansen et al., 1998). The substrate to inoculum ratios (VS weight basis) were 0.8 for SRAS+I, and 0.08 for Spond+I, which were sufficient considering that our goal was to combine a high digestibility of VS with methane yield.

The methane yield from RAS-sludge (SRAS+I) was higher than from pond-sludge (Spond+I) (Table 4.7). Although the inoculum to substrate ratio (VS weight basis) in Spond+I was 10 times higher than in SRAS+I, the fraction of VS and COD removed from pond-sludge was smaller than for RAS-sludge. This was not high; possible causes might include a high hydrogen sulfide concentration during digestion or because of chemical and drug residues present in the pond-sludge. A 50% inhibition occurred with H_2S concentrations of 60 to 240 mg l^{-1} in the digester (Speece, 1996). In addition, the pond-sludge accumulated over a 2 months period, hence a large fraction of easily degradable VS was already mineralized leaving only organic matter that is difficult to digest, e.g. lignin compounds (Stinson and Ham, 1995). Typically, one kg of commercial striped catfish feed contains more than 200 g of soybean and 400g wheat flour (Hien et al., 2010). Non–starch polysaccharides (NSP) containing lignin, hemicellulose and cellulose, which have low digestibility, are present in soybean meal and wheat flour. Meriac et al. (2014b) reported that one kg of rainbow trout feed comprised 150 g soybean meal and 175 g wheat flour. The COD of the NSPs in the trout's faeces represented 65 % of the total COD in the faeces. Presence of soybean meal in fish diets could also explain why decomposition of collected fish faeces was 58% less than for faeces based on soybean meal free diets (Mirzoyan, 2009). Overall, the methane production potential of solids collected from striped catfish, trout, salmon and striped bass ponds is similar (Gebauer, 2004; Gebauer and Eikebrokk, 2006; Kugelman and Van Gorder, 1991; Lanari and Franci, 1998) (Table 4.11). When compared to waste from terrestrial animals, the methane yield (ml CH_4 g^{-1} VS) from striped catfish sludge collected in RAS is lower than for piglet manure, cattle slaughter waste, and duckweed while it is higher than for manure from sow, cow, buffalo, rabbit, sheep, goat, chicken, slaughter waste from pig and fish, household waste and grass and cassava residues (Cu

et al., 2015) because manure from terrestrial animals is richer in nutrients and COD, and contains less ash.

The methane and carbon dioxide fraction in biogas depends on the proximate composition of the sludge. Wellinger et al. (2013)reported a yield of 60% CH_4: 40% $CO_2$for protein, 72% CH_4: 28% CO_2 for lipid and 50% CH_4 : 50% CO_2 for carbohydrate. Striped catfish feed is a mixture of ingredients with specific apparent digestibility coefficients (ADC) (Hien et al., 2010; Hung et al., 2003). The high C and low N fractions in striped catfish pond-sludge accounted for the observed ratio in the biogas produced of 47% CH_4 and 50% CO_2, while the higher N fraction in RAS-sludge increased this ratio to 53% CH_4: 44% CO_2.

One kg striped catfish produced in RAS could produce 33.5 l CH_4 which represents 0.33 kWh potential energy yield per kg fish produced. With pond-sludge, 13.9 l $CH_4$was obtained per kg fish produced, which represents a potential energy yield of 0.14 kWh per kg fish produced. Assuming 300 tonnes striped catfish is annually produced per ha in ponds in the Mekong delta (MARD, 2014a), 42,000 kWh can be obtained per ha from pond-sludge. If all striped catfish in the Mekong delta would be produced in RAS, then the potential energy yield would be 99,000 kWh per ha. One year has 8,760 hours, thus the potential energy yield corresponds to a constant energy supply slightly above 11.3kW. The electricity yield from methane is around 30% (Henze et al., 1997), so a 3.4 kW power source could be realized. It can compensate about 10-12% electricity consumption for aeration and pump in RAS for striped catfish culture.

Table 4. 10: Nutrient content of different types of compost from animal waste.

Compost	pH	EC mS cm^{-1}	VS %	TC %	C/N	TP %	TN %	Ca mg kg^{-1}	Mg mg kg^{-1}	K mg kg^{-1}	Reference
Spond + RS	7.3	2	31.6	10	14	0.2	0.8	4,823	2,136	9,167	[1]
Spond + RS	7.4	2.4	-	9	-	0.4	0.9	84	2,540	11,600	[2]
SRAS + RS	7.6	3.2	46.5	18	13	1.1	1.4	4,985	1,474	11,320	[3]
Scattle manure	7.7	7.4	19.2	10	-	0.9	1.1	13	5.6	12,000	[4]
Spoultry manure	8.0-8.7	3.6-4.3	70-75	35-37	9-13	-	2.7-4.1	-	-	-	[5]
Spig manure	-	-	63-72	58-63		1.5-3.8	1.7-3	-	-	13,400	[6]
Ssewage	6.8-7.2	3.7-3.8	48-53	18-23	10-11	2.5-2.7	1.9-2.3	-	-	-	[7]

Spond + RS: sludge in striped catfish ponds mixed with rice straw (this study) [1] Spond + RS: sludge in traditional striped catfish ponds mixed with rice straw (Phung et al. (2009) [2] SRAS + RS: sludge in striped catfish RAS mixed with rice straw (this study) [3] Scattle manure: Beef cattle feedlot manure (result of experiment in 1992) (Eghball et al. (1997) [4] Spoultry manure: Poultry manure + rice straw+oilseed rape cake (Abdelhamid et al. (2004) [5] Spig manure: Pig manure + rice straw (Tran et al. (2011) [6] Ssewage: Sewage + rice straw(Roca-Pérez et al. (2009) [7].

Table 4. 11: Methane production from different types of aquaculture sludge.

Sludge	T(°C)	HRT (day)	TS (%)	Salinity	Digestion efficiency (%)			CH_4 (%)	CH_4 (l g^{-1} COD add)	Reference
					TS	VS	COD			
Spond	36	60	6	Fresh	11.6	38.3	37.2	46.8	0.125	[1]
SRAS	36	60	6	Fresh	32.7	59.6	57.5	52.7	0.201	[2]
SAtlantic salmon RAS	35	10-20	4-6	Fresh	-	-	57-71	36-71	0.13-0.16	[3]
Strout	24-25	22-38	1.4-2.4	Fresh	-	93-97	-	>80	0.20-0.25	[4]
Ssalmon RAS	35	30	8.2-10.2	Brackish	-	47-62	35-55	49-58	0.11-0.18	[5]
Sstriped bass RAS	30	6-8	0.4	Brackish	-	92-98	99.6	4-53	0.04-3.6	[6]

Spond: sludge in traditional striped catfish ponds this study [1],SRAS: sludge in striped catfish RAS this study [2],SAtlantic salmon RAS : sludge Atlantic Salmon RAS Kugelman and Van Gorder (1991)[3], Strout: Rainbow trout RAS Lanari and Franci (1998) [4],Ssalmon RAS: sludge in Salmon RAS Gebauer (2004) [5], Sstriped bass RAS: sludge striped bass RAS Mirzoyan (2009)[6].

4.4.4. Research constraints

Small quantities of compost were produced under controlled indoor conditions. Compost yield under open field conditions should also be evaluated to predict yields under field conditions. Quality and quantity of compost from sludge in striped catfish ponds in this study can not assume for during a full production cycle.

4.5. Conclusions and recommendations

Sludge production in striped catfish ponds was 1.2 kg per kg fish produced, which is mainly due to a high mineral content in the sludge, originating from suspended soil particles in the daily water intake. Although 6 times less sludge is produced in RAS, the concentration of VS in the sludge is much higher, making it a good resource to produce compost or energy from methane. Nevertheless, the quality and quantity of methane from striped catfish sludge was lower than for animal manures and the resulting electricity yield is low. Therefore, composting the pond-sludge or RAS-sludge is presently considered the best option to reuse part of the nutrients trapped in the sludge from striped catfish culture systems. Another option that still could be explored is denitrification. A disadvantage of denitrification is that volatile solids are mainly volatilized, while advantages are that water exchange with the river can be reduced further and that less liming material is needed.

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CHAPTER 5

Effect of an upflow-sludge-blanket denitrification reactor on environmental sustainability of striped catfish production (*Pangasianodon hypophthalmus*) in recirculating aquaculture system

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Abstract

The effect of integrating an upflow-sludge-blanket denitrification reactor (USB) in a recirculating aquaculture system (RAS) for striped catfish *(Pangasianodon hypophythalmus, Sauvage,1878)* production on water quality, water exchange, fish performance and environmental sustainability was assessed. Four identical RAS were used, of which 2 RAS served as control treatment (RAS) and 2 RAS were extended with a USB denitrification reactor (RAS+USB). Fish performance (e.g. growth, survival, feed conversion ratio (FCR)) and fish quality (fillet percentage, fillet color and off-flavour) were similar in RAS and RAS+USB treatment ($P > 0.05$). Per kg fish produced (RAS vs. RAS+USB), less water (175 l vs. 77) and less $NaHCO_3$ (176 vs. 86 g) were consumed in the RAS+USB treatment. Also, less dry matter (288 vs. 80 g), less nitrogen (21 vs.7 g) and less phosphorous was discharged (10 vs. 9 g) ($P < 0.05$). In the RAS-USB treatment 40% of feed dry matter was unaccounted and 9% of feed nitrogen was lost. Integrating an USB-reactor in RAS reduced nutrient discharge with effluent from RAS, increased volatilization of N_2 gas and concentrated phosphorous in the sludge blanket, from where it can be discharged for further treatment. The low water consumption in RAS and the very low in RAS+USB treatment allow a better control on biosecurity than what is presently possible in pond culture systems.

Keywords: *denitrification, recirculating aquaculture systems, sustainability, striped catfish, pH control.*

5.1. Introduction

When the striped catfish *(Pangasianodon hypophythalmus, Sauvage, 1878)* culture developed in the late 20th century, various culture systems were used, including cages, pens and ponds, relying on river trash fish as an important food input (Phuong and Oanh, 2010). Quite quickly, the preferred culture system became deep ponds receiving formulated feed and exchanging water with the river to maintain water quality (Nhut et al., submitted-a).

Presently, about 1.1 million metric tons is produced annually in the Mekong delta using 5000 ha of ponds divided over 5400 farms (MARD, 2014a). Striped catfish ponds range in size from 0.08 to 2.2 ha, are 2 to 6m deep and are situated close to the river or channels to facilitate water exchange, delivery of feed from the feed factory and transport of fingerlings to the farmers and market sized fish to the processing plant. Pond production varies between 70 and 850 tons ha^{-1} crop^{-1} (Phan et al., 2009). Striped catfish diets usually contain 90% dry matter (DM), 22-30% crude protein (CP) and 1-1.35% total phosphorus (TP), realizing a feed conversion ratio of 1.7 – 1.9 (Bosma et al., 2009; Phan et al., 2009). The fish are produced with a nutrient utilization efficiency of 38% for nitrogen (N), 14% for phosphorous (P) and 29% for DM administrated with the feed (Nhut et al., submitted-a). On average, 19 g N, 17g P and 360 g DM is discharged per kg fish produced (Nhut et al., submitted-a). The production of one kg of fish in pond culture requires 0.043 - 0.09 kWh energy (Bosma et al., 2009) and a water exchange of 2.5 - 9.1 m^3 with the river (Anh et al., 2010; Bosma et al., 2009; Phan et al., 2009).

Western consumers demand that farmed fish products are sustainably produced (Verreth and Oberdieck, 2009). Therefore, the sector initiated research on sustainability. One way to improve sustainability is the use of Recirculation Aquaculture System (RAS) technology. Nhut et al. (submitted-b) showed that striped catfish can be successfully grown in RAS, using only 146 l of water per kg fish produced, with a nutrient utilization efficiency of 48% for N and 21% for P, a discharge of 14 g N and 12 g P per kg fish produced, and a survival of 94% (Nhut et al., submitted-b). The excellent fish performance and relatively low environmental impact of striped catfish production in RAS can be further improved by integrating a denitrification reactor within the RAS (Eding et al., 2009; Timmons and Ebeling, 2010; van Rijn et al., 2006). A denitrification reactor can be fueled with the carbon (C) in faecal solid

waste to remove nitrate nitrogen (NO_3-N) form the water of a RAS. Advantages of such a denitrification reactor include a further reduction of the solid fecal waste discharge and a lower water exchange than in conventional RAS while a favorable water quality can be maintained. An additional advantage of denitrification in RAS is that less sodium bicarbonate ($NaHCO_3$) is required to maintain alkalinity and pH than in a conventional RAS (Eding et al., 2009; Timmons and Ebeling, 2010; van Rijn et al., 2006). In this study, an upflow-sludge-blanket denitrification reactor (USB-DR) was integrated into a conventional striped catfish RAS (further referred to as RAS+USB). The objective was to compare and quantify striped catfish performance, water quality and waste discharge in RAS without (RAS) and RAS with denitrification (RAS+USB).

5.2. Materials and Methods

5.2.1. Experimental design

Four identical RAS were used, two conventional RAS and two conventional RAS extended with an USB-DR (RAS+USB) (Figure 5.1). In this experiment the RAS was the experimental unit.

5.2.2. Experimental RAS and system maintenance

Experimental RAS. Each RAS contained an 850-l circular PVC tank with a central bottom drain and a net cover to prevent fish from escaping. From the fish tank water was flowing (2.4 m^3 h^{-1}) by gravity to a 260-l swirl separator (inlet pipe inner diameter 63 mm; inner diameter separator 0.85m, inner surface area 0.57 m^2, hydraulic surface load (HSL) 118 m^3 m^{-2} day^{-1}). The swirl separator in each RAS+USB was equipped with a stirrer (30 seconds stirring, 30 minutes pause, stirrer velocity 7 rpm) to prevent solid waste from clogging and to prevent degrading sludge to be washed out due to gas bubble formation causing the waste to float. In conventional RAS, water from the swirl-separator flowed to the central bottom inlet of a 303-l moving bed biofilm reactor, with an installed biofilter surface area of 167m^2 (*Bio-media type Helix 12mm PN10; specific biofilter surface area 834 m^2 m^{-3}, Fleuren & Nooijen company, the Netherlands*). A 8l-bottle connected with bottom outlet of swirl separator to harvest sludge every 3 hours.

From the moving bed biofilter, the water flowed to a 580-l sump, from where it was pumped (*250W/50Hz, Ebara, Italy*) across a 1.3 m^3 trickling filter (dimensions: height 140 cm, cross sectional area 0.93 m^2; *Bio-blok®-200, EXPO-NET, Denmark*, specific biofilm surface area 200 m^2 m^{-3}, HSL 150 m^2 m^{-3} d^{-1}). The inflow water to the trickling filter was spread homogeneously over the cross-sectional area through a PVC water distribution grid. The outflow water of the trickling filter was collected in a 200-l sump from where it flowed back to the fish tank, while surplus water returned to sump. A water inlet valve, connected to a water meter (GMK-15, range accuracy (m^3h^{-1}) ± 2%, ASAHI company, Bangkok, Thailand) to record replacement water, was connected to sump. The supply water came from a 200 m^3 overhead storage tank.

Upflow Sludge Blanket Denitrification Reactor (USB-DR). The USB-DR is a 2.15 m high transparent cylinder with a 0.38 m inner diameter. The solid waste accumulating at the bottom of the swirl separator was pumped (Masterflex peristaltic pump console drive model; 6-600 rpm; pump house Masterflex easy load II; tubing masterflex 18 Norprenea; Applikon, Schiedam, the Netherlands) with a flow of 807 l d^{-1} to the bottom section of the USB-DR. The flow through the USB-DR concurred with a HSL of 7.1 m^3 m^{-2} day^{-1}. Water overflowed at the top of the USB-DR into a collection channel from where it flowed back into the swirl separator through central tube by gravity. Difference in height of top of the USB-DR and top of swirl separator was a meter (Figure 5.2). In this way, solids escaping from the USB-DR could be recaptured. The stirrer in the USB-DR was a rectangular 210 x 39 cm open frame connected with a central rod to a rotor. Formation of nitrogen gas bubbles could be observed through the transparent wall of the reactor. The stirring promoted nitrogen gas removal from the sludge bed and ensured mixing of reactor influent with the sludge blanket.

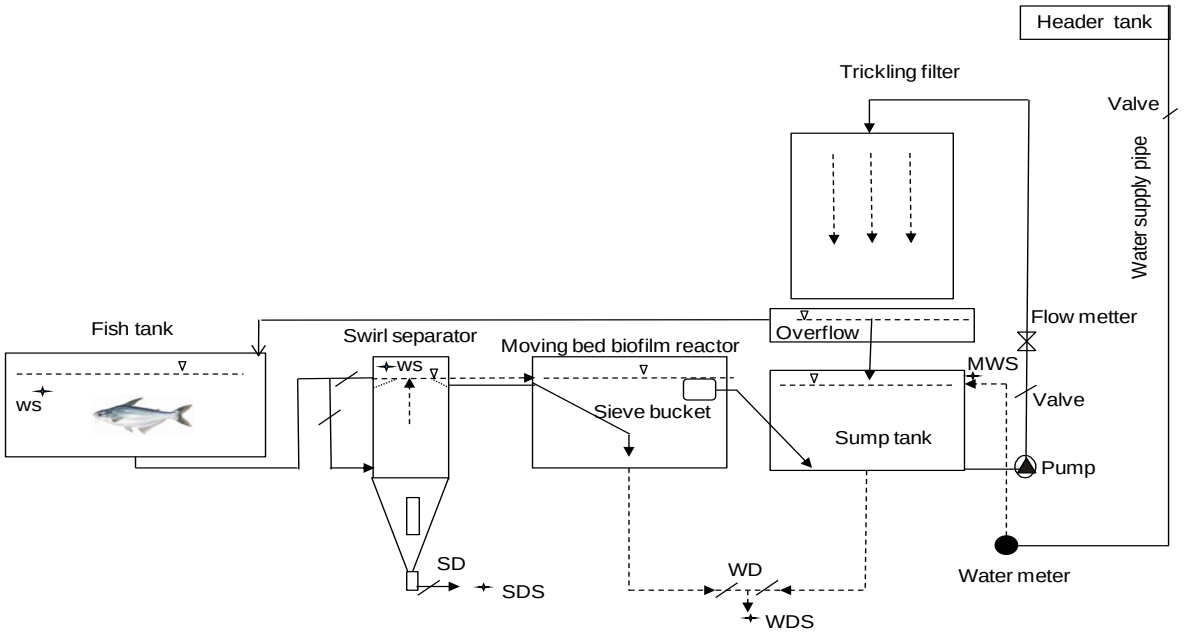


Figure 5. 1: Experimental set up of a conventional Recirculating Aquaculture System (RAS-treatment). WS: water sampling; SD: sludge discharge; SDS: sludge discharge sampling; WD: water discharge; WDS: Water discharge sampling; (+) sampling point.

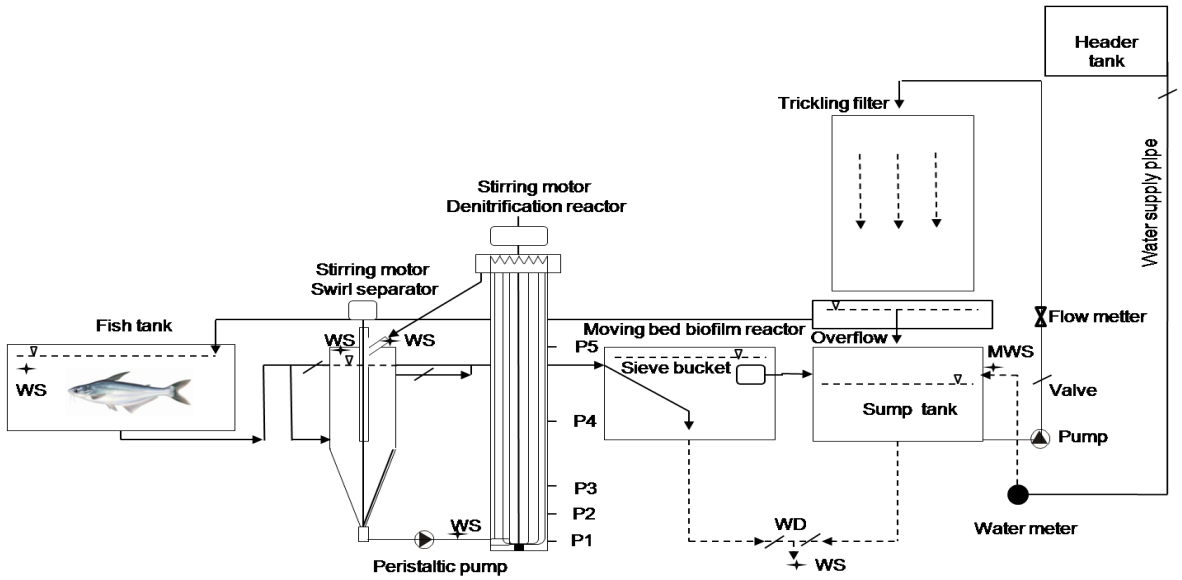


Figure 5. 2: Experimental set up of a conventional recirculating aquaculture system with an upflow sludge blanket denitrification reactor (RAS + USB). P1, 2, 3, 4, 5: are valves to discharge or sample USB-DR sludge. WS: water sampling; WD: water discharge; MWS: Water makeup sampling; (+) sampling point.

Table 5. 1: RAS and RAS + USB volume, flow rate and retention time through RAS components.

System component	Volume (L)	Flow rate (l/h)	Retention time (h)
Fish tank	850	2400	0.35
Swirl separator	260	2434	0.11
Moving Bed Biofilm Reactor	303	2400	0.13
Sump 1	580		
Sump 2	200		
USB - Denitrification reactor	243	33.6	7.23
Total system volume RAS	2193		
Total system volume RAS+USB	2436		

System maintenance. The biofilters in each RAS were fully matured at the start of the experiment, as prior to the experiment, striped catfish had been cultured non-stop for 210 days in the systems. Before starting the experiment the solid waste that had accumulated at the bottom of the biofilters was removed. The water meter of each system was checked daily at 8.00 a.m. to quantify the amount of water added during the experiment. Through water exchange the NO_3-N concentration was kept below 100 mg l^{-1}. Daily, sludge accumulated at the bottom of moving bed biofilm reactor, sump (1) and sump (2) was siphoned out and poured back to the swirl separator by hand to recapture at 8 a.m. to reduce passive denitrification. At the same time, the swirl separators were cleaned by hand to make sure that all remaining sludge was removed (RAS) or entered the denitrification reactor (RAS+USB).

5.2.3. Fish and feeding

Fish. Each RAS was stocked with genetically improved striped catfish fingerlings obtained from Research Institute for Aquaculture No2 (RIA 2, Ho Chi Minh City, Vietnam) that had been grown in RAS. Before the start of the experiment, the fish were acclimated to the conditions in the culture systems for two weeks. At the start of the experiment, the fish biomass was adjusted to ~125 kg per system. Detailed information on duration of the experiment and fishes stocked are presented in Table 5.1.

Table 5. 2: Experimental conditions for the two treatments: conventional recirculating aquaculture systems (RAS) and the conventional RAS extended with an upflow sludge blanket denitrification reactor (RAS+USB).

Treatment	RAS	RAS + USB
Replicates	2	
Experimental period (day)	56	
Number of fish stocked per system (#)	202 ± 4.2	201 ± 1.4
Initial individual fish weight (g)	621.8 ± 13.0	623.5 ± 5.0

Feed and feeding. Fish were fed an 8-mm extruded floating pellet (Vietthang company, Vietnam) by hand at 1:00, 5:00, 9:00, 13:00, 17:00 and 21:00 hours, carefully looking for signs of satiation. The system in which fish had the smallest appetite at that particularly feeding time, determined the amount of feed given to the other systems. By doing this consequently fish in each system received the same amount of feed. The proximate composition of the feed is given in Table 5.2.

Table 5. 3: Feed composition as analyzed (in g kg^{-1} feed on wet weight (ww)).

Parameter	Unit	g kg^{-1} feed
Dry matter	g kg^{-1} feed	894.0
Crude protein	g kg^{-1} feed	263.0
Crude fat	g kg^{-1} feed	52.2
Nitrogen-free extract (NFE)[(1)]	g kg^{-1} feed	448.0
Fiber	g kg^{-1} feed	57.3
Ash	g kg^{-1} feed	73.5
Acid insoluble ash (AIA)	g kg^{-1} feed	18.0
Total-P	g kg^{-1} feed	13.2
Chemical oxygen demand (COD)	g kg^{-1} feed	1202.0

[(1)] NFE (g/kg feed, ww) = 1000 - protein – fat – fiber – ash – water. [(2)] COD content of the diet was calculated as described in Dalsgaard and Pedersen (2011). COD_{feed} = crude protein (g kg^{-1}) * 1.77 + Crude fat (g kg^{-1}) * 2.88 + NFE (g kg^{-1}) * 1.16 + Fiber (g kg^{-1}) *1.16.

5.2.4. Measurements and analysis

Sampling

Feed and fish were sampled at the start (15 fish from base population) and end (5 fish per experimental unit) of the experiment for proximate analysis. Fish were not fed for 24 hours prior to sampling, and sampled fish was euthanized by 50 mg l^{-1}tricaine methanesulfonate (98%) (Sigma-Aldrich, Missouri, US). Per system, at stocking and harvest, fish were counted and batch weighed. At harvest, 15 fish per experimental system were sampled for determination of fillet yield, fillet coloration and off-flavour. Per RAS unit, every four hours the solid waste accumulated at the bottom of the swirl separator was collected and quantified (reported on a daily basis). The composition of the solid waste collected was determined weekly on a pooled and homogeneously mixed sample of the solid waste collected during that week.

Water samples were taken weekly from the overhead storage tank and collection locations in each recirculation system (sampling points are shown in Figure 5.1 and 5.2) and stored at 4^{o}C to laboratory to analyse total alkalinity, total ammonia nitrogen (TAN), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), total phosphorus (TP), chemical oxygen demand (COD), total nitrogen (TN) and total suspended solids (TSS). Temperature, oxygen dissolved (DO) and pH of water were measured in overhead storage tank and fish tanks directly. Water discharged from each recirculation system was separately stored at 4^{o}C and analysed for TN, TP, COD and TSS.

Weekly, a 24-hour sampling was conducted in each system, taking samples at 9:00, 13:00, 17:00, 21:00; 1:00 and 5:00 9:00 hours. The first sample of each 24-hour measurement was taken at 9.00. The 24-hour water samples were taken from effluent, the fish tanks and the inlet and outlet of each USB-DR. Water samples were analyzed for COD, TA, TKN, TAN, NO_2-N, NO_3-N, TSS, PO_4-P and TP according to standard methods (APHA, 1999). During each 24-hour measurement no system water was discharged and no make-up water added.

Analysis

Water analysis. Dissolved oxygen (mg l^{-1}), pH and water temperature (^{o}C) were measured daily at 9.00 a.m. in the reservoir, effluent of the fish tanks and in the inlet and outlet of each USB-DR (multi-parameter meter HI9828, Hanna Instruments, Rhode Islands, USA).

Water samples from fish tanks, USB inlet, USB outlet and discharge water were analyzed (APHA, 1999) for COD (dichromate reflux), total alkalinity (TA; titration with sulfuric acid and methyl orange indicator), Kjeldahl N (TKN; Kjeldahl method), total ammonia nitrogen (TAN; colorimetric method), nitrite nitrogen (NO_2-N; colorimetric method with diazotized sulfanilamide), nitrate nitrogen (NO_3-N;cadmium reduction to nitrite and measurement of nitrite), TSS (dried to constant weight at 103 - 105 ^{o}C), orthophosphate (PO_4-P;ammonium molybdate and potassium antimonyl tartrate method) and TP (photometric method).

Fish, feed and sludge analysis. Whole fish was analyzed for DM, CP, CL, TP and ash. Whole fish samples for composition analysis ensured empty feed in stomach and intestine, individual fish was minced and homogenized for composition analysis. Dead fish during culture were collected, weighted and counted and analyzed body composition. The fifteen fish collected per system at the end of the culture period were filleted by hand by an employee of the processing Vinh Hoan company[2], and fillet yield and color grade were determined according to Sang et al. (2012). The grade of fillet coloration was defined as white (score 1), pink (score 2) and yellow (score 3). Off-flavor of fillet was determined by an employee of a processing company, and was defined as good-flavor (score 1) and off-flavor (score 2). Feed was minced and homogenized for proximate composition (DM, TP, CP, CL, carbohydrate, acid insoluble ash (AIA) and ash). Sludge was homogenized to analyze DM, TP, chemical oxidation demand (COD), Kjeldahl N and ash. The DM was calculated by gravimetric analysis after drying at 105 ^{o}C for 24 hours (Foy and Rosell, 1991). The ash in whole fish, feed and sludge were analyzed according to APHA (1999).The TKN was analyzed by the Kjeldahl method (Foy and Rosell, 1991). The CP in feed was calculated by 6.25 x TKN. The CL in whole fish and feed were analyzed by acid-hydrolysis Soxhlet method (AOAC, 2000). The carbohydrate in feed was determined as the difference in DM content minus CP, ash and fat. The AIA in feed was analyzed according to AOAC (2000).

[2]VinhHoan Corporation, National Road 30, Ward 11, Cao Lanh City, Dong Thap Province, Vietnam.

The TP in whole fish, feed and sludge was analyzed spectrophotometrically following Kitson and Mellon (1944). COD in sludge was analyzed according to APHA (1999).

5.2.5. Calculations and statistics

Calculations

Details of calculations of resource use, nutrient utilization efficiency, waste discharged, fish performance indicators, fish quality at harvest, COD in feed and fish, nutrient mass balance at fish and system level, and USB-DR performance indicators are given in Table 5.4.

Table 5. 4: Units and formulas used to calculate resource use, nutrient utilization efficiency, waste discharged, fish performance indicators, fish quality at harvest, COD in feed and fish, nutrient mass balance at fish and system level, and USB-DR performance indicators.

Parameter	Unit	Formulas
Resource use		
Fingerlings use (FU)	# kg^{-1}fish	$FU = N_{tot\ initial} / (W_{tot\ final} - W_{tot\ initial})$
Water use (WU)	l kg^{-1}fish	$WU = V_{tot\ inflow} / (W_{tot\ final} - W_{tot\ initial})$
	l kg^{-1}feed	$WU = V_{tot\ inflow} / FC$
$NaHCO_3$ use (CU)	g kg^{-1}fish	$CU = M_{tot\ NaHCO3} / (W_{tot\ final} - W_{tot\ initial})$
	l kg^{-1}feed	$CU = M_{tot\ NaHCO3} / FC$
Energy use (EU)	kWh kg^{-1}fish	$EU = E_{tot\ electricity} / (W_{tot\ final} - W_{tot\ initial})$
	kWh kg^{-1}feed	$EU = E_{tot\ electricity} / FC$
Nutrient utilisation efficiency		
Nutrient in fish biomass (MR) (B =initial, final or dead fish, R=DM, N, P or COD)	g	$MR = 1000 * C_{R\ in\ fish} / 100 * W_{tot\ B}$
Utilisation efficiency (XR) (R = DM, N, P or COD)	%	$XR = [(MR_{final} - MR_{initial}) /1000] / [C_{R\ in\ feed} /100* FC] * 100$

Table 5.4 (continued- 1): Units and formulas used to calculate.

Parameter	Unit	Formulas
Waste discharged		
Waste removed (WR)	g kg^{-1}fish	$WR = [(C_{R\ effluent} * V_{tot\ effluent}) + (C_{R\ in\ dm\ sludge} / 100 * W_{tot\ dm\ sludge}) - (C_{R\ influent} * V_{tot\ influent})] / (W_{tot\ final} - W_{tot\ initial})$
Fish performance indicators		
Total initial fish biomass ($W_{tot\ initial}$)	kg	$W_{tot\ initial} = N_{tot\ initial} * W_{I\ initial}$
Total final fish biomass ($W_{tot\ final}$)	kg	$W_{tot\ final} = N_{tot\ final} * W_{I\ final}$
Initial density (ID)	kg m^{-3}	$ID = W_{tot\ initial} / V_{tot\ fish\ tank}$
Final density (FD)	kg m^{-3}	$FD = W_{tot\ final} / V_{tot\ fish\ tank}$
Total dead fish biomass ($W_{tot\ mortality}$)	kg	$W_{tot\ mortality}$ = cumulative weight of dead fish in kg
Survival (SR)	%	$SR = 100 * N_{tot\ final} / N_{tot\ initial}$
Geometric mean body weight (Wg)	g	$W_g = e^{[(\ln(WI\ final * 1000) + \ln(WI\ initial * 1000))/2]}$
Specific growth rate (SGR)	% bwd^{-1}	SGR = 100 * (lnWI final - lnWI initial) / D
Feed conversion ratio (FCR)	-	FCR = FC / (Wtot final - Wtot initial)
Metabolic feeding rate (MFR)	g $kg^{-0.8}d^{-1}$	[1000 * FC / {(Ntot.initial + Ntot.final) / 2}] * {(Wg / 1000)-0.8} / D
Metabolic growth rate (MGR)	g $kg^{-0.8}d^{-1}$	(WIfinal - WIinitial) * {(Wg / 1000)-0.8} / D
Fish quality at harvest		
Fillet percentage (FY)	%	FY = 100 * (FW / WI final * 1000)
Fillet colouration (FIC)	#	FIC = {(1*nwhite) + (2*npink) + (3*nyellow)} / n
Off-flavor (OFL)	#	OFL = {(1*n good-flavor) + (2*noff-flavor)} / n
COD in feed and fish		
COD crude proteina (CODCP)	g O_2 g^{-1}CP	CODCP = 1.77* CP
COD crude fata (CODCF)	g O_2 g^{-1}CF	CODCF = 2.88* CF
COD Nitrogen free extract (NFE) (CODNFE)a	g O_2 g^{-1} NFE	CODNFE = 1.16*NFE
Nutrients mass balance		
At fish level		
Nutrient in feed (NRfeed) (R = N, P, DM or COD)	g kg^{-1}feed	NRfeed = [(CR feed / 100) * FC * 1000] / FC
Nutrient retained in fish (NRfish retained)	g kg^{-1}feed	NRfish retained = (MRfinal – MRinitial) /FC
Nutrient in dead fish (NRfish mortality)	g kg^{-1}feed	NRfish mortality = (CR mortality /100) * Wtot mortality * 1000 / FC

Table 5.4 (continued- 2): Units and formulas used to calculate.

Parameter	Unit	Formulas
At system level		
Input (NRinput)	g kg^{-1}feed	NRinput = NRno retained + NRinfluent
Nutrient no retained in fish (NRno retained)	g kg^{-1}feed	NRno retained = NRfeed – (NRfish retained + NR mortality)
Nutrient in influent (NR influent)	g kg^{-1}feed	NRinfluent = CR inflow * Vtot inflow / 1000 / FC
Output (NRoutput)	g kg^{-1}feed	NRoutput = NReffluent + NRsludge + NRno measured
Nutrient in effluent (NReffluent)	g kg^{-1}feed	NReffluent = CR outflow * Vtot outflow / 1000 / FC
Nutrient in sludge (NRsludge)	g kg^{-1}feed	NRsludge = (CR dm sludge / 100) * Mtot dm sludge / FC
Final sludge in USB (NRfinal USB)	g kg^{-1}feed	NRfinal USB = [CR wet sludge * Vtot wet sludge /1000] / FC
Removal in USB ($NR_{removal\ USB}$)	g kg^{-1} feed	$NR_{removal\ USB} = FR_{USB} * [C_{R\ inlet\ USB} - C_{Routlet\ USB}] / FC$
Nutrient no measured ($NR_{no\ measured}$)	g kg^{-1} feed	$NR_{no\ measured} = NR_{input} - NR_{effluent} - NR_{sludge}$
USB-DB performance		
Hourly nutrient removal ($NR_{USBhr\ removal}$)	g kg feed hr^{-1}	$NR_{hr\ removal} = FR_{USB\ T} * [C_{R\ inlet\ USB\ T} - C_{Rout\ USB\ T}] / FC_T$
NO_3-N removal ($NR_{USB\ NO3\text{-}N\ removal}$)	g kg^{-1} feed	$NR_{USB\ NO3\text{-}N\ removal} = FR_{USB} * [(C_{NO3\text{-}N\ inlet\ USB} - C_{NO3\text{-}N\ outlet\ USB}) + 5/3\ (C_{NO2\text{-}N\ inlet\ USB} - C_{NO2\text{-}N\ outlet\ USB})] / FC$
Solid removal (SR)	g kg^{-1} feed	$SR = [M_{tot\ dm\ sludge}$in RAS without USB $- NR_{final\ USB}] / FC$

[a]Based on Dalsgaard and Pedersen (2011). $\mathbf{C_{NO2\text{-}N\ inlet\ USB}}$**:** NO_2-N concentration in inlet USB (g m^{-3}), $\mathbf{C_{NO2\text{-}N\ outlet\ USB}}$: NO_2-N concentration in outlet USB (g m^{-3}), $\mathbf{C_{NO3\text{-}N\ inlet\ USB}}$**:** NO_3-N concentration in inlet USB (g m^{-3}), $\mathbf{C_{NO3\text{-}N\ outlet\ USB}}$: NO_3-N concentration in outlet USB (g m^{-3}), $\mathbf{C_{R\ effluent}}$: nutrient (DM, N, P or COD) concentration in effluent water (g m^{-3}), $\mathbf{C_{R\ in\ dm\ sludge}}$ **:** nutrient (DM, N, P or COD) concentration in dry matter sludge (%),$\mathbf{C_{R\ in\ feed}}$: nutrient (DM, N, P or COD) concentration in feed (%), $\mathbf{C_{R\ in\ fish}}$: concentration of nutrient (DM, N, P or COD) in initial or final fish (%), $\mathbf{C_{R\ influent}}$: nutrient (DM, N, P or COD) concentration in influent water (g m^{-3}), $\mathbf{C_{R\ inlet\ USB}}$: nutrient (N, P or COD) concentration inlet of USB (g m^{-3}), $\mathbf{C_{R\ inlet\ USB\ T}}$: nutrient (N, P or COD) concentration inlet of USB at time of determination (g m^{-3}), $\mathbf{C_{Routlet\ USB\ T}}$: nutrient (N, P or COD) concentration outlet of USB at time of determination (g m^{-3}), $\mathbf{C_{R\ mortality}}$: nutrient concentration in fish mortality (%),$\mathbf{C_{Routlet\ USB}}$: nutrient (N, P or COD) concentration outlet of USB (g m^{-3}), $\mathbf{C_{R\ wet\ sludge}}$: nutrient concentration in wet sludge (g m^{-3}), **COD:** chemical oxygen demand, **D**: days of culture period (d), $\mathbf{E_{tot\ electricity}}$: total electricity consumption including light, air-blower, water pumping and other activities during full production cycle per experimental unit (kWh), **FC:** cumulative feed input (kg), $\mathbf{FC_T}$: cumulative feed at time of determination (kg), $\mathbf{FR_{USB}}$: flow rate USB (l d^{-1}), $\mathbf{FR_{USB\ T}}$: flowrate of USB at time of determination (l d^{-1}), **FW**: average weight of complete skinless fillet from one individual after removing fat and red muscle following standard process for export markets (g), $\mathbf{M_{tot\ NaHCO3}}$: total amount of $NaHCO_3$ applied (g), $\mathbf{M_{tot\ sludge}}$: total weight of dry sludge (g), $\mathbf{MR_{final}}$: total amount of nutrient in final fish biomass (g), $MR_{initial}$: total amount of nutrient in initial fish biomass (g), **n**: number of fish in sample (#), $\mathbf{n_{good\text{-}flavor}}$: number of fish in sample with no off-flavor (#), $\mathbf{n_{off\text{-}flavor}}$: number of fish with off-flavor (#),$\mathbf{n_{pink}}$: number of fish with pink fillet (#), $\mathbf{N_{tot\ final}}$: total number of fish harvested (#), $\mathbf{N_{tot\ initial}}$: total number of fish stocked (#), $\mathbf{n_{white}}$: number of fish with white fillet (#), $\mathbf{n_{yellow}}$: number of fish with yellow fillet (#),$\mathbf{V_{tot\ effluent}}$: total effluent water volume (l), $\mathbf{V_{tot\ inflow}}$: total water volume

during culture period (l), $\mathbf{V_{tot\ influent}}$: total influent water volume (l), $\mathbf{V_{tot\ wet\ sludge}}$: total sludge volume of wet sludge (l), $\mathbf{W_{I\ final}}$: individual final body weight (kg), $\mathbf{W_{I\ initial}}$: individual initial body weight (kg),$\mathbf{W_{tot\ B}}$: total initial or final fish biomass (kg), $\mathbf{W_{tot\ dm\ sludge}}$: total amount of dry matter sludge (g), $\mathbf{W_{tot\ mortality}}$: total mortality fish biomass (kg).

Statistics

Water quality parameters were averaged over the culture period. The daily sludge production and waste discharge were summed over the culture period, as were water use, chemicals applied and energy use. Fish growth parameters and mass balances were calculated by experimental unit. Results for RAS and RAS+USB were compared by one-way ANOVA. Significant difference is given based on Tukey test. Differences in water quality parameters, fish performance parameters, nutrient inputs or outputs, and resource utilization parameters (consumption or use of fingerling, water, electricity and sodium bicarbonate, nutrient retained in fish (DM, N, P, COD) and nutrient discharge (DM, N, P, COD)) between RAS and RAS+USB were analyzed by one-way ANOVA. Daily and weekly measurements were averaged over the culture period before ANOVA. The daily and weekly measurements were used to calculate the nutrient mass balances (Table 5.4).

5.3. Results

5.3.1. Water quality

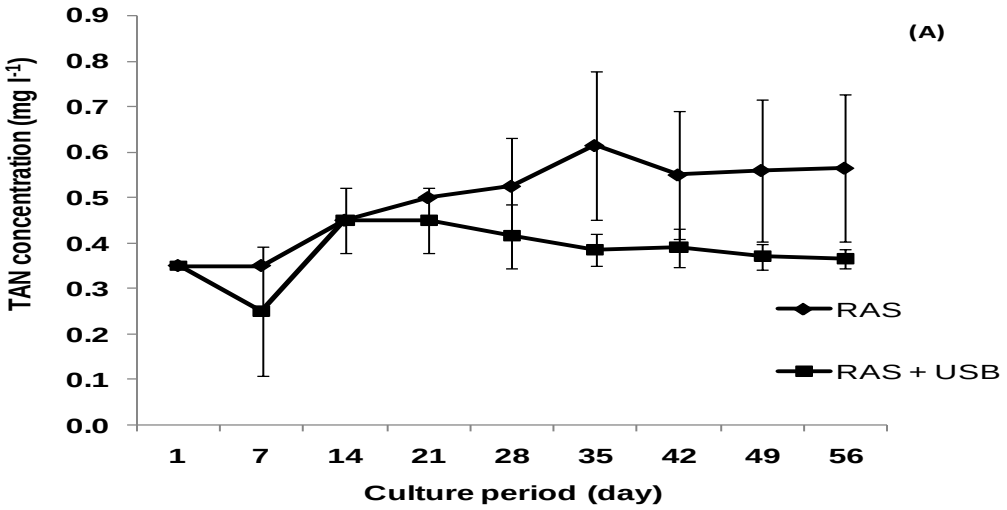
Water temperature, pH, TAN (Figure 5.3A) NO_2-N (Figure 5.3B) and hydrogen sulphide concentrations in the fish tanks of RAS and RAS+USB were similar ($P > 0.05$), while dissolved oxygen, TN, NO_3-N (Figure 5.3C), TA (Figure 5.3D) and COD were lower, and TSS (Figure 5.3E) and TP (Figure 5.3F) were higher in RAS+USB than in RAS ($P < 0.05$) (Table 5.5). The highest concentrations of NO_3-N were observed during experimental week 2 in RAS and week 4 in RAS+USB (Figure 5.3C). TA was higher in RAS+USB than in RAS already from the first days of the experiment ($P < 0.05$) (Figure 5.3D).

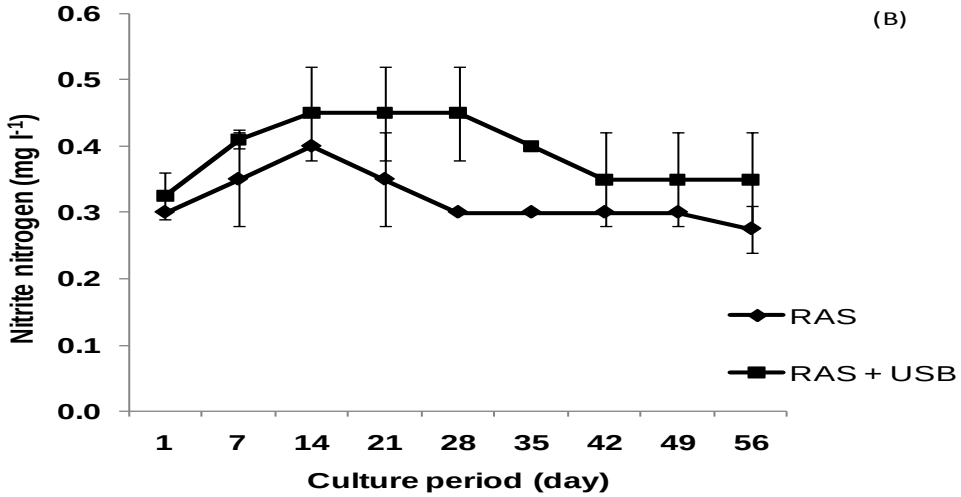
Table 5. 5: Weekly water quality in RAS and RAS + USB. Values are mean ± S.D.; n=2.

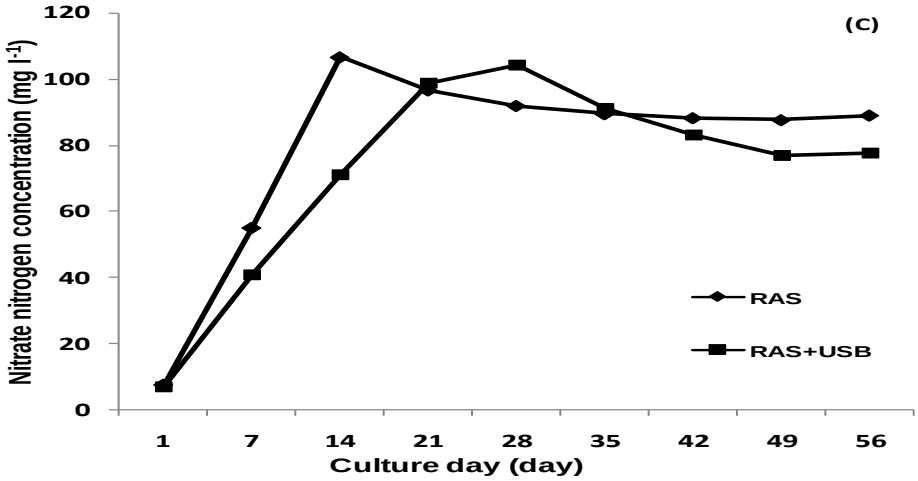
		RAS			RAS + USB			
Parameter	Unit	Mean	±	SD	Mean	±	SD	p-value
Temperature	^{o}C	28[a]	±	0.1	28.1[a]	±	0.1	0.558
pH of water								
Influent	mg l^{-1}	8.3	±	0.1	8.3	±	0.1	
Fish tank	mg l^{-1}	7.2[a]	±	0.1	7.3[a]	±	0.1	0.259
Inlet USB	mg l^{-1}				7.6	±	0.1	
Outlet USB	mg l^{-1}				7.9	±	0.1	
Dissolved oxygen								
Influent	mg l^{-1}	3.8	±	0.2	3.8	±	0.2	
Fish tank	mg l^{-1}	4.7[a]	±	0.1	4.4[b]	±	0.1	0.047
Inlet USB	mg l^{-1}				1.5	±	0.1	
Outlet USB	mg l^{-1}				0.6	±	0.1	
Total ammonia nitrogen								
Influent	mg l^{-1}	0.04	±	0.06	0.04	±	0.06	
Fish tank	mg l^{-1}	0.33[b]	±	0.1	0.43[a]	±	0.1	0.221
Inlet USB	mg l^{-1}				1.74	±	0.1	
Outlet USB	mg l^{-1}				0.21	±	0.1	
Nitrite nitrogen								
Influent	mg l^{-1}	0.04	±	0.03	0.04	±	0.03	
Fish tank	mg l^{-1}	0.43[b]	±	0.2	0.47[a]	±	0.1	0.780
Inlet USB	mg l^{-1}				0.52	±	0.1	
Outlet USB	mg l^{-1}				2.03	±	0.1	
Nitrate nitrogen								
Influent	mg l^{-1}	1.2	±	1.24	1.2	±	1.24	
Fish tank	mg l^{-1}	82.9[a]	±	2	62.8[b]	±	4	0.001
Inlet USB	mg l^{-1}				60.1	±	1	
Outlet USB	mg l^{-1}				39.3	±	1.3	
Total nitrogen								
Influent	mg l^{-1}	3.5	±	0.7	3.5	±	0.7	
Fish tank	mg l^{-1}	84.9[a]	±	0.3	65.2[b]	±	0.3	0.001
Inlet USB	mg l^{-1}				90.1	±	2.2	
Outlet USB	mg l^{-1}				68.3	±	2.1	

Table 5.5 (continued- 1): Weekly water quality in RAS and RAS + USB. Values are mean ± S.D.; n=2.

		RAS			RAS + USB			
Parameter	Unit	Mean	±	SD	Mean	±	SD	p-value
Total alkalinity								
Influent	mg l^{-1}	258.6	±	5.3	258.6	±	5.3	
In fish tank	mg l^{-1}	113.7[b]	±	3.0	147.9[a]	±	3.3	0.009
Inlet USB	mg l^{-1}				154.1	±	6.8	
Outlet USB	mg l^{-1}				245.7	±	6.1	
Chemical oxygen demand								
Influent	mg l^{-1}	5.32	±	0.9	5.32	±	0.9	
Fish tank	mg l^{-1}	41.1[a]	±	2.4	31.8[b]	±	1.9	0.049
Inlet USB	mg l^{-1}				309.8	±	2.5	
Outlet USB	mg l^{-1}				233.8	±	3.1	
Total suspended solids								
Influent	mg l^{-1}	3.2	±	0.5	3.2	±	0.5	
Fish tank	mg l^{-1}	49.9[b]	±	1.0	65.7[a]	±	2	0.010
Inlet USB	mg l^{-1}				162.7	±	1	
Outlet USB	mg l^{-1}				40.2	±	2.1	
Total phosphorus								
Influent	mg l^{-1}	1.74	±		1.25			
Fish tank	mg l^{-1}	18.2[b]	±	1.6	24.3[a]	±	0.6	0.038
Inlet USB	mg l^{-1}				33.2	±	2.9	
Outlet USB	mg l^{-1}				29.4	±	1.5	
Hydrogen sulphide								
Influent	mg l^{-1}	0			0			
Fish tank	mg l^{-1}	0.16[b]	±	0.01	0.24[a]	±	0.1	0.301
Inlet USB	mg l^{-1}				0.32	±	0.1	
Outlet USB	mg l^{-1}				0.51	±	0.1	


(A)
TAN concentration (mg l-1)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
7
14
21
28
35
42
49
56
Culture period (day)
RAS
RAS + USB


(B)
Nitrite nitrogen (mg l-1)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
7
14
21
28
35
42
49
56
Culture period (day)
RAS
RAS + USB


(C)
Nitrate nitrogen concentration (mg l-1)
120
100
80
60
40
20
0
1
7
14
21
28
35
42
49
56
Culture day (day)
RAS
RAS+USB

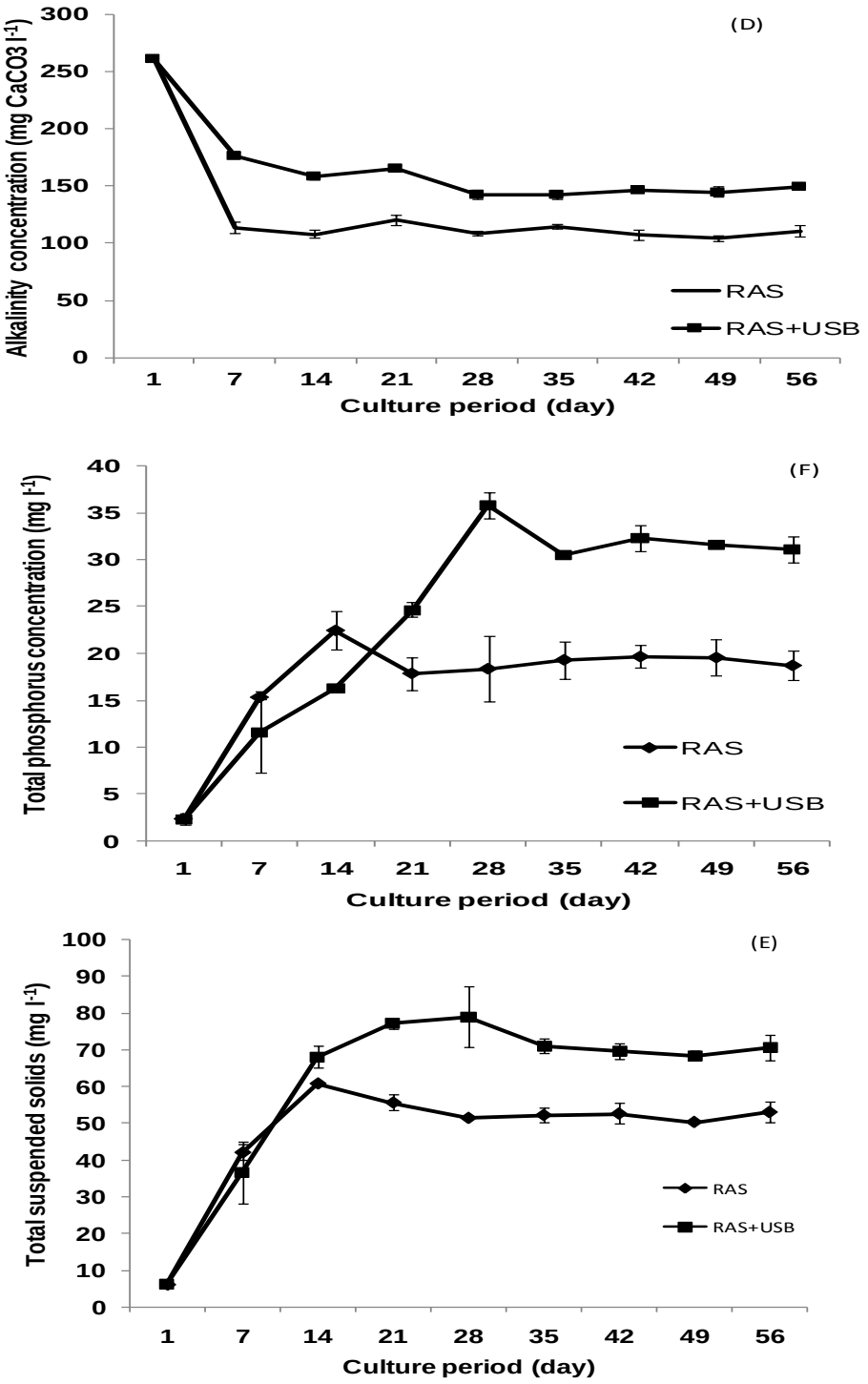


Figure 5. 3: Weekly water quality in RAS without denitrification reactor (RAS) and RAS with denitrification reactor (RAS+USB): Total ammonia nitrogen (A), nitrite nitrogen (B), nitrate nitrogen (C), total alkalinity (D), total phosphorous (F) and total suspended solids concentration (E). Values are mean ± S.D.; n=2.

5.3.2. Fish growth performance

Survival, specific growth rate and final body weight of striped catfish in RAS and RAS+USB were similar ($P > 0.05$) (Table 5.6). Also, the percentage fillet yield, meat colour and off-flavour were similar between RAS and RAS+USB ($P > 0.05$).

Table 5. 6: Fish growth performance and fish quality in RAS and RAS+USB. Values are mean ± S.D.; n=2.

Parameter		RAS			RAS + USB			p-value
	Unit	Mean	±	S.D.	Mean	±	S.D.	
Fish growth performance								
Culture period	d	56.0	±	0.0	56.0	±	0.0	-
Initial BW	g	621.8[a]	±	13.0	623.5[a]	±	5.0	0.879
Final BW	g	921.0[a]	±	198	949.0[a]	±	173.0	0.059
Initial number fish	#	202.0[a]	±	4.2	201.0[a]	±	1.4	0.782
Final number fish	#	199.0[a]	±	3.5	196.0[a]	±	2.8	0.890
Initial biomass	kg	125.6[a]	±	1.0	125.3[a]	±	1.1	0.860
Final biomass	kg	183.2[a]	±	1.3	186.0[a]	±	3.0	0.120
Initial density	kgm^{-3}	147.7[a]	±	0.1	147.4[a]	±	0.1	0.086
Final density	kgm^{-3}	230.0[a]	±	4.2	230.6[a]	±	3.3	0.890
Total feed	kg	80.2	±	0.0	80.2	±	0.0	-
Survival	%	98.5[a]	±	0.3	97.2[a]	±	2.1	0.672
Geometric mean body weight	g	756.7[a]	±	12.0	769.2[a]	±	3.6	0.295
Specific growth rate	% bwd^{-1}	0.7[a]	±	0.0	0.8[a]	±	0.0	0.084
Feed conversion rate	kg kg^{-1}	1.39[a]	±	0.1	1.34[a]	±	0.1	0.096
Metabolic feeding rate	g $kg^{-0.8}d^{-1}$	9.0[a]	±	0.1	8.9[a]	±	0.1	0.233
Metabolic growth rate	g $kg^{-0.8}d^{-1}$	6.7[a]	±	0.2	7.2[a]	±	0.1	0.063
Fish quality								
Fillet percentage	%	37.1[a]	±	0.3	37.2[a]	±	0.1	0.682
Meat colouration	-	1.0	±	0.0	1.0	±	0.0	-
Off flavour	-	1.0	±	0.0	1.0	±	0.0	-

RAS: recirculating aquaculture system without USB; RAS+USB: recirculating aquaculture with a denitrification reactor (USB). Mean with different superscript within each row are significantly different ($P < 0.05$). S.D.: standard deviation.

5.3.3. Nutrient mass balances

5.3.3.1. Nitrogen mass balance

On average, 49% of the N in the feed was retained in fish biomass ($P > 0.05$). The amount of N discharged with effluent in RAS+USB was 25% of the N discharge in RAS ($P < 0.05$) (Table 5.7).

Table 5. 7: Nitrogen mass balance in RAS and RAS+USB. Values are mean ± S.D.; n=2.

	Unit	RAS				RAS + USB				p-value
Parameter		Mean	±	SD	%	Mean	±	SD	%	
At fish level										
-N in feed	g kg^{-1}feed	42.1	±	0.0	100.0	42.1	±	0.0	100.0	-
-N in fish retained	g kg^{-1}feed	20.6[a]	±	1.0	48.9	20.7[a]	±	0.7	49.2	0.142
-N in fish mortality	g kg^{-1}feed	0.2[a]	±	0.1	0.5	0.2[a]	±	0.3	0.5	0.979
-N not retained in fish	g kg^{-1}feed	21.3[a]	±	1.0	50.6	21.2[a]	±	1.0	50.3	0.179
At system level										
N input	g kg^{-1}feed	21.9			100.0	21.5			100.0	-
-N not retained in fish	g kg^{-1}feed	21.3[a]	±	1.0	97.5	21.2[a]	±	1.0	98.6	0.179
-N in influent	g kg^{-1}feed	0.6[a]	±	0.0	2.5	0.3[b]	±	0.01	1.4	0.001
N output										
-N in daily sludge removal*	g kg^{-1}feed	4.9	±	0.1	22.4	No discharge at al				
-N in effluent	g kg^{-1}feed	10.7[a]	±	0.8	48.9	2.6[b]	±	0.0	12.1	0.003
-N in final sludge in USB	g kg^{-1}feed				0.0	2.9	±	0.2	13.5	-
-N removal in USB	g kg^{-1}feed				0.0	12.3	±	0.1	56.3	-
-N not measured	g kg^{-1}feed	6.3[a]	±	1.0	28.8	3.7[a]	±	0.6	18.1	0.594

* Throughout the experiment sludge that settled in the swirl separator was discharged with an interval of four hours. RAS: recirculating aquaculture system without USB; RAS+USB: recirculating aquaculture with an USB-denitrification reactor. Mean with different superscript within each row are significantly different ($P < 0.05$).S.D.: standard deviation.

5.3.3.2. Phosphorus mass balance

On average, 21% of P fed was retained in fish biomass ($P > 0.05$). At the end of the experiment, 54% of the dietary P in the RAS +USB treatment not retained in fish biomass, accumulated in the USB-DR (assuming that all sludge P is originating from dietary P). In RAS, 51% of the dietary P not retained in fish biomass was removed with the sludge (Table 5.8).

Table 5. 8: Phosphorus mass balance in RAS and RAS+USB. Values are mean ± S.D.; n=2.

	Unit	RAS				RAS + USB				p-value
Parameter		Mean	±	SD	%	Mean	±	SD	%	
At fish level										
-P in feed	g kg^{-1}feed	13.2	±	0.0	100.0	13.2	±	0.0	100.0	
-P in fish retained	g kg^{-1}feed	2.6[a]	±	0.1	19.7	2.9[a]	±	0.2	22.0	0.183
-P in fish mortality	g kg^{-1}feed	0.3[a]	±	0.1	2.3	0.3[a]	±	0.1	2.3	0.982
-P not retained in fish	g kg^{-1}feed	10.3[a]	±	0.1	78.0	10.0[a]	±	0.2	75.8	0.233
At system level										
P input	g kg^{-1}feed	10.7			100.0	10.2			100.0	
-P not retained in fish	g kg^{-1}feed	10.3[a]	±	0.1	96.3	10.0[a]	±	0.2	98.0	0.233
-P in influent	g kg^{-1}feed	0.4[a]	±	0.0	3.7	0.2[b]	±	0.0	2.0	0.001
P output										
-P in daily sludge removal[*]	g kg^{-1}feed	5.3	±	1.0	49.6	No discharge at al				
-P in effluent	g kg^{-1}feed	2.5[a]	±	0.2	30.8	1.2[b]	±	0.1	11.8	0.001
-P in final sludge in USB	g kg^{-1}feed				0.0	5.4	±	0.3	52.9	
-P not measured	g kg^{-1}feed	2.9[a]	±	0.9	27.1	3.6[a]	±	0.2	35.3	0.209

[*] Throughout the experiment sludge that settled in the swirl separator was discharged with an interval of four hours. RAS: recirculating aquaculture system without USB; RAS+USB: recirculating aquaculture with an USB-denitrification reactor. Mean with different superscript within each row are significantly different ($P < 0.05$). S.D.: standard deviation.

5.3.3.3. Dry matter mass balance

On average, 36% of the DM fed was retained in fish biomass ($P > 0.05$). About 2.2 times more DM was discharged with the effluent in RAS than in RAS+USB ($P < 0.05$) (Table 5.9).

Table 5. 9: Dry matter mass balance in RAS and RAS + USB. Values are mean ± S.D.; n=2.

Parameter	Unit	RAS Mean	±	SD	%	RAS + USB Mean	±	SD	%	p-value
At fish level										
-DM in feed	g kgfeed^{-1}	894.0	±	0.0	100.0	894.0	±	0.0	100.0	-
-DM in fish retained	g kgfeed^{-1}	307.0^{a}	±	11.4	34.3	331.7^{a}	±	7.7	37.1	0.124
-DM in fish mortality	g kgfeed^{-1}	2.7^{a}	±	0.1	0.3	2.8^{a}	±	3.9	0.3	0.984
-DM not retained in fish	g kgfeed^{-1}	584.3^{a}	±	11.4	65.4	559.5^{a}	±	11.7	62.6	0.163
At system level										
DM input	g kgfeed^{-1}	584.9			100.0	559.8			100.0	
-DM not retained in fish	g kgfeed^{-1}	584.3^{a}	±	11.4	9.9	559.5^{a}	±	11.7	99.9	0.163
-DM in influent	g kgfeed^{-1}	0.6^{a}	±	0.0	0.1	0.3^{b}	±	0.1	0.1	0.001
DM output										
-DM in daily sludge removal*	g kgfeed^{-1}	201.0	±	7.2	34.4	No discharge at al				
-DM in effluent	g kgfeed^{-1}	7.1^{a}	±	0.5	1.2	3.2^{b}	±	0.1	0.6	0.006
-DM in final sludge in USB	g kgfeed^{-1}				0.0	56.8	±	5.4	10.1	-
-DM removal in USB	g kgfeed^{-1}				0.0	144.5	±	5.4	25.8	-
-DM not measured	g kgfeed^{-1}	376.8^{a}	±	11.7	64.4	355.3^{a}	±	11.6	63.5	0.202

* Throughout the experiment sludge that settled in the swirl separator was discharged with an interval of four hours. RAS: recirculating aquaculture system without USB; RAS+USB: recirculating aquaculture with an USB-denitrification reactor. Mean with different superscript within each row are significantly different ($P < 0.05$). S.D.: standard deviation.

5.3.3.4. COD mass balance

On average, 49% of the COD supplied with the feed was retained in harvested fish biomass ($P > 0.05$). In RAS, 27.3 and 3.7% of the COD not retained in fish biomass was discharged with sludge and effluent, respectively. The COD discharged with the RAS effluent was 3.6 times higher than the discharge from RAS+USB ($P > 0.05$). At the end of the experiment, the sludge remaining in the USB-DR was 14.1% of the COD not retained in fish biomass (Table 5.10). Only 1% of the COD waste input was discharged with the effluent in the RAS+USB.

Table 5. 10: COD mass balance in RAS and RAS+USB. Values are mean ±S.D.; n=2.

Parameter	Unit	RAS				RAS + USB				p-value
		Mean	±	SD	%	Mean	±	SD	%	
At fish level										
-COD in feed	g kg^{-1}feed	1202	±	0.0	100	1202	±	0.0	100	
-COD in fish retained	g kg^{-1}feed	574.0[a]	±	3.8	47.8	594.9[a]	±	11.7	49.5	0.140
-COD in fish mortality	g kg^{-1}feed	23.5[a]	±	25.2	2.0	26.1[a]	±	8.7	2.2	0.456
-COD not retained in fish	g kg^{-1}feed	604.5[a]	±	29.0	50.2	581[a]	±	2.9	48.3	0.888
At system level										
COD waste input	g kg^{-1}feed	605.5			100.0	581.4			100	
-COD not retained in fish	g kg^{-1}feed	604.5[a]	±	29	99.8	581[a]	±	4.3	99.9	0.888
-COD in influent	g kg^{-1}feed	1.0[a]	±	0.1	0.2	0.4[b]	±	0.0	0.1	0.001
COD output										
-COD in daily sludge removal*	g kg^{-1}feed	165.1	±	19	27.3	No discharge at al				
-COD in effluent	g kg^{-1}feed	22.4[a]	±	0.4	3.7	6.3[b]	±	0.02	1.1	0.003
-COD in final sludge in USB	g kg^{-1}feed					81.8	±	5.0	14.1	
-COD removal in USB	g kg^{-1}feed					34.4	±	1.1	5.9	
-COD not measured	g kg^{-1}feed	418[a]	±	29.5	69.0	458.9[b]	±	3.7	78.9	0.011

* Throughout the experiment sludge that settled in the swirl separator was discharged with an interval of four hours. RAS: recirculating aquaculture system without USB; RAS+USB: recirculating aquaculture with an USB-denitrification reactor. Mean with different superscript within each row are significantly different ($P < 0.05$). S.D.: standard deviation.

5.3.4. Sustainability indicators

Less water, energy and $NaHCO_3$ was used per kg fish produced or per kg feed consumed in RAS+USB than in RAS ($P < 0.05$). Fingerling use was similar between RAS and RAS+USB ($P > 0.05$). No antibiotics were applied during the experimental period (Table 5.11). The percentages of DM, N, P and COD retained in fish biomass (including dead fish) were similar between RAS and RAS+USB ($P > 0.05$). However, significantly smaller quantities of DM,

N, P and COD were discharged per kg fish produced and per kg feed consumed in RAS+USB than in RAS ($P < 0.05$) (Table 5.11).

Table 5. 11: The key sustainability indicators of striped catfish culture in RAS and RAS + USB. Values are mean; n=2.

Parameter	Unit	RAS			RAS + USB			p-value
		Mean	±	SD	Mean	±	SD	
Resource utilisation efficiency								
Fingerling use	# kg^{-1}fish	3.7^{a}	±	0.1	3.3^{a}	±	0.2	0.090
	# kg^{-1}feed	2.5^{a}	±	0.1	2.5^{a}	±	0.2	0.782
Water use	l kg^{-1}fish	175.4^{a}	±	1.8	77.1^{b}	±	3.9	0.003
	l kg^{-1}feed	126.2^{a}	±	1.5	37.7^{b}	±	1.9	0.001
$NaHCO_3$ use	g kg^{-1}fish	175.6^{a}	±	6.5	86.3^{b}	±	5.1	0.003
	g kg^{-1}feed	119.2^{a}	±	1.6	64.6^{b}	±	1.3	0.001
Antibiotic use		No			No			
Nutrient utilisation efficiency								
DM retained in fish	%	34.6^{a}	±	1.3	37.4^{a}	±	0.9	0.124
N retained in fish	%	49.4^{a}	±	2.4	49.7^{a}	±	1.5	0.142
P retained in fish	%	20.0^{a}	±	0.7	24.3^{a}	±	1.4	0.183
COD retained in fish	%	49.8^{a}	±	0.3	50.0^{a}	±	2.3	0.230
Waste discharge								
DM discharge	g kg^{-1}fish	288.4^{a}	±	59.1	80.0^{b}	±	0.7	0.001
	g kg^{-1}feed	207.5^{a}	±	87.0	59.7^{b}	±	0.2	0.001
N discharge	g kg^{-1}fish	20.9^{a}	±	0.6	7.0^{b}	±	0.7	0.001
	g kg^{-1}feed	15.0^{a}	±	0.5	5.2^{b}	±	0.3	0.002
P discharge	g kg^{-1}fish	10.3^{a}	±	0.4	8.6^{b}	±	0.1	0.023
	g kg^{-1}feed	7.4^{a}	±	0.3	6.4^{a}	±	0.3	0.072
COD discharge	g kg^{-1}fish	259.2^{a}	±	24.1	101.2^{b}	±	6.3	0.012
	g kg^{-1}feed	186.5^{a}	±	20.8	75.5^{b}	±	0.7	0.021

RAS: recirculating aquaculture system without USB; RAS+USB: recirculating aquaculture with a denitrification reactor (USB). Mean with different superscript within each row are significantly different ($P < 0.05$). S.D.: standard deviation.

5.4. Discussion

5.4.1. Water quality

The water temperature of 28^{o}C was in the optimal range for denitrification (Timmons and Ebeling, 2010) (Park, 2000) and is also favourable for feeding and appetite of striped catfish (Phuc et al., 2015). From the start of the experiment no water exchange was applied until the maximum allowable nitrate concentration of 100 mg nitrate-N l^{-1} was reached. This concentration was reached two weeks earlier in RAS (week 2) when compared with RAS+USB. Nitrate-N concentration in the fish tanks of RAS and RAS+USB was maintained between 60 and 100 mg l^{-1} (Figure 5.3c) from the second week of the experiment onwards and is significantly lower than the 140 mg/l advised for African catfish (Bovendeur et al., 1987; Schram et al., 2014). However, from this experiment it cannot be concluded that the nitrate-N concentration did not affect growth performance in both treatments. During this experiment, the total alkalinity was maintained above 100 mg l^{-1} by sodium bicarbonate addition. From the second week of the experiment onwards, the total alkalinity in RAS+USB was overall 30 mg l^{-1} higher than in RAS (Figure 5.3D). In RAS+USB on average, 0.65 equivalents of alkalinity (54.6 g $NaHCO_3$) was added per kg feed, which was 0.78 alkalinity equivalents less (64.6 g$NaHCO_3$) when compared with RAS. The lower alkalinity supply in RAS+USB can be explained by the 12.3 g nitrate-N removal per kg feed due to denitrification (Table 5.7) when assuming denitrifiers assimilate ammonium and 0.91 alkalinity equivalents is produced per mole Nitrate-N removed (Henze, 1991).

5.4.2. Fish performance and quality

As expected, to maintain the Nitrate-N concentration below 100 mgl^{-1} water renewal in RAS+USB was much lower (77 versus 175 l kg^{-1} feed) than in RAS (Table 5.11, $P > 0.05$) with no apparent effect on growth, survival, FCR and product quality (Table 5.6, $P > 0.06$). Similar effects in RAS with or without a denitrification reactor were reported for Nile tilapia (Eding et al., 2009). Nitrifying biofilters in RAS are potential production sites of geosmin and 2-methylisoborneol (MIB), substances that cause off-flavour (Guttman and van Rijn, 2008). Occurrence of “earthy’’ or ‘’musty’’ off-flavour due to geosmin and MIB, respectively, is

commonly reported in RAS with nitrifying biofilters (Bai et al., 2013; Burr et al., 2012; Guttman and van Rijn, 2008, 2009; Schrader et al., 2010; Tucker, 2000; Tucker and van der Ploeg, 1999). It was therefore unexpected that although the biofilters in all systems had been operating for more than 210 days before the start of the experiment, no off flavour was detected in harvested fish. Nevertheless, in a pilot outdoor commercial RAS relying on a combination of septic tank and nitrification for water purification and targeting a production of 100 kg m^{-2} (data not presented), off-flavour developed. If this happens, integration of denitrification in RAS might reduce geosmin and MIB concentrations (Guttman and van Rijn, 2009). Because no off-flavour developed during our experiment, the effect of denitrification on off-flavour could not be verified, and therefore, follow-up experiments are recommended.

H_2S will be produced under low redox conditions and nitrate availability (10 – 50 mg l^{-1}) in an USB-DR (Timmons and Ebeling, 2010). Linh (2011) reported that a H_2S concentration above 0.96 mg l^{-1} concurs with a higher percentage of striped catfish getting slightly yellow fillets. This was not the case in our study with 100% white fillet (Table 5.6), where in all systems the H_2S concentration in fish tank remained below 0.24 mg l^{-1} (Table 5.5).

5.4.3. Nutrient mass balance and USB-DR efficiency

The retention into fish biomass of N, P, DM and COD supplied with feed was 49, 22 – 24, 34 – 37 and 48 – 50%, respectively (Tables 5.7 through 5.10), which is higher than for nutrient retention efficiencies realized in the same fish size (600-900g $individual^{-1}$) in traditional ponds (Nhut et al, submitted-a). Reported nutrient retention efficiencies for channel catfish in ponds (Gross et al., 1998; Gross et al., 2000), tilapia in RAS, excepting for P (Eding et al., 2009), trout in race ways (d'Orbcastel et al., 2009b) and shrimp in ponds (Thakur and Lin, 2003) were also lower than the efficiencies realized in this experiment. Higher efficiencies of nutrient retention can be achieved when fish raises from 10 to 900 g per individual.

Of the nitrogen supplied with the feed, 7 % accumulated and 29% was removed in the USB-DR. Only 12% (6.9% final sludge in 6.1% USB-DR + 6.1% effluent – 1% influent) of the nitrogen fed was discharged from RAS + USB with the effluent, compared to 36% from RAS (sludge 12% + effluent 25% - 1% influent) (Table 5.7). The nutrients removed from RAS with the sludge, or from RAS+USB with the sludge accumulating in the denitrification

reactor can be collected and reused through composting (Phung et al., 2009). In consequence, in RAS a higher fraction of the nitrogen supplied with the feed can be re-used as compost than in RAS+USB. However, the amount of nitrogen discharged with the effluent in RAS is more than two times higher than the nitrogen potentially recuperated as compost.

In RAS, 15% of the nitrogen supplied with feed, remained unaccounted. In RAS+USB this was 9% (Table 5.7). The assumption is that this is mainly due to volatilisation of N_2-gas through passive denitrification occurring in the swirl separator and in biofilters, sumps and pipes of each system. (van Rijn et al., 2006) contributed 9 – 21% nitrogen loss to passive denitrification in RAS, which concurs with our results. Nitrogen loss due to passive denitrification in our RAS, from which sludge was removed every 4 hours, was low. Removing sludge less frequently, resulted in unaccounted nitrogen removal percentages of 23.3% for Indian major carp (Adhikari et al., 2014), 65% for African catfish (Bovendeur et al., 1987), 57% for channel catfish (Boyd, 1985) and 36% for shrimp (Thakur and Lin, 2003). The present experiment lasted 56 days, during which time in the trickling tower was not cleaned. This was also the case in a previous experiment, which lasted 207 days, concurring with 25% unaccounted nitrogen loss (Nhut et al., submitted-b).

The USB-DR performed trapping P in sludge (5.4 g P kg^{-1} feed). Incorporation of an USB-DR had no effect on the percentage of dietary P retained in fish biomass, which was on average 23 % in RAS and RAS+USB (Table 5.8). In RAS, all P removed with sludge collected in the swirl separator (40% of dietary P, Table 5.8) can be re-used through composting (Nhut et al., submitted-c). The same most likely holds for the similar amount of sludge accumulating in the USB-DR (41% of dietary P, Table 5.8), making RAS and RAS+USB potentially equally efficient for re-using P not retained in fish biomass for crop production (Da et al., 2015; Phung et al., 2009). Twenty two percent of dietary P in RAS and 27% in RAS+USB (Table 5.8) remained unaccounted. In part, this might be due to sludge accumulation in the biofilters and pipes (van Rijn et al., 2006), which was not quantified in this study. This should be checked in a follow-up experiment, and if containing a substantial amount of nutrients, could also be composted or transferred to the denitrification reactor. The principal advantage of incorporating a denitrification reactor in RAS is that 52 % less P is discharged with effluent (Table 5.8). This reduction concurs with a 70% reduction in water

use (Table 5.11) and a 70% higher P concentration in RAS+USB compared to RAS (Figure 5.3F).

About 36% of feed DM was retained in fish biomass in RAS and RAS+USB ($P > 0.05$) (Table 5.9). In RAS, 22 % of feed DM was discharged as sludge and 1% was discharged with the effluent. In RAS+USB, no sludge was discharged, 0.6% of feed DM was discharged with effluent, and 7% accumulated in the denitrification reactor. The rest of DM fed, 42% in RAS and 56% in RAS+USB were not explained by measurements but was due to respiration by biological degradation and accumulation in the system volume. The apparent DM digestibility of the striped catfish feed was 72% (data not reported), hence the 22.5% of dietary DM collected in the swirl separator in RAS (Table 5.9) represented 80% of the undigested feed. The observed DM loss due to respiration and denitrification in RAS and RAS+USB of 41% ($P > 0.05$, Table 5.9) concurs with (Heinsbroek and Kamstra, 1990) for European eel in RAS, but is higher than reported by (Bovendeur et al., 1987) for African catfish in RAS. The mass balance of COD reveals a similar pattern, with about 50% of the COD in the feed was not retained in fish biomass in RAS and RAS+USB ($P > 0.05$, Table 5.10). In RAS+USB, only 0.5% of the COD in the feed was discharged with effluent, while 37 – 43% was unaccounted, mainly due to accumulation in components of system, respiration and breakdown of organic matter in the systems (Heinsbroek and Kamstra, 1990).

The denitrification reactor in RAS+USB removed 12 g N per kg feed applied, representing 29% of the feed input and 58% of the N not retained in fish biomass (Table 5.7) which is excellent. Meriac (2014), using a conceptually similar RAS+USB system for trout as in this study, removed 48% of the dietary N not retained in fish biomass. The N removal efficiency of a USB-DR can be highly variable, as it is influenced by numerous factors, including sludge retention time, quantity of raw sludge material, quality and quantity of carbon resource, mixing frequency in the reactor, salinity and temperature (van Rijn et al., 2006). The daily production of sludge per kg feed was about 165 COD g (Table 5.10) and 21.5 g N was not retained in fish biomass (Table 5.7), resulting in a COD/N ratio of 7.6 in the USB-DR input. This is considered input ratio for a denitrification reactor is slightly higher than optimal value for complete nitrate reduction to elemental nitrogen (van Rijn et al., 2006). In the USB, 34.4 g COD (Table 5.10) and 12.2 g N (Table 5.7) were removed to ending up in

yield of bacteria biomass production, corresponding to a COD/N ratio of 2.8 of bioavailable waste. This is close to the theoretical COD/N ratio of 2.86 reported by (Henze et al., 1997).

5.4.4. Sustainability indicators

Integrating an USB-DR in RAS resulted in a 70% reduction in water use and a 54% smaller input of $NaHCO_3$ (Table 5.11). The latter was expected as per mole N denitrification produces 1 equivalent alkalinity while nitrification consumes 1.98 equivalents alkalinity (Henze et al., 1997). At the current cost price of $NaHCO_3$ (0.53 \$ kg^{-1} $NaHCO_3$) this results in a saving of 56 \$ US per MT striped catfish produced.

The water use in RAS+USB was 77 l kg^{-1} fish produced, which is very low. Water use in ponds in the Mekong delta is 53 – 158 times higher (Anh et al., 2010; Bosma et al., 2009; Phan et al., 2009). The ASC standard allows a 132 times higher water use for striped catfish production in ponds (ASC, 2012) than the realized water use in RAS+USB. An important advantage of a low water exchange in RAS and RAS+USB is that it reduces risks for contamination (e.g. disease, toxins) and thus improves biosecurity (Nhut et al., submitted-b).

Electricity use for pumping (for water exchange, water recirculating and sludge movement), aeration, stirring of sludge blanket and light was 18% higher in RAS+USB than in RAS. This is slightly less than the increased electricity consumption due to additions of an USB-DR to a tilapia RAS in the Netherlands, where the water was also heated (Eding et al., 2009). The latter was not necessary in the Mekong delta where the water temperature is close to optimal temperature for striped catfish culture. It should be noted that the electricity consumption in this experiment was higher than required, as the biofilters were larger than necessary, operating at an internal flow rate of 41 m^3 per kg feed applied, where a flow rate of 8 – 10 m^3 kg^{-1}feed is sufficient for raising striped catfish in RAS (Nhut et al., submitted-b).

In both RAS and RAS+USB no chemicals and drugs were applied. The average survival during the experiment was 98% (Table 5.6, $P > 0.05$). High mortalities are reported in pond culture, due to 15 types of diseases/syndromes (Phan et al., 2009). Twelve antibiotics and 22 chemicals are commonly used to reduce mortality due to disease (Bosma et al., 2009; Rico et

al., 2013; Rico and Van den Brink, 2014). Producing in RAS and RAS+USB can be part of a strategy to reduce losses due to disease and chemical and antibiotic use in striped catfish culture. Three times less N and 1.6 times less P were discharged per kg fish produced from RAS+USB than from traditional ponds (De Silva et al., 2010). Considering N and P discharge, the RAS+USB performed better than trout in flow through tanks, and channel catfish and shrimp in ponds (d'Orbcastel et al., 2009b; Eding et al., 2009; Gross et al., 1998; Gross et al., 2000; Thakur and Lin, 2003), and was comparable to the discharge from a tilapia RAS integrated with USB (Eding et al., 2009).

5.5. Conclusions and commendations

Striped catfish production and fish quality indicators in RAS+USB and RAS were comparable. With a water consumption of 38 l per kg feed, the NO_3-N concentration was maintained below 100 mgl^{-1} in RAS+USB. The low water exchange facilitates maintaining biosecurity and contributes to minimizing pollution from aquaculture. Because the small volume of waste flows, wastes are easy to collect and treat before discharge. Future research should focus on RAS+USB upscaling to commercial size.

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CHAPTER 6

General discussion

The aim of the thesis was to document improvements in sustainability of striped catfish production through the application of environmentally friendly production methods and waste treatment techniques.

6.1. Sustainability indicators in striped catfish culture systems

To establish a baseline on sustainability of striped catfish production in 3-5 m deep ponds in the Mekong delta, water and nutrient flows in four ± 1 ha-production ponds were monitored during a full production cycle (Chapter 2). The obtained information allowed to calculate 20 sustainability indicators either expressed per kg striped catfish produced or per kg feed consumed. These sustainability indicators, expressed per kg fish produced are summarized in Table 6.1. The same sustainability indicators were calculated for production in flow-through tanks (FT), RAS (Chapter 3), RAS with a denitrification reactor (Chapter 5) and an outdoor RAS-pond trial[3]. In addition, the effect of composting and biogas production from solid waste collected in ponds and RAS on the overall nutrient use efficiency was explored (Chapter 4).

Table 6. 1: Sustainability indicators in striped catfish culture systems.

Indicator	Unit	Pond	FT	RAS	RAS +USB	RAS-pond
Resource utilization efficiency						
Mortality	%	36.3	4.2	6.2	2.8	18.2
Fingerlings	# kg^{-1}fish	1.9	1.6	1.8	3.3	1.5
Feed	kg feed kg^{-1}fish	1.6	1.3	1.3	1.3	1.4
Water use	l kg^{-1}fish	4,900	14,750	146.3	77.1	419
Energy use	kWh kg^{-1}fish	0.1	6.7	13.6	9.6	0.8
Chemical						
Lime ($CaCO_3$)	g kg^{-1}fish	28.2	0	0	0	11.3
Salt (NaCl)	g kg^{-1}fish	15.8	12.0	15.2	14.7	16.4
$NaHCO_3$	g kg^{-1}fish	0	0	43.6	86.3	0
$CuSO_4$	g kg^{-1}fish	0.1	0	0	0	0
Iodine	g kg^{-1}fish	0.4	0	0	0	0
Antibiotic	g kg^{-1}fish	1.5	0	0	0	0.02

[3] The trial was done in a 200 m^2 1.8 m deep tank, in which a moving bed biofilter and septic tank was installed. The recirculation flow was created through aeration and airlifts. The trial was successful, reaching production targets. Because there were no replicates, the results were not presented in peer reviewed manuscripts.

Table 6.1 (continued- 1): Sustainability indicators in striped catfish culture systems.

Indicator	Unit	Pond	FT	RAS	RAS +USB	RAS-pond
Labour	hr $kgfish^{-1}$	0.1	1.4	1.6	1.5	0.1
Fillet percentage	%	35.4	37.8	37.2	37.2	37.8
Color grade	1-3	1.3	1.0	1.0	1.0	1.0
Off-flavor	-	No	No	No	No	No
Nutrient utilization efficiency						
Nitrogen	% feed input	38.3	49.3	48.1	49.7	49.4
Phosphorus	% feed input	14.3	20.0	20.7	24.3	23.2
Dry matter	% feed input	28.5	31.8	31.4	37.4	35.5
COD	% feed input	-	47.0	48.5	50.0	54.1
Waste discharge						
Nitrogen	g kg^{-1}fish	18.5	29.0	13.7	7.0	10.6
Phosphorus	g kg^{-1}fish	16.7	14.5	11.9	8.6	9.7
Dry matter	g kg^{-1}fish	359.6	826.2	348.7	80	86.4
COD	g kg^{-1}fish	-	826.1	199.5	101.2	126.2
Compost						
Available nitrogen	% compost DM	0.8	-	1.4	-	-
Available phosphorus	% compost DM	0.2	-	1.1	-	-
Ash	% compost DM	68.4	-	53.5	-	-
Methane						
Methane	% CH_4 in biogas	46.8	-	52.7	-	-
	L CH_4 g^{-1} VS added	0.2	-	0.3	-	-

FT: flow-through; RAS: recirculating aquaculture system; RAS+USB: RAS integrated with a denitrification reactor; DM: dry matter; VS added: volatile solids in initial RAS-sludge or pond-sludge (Chapter 5). The value in pond is mean of four ponds (Chapter 2). The value in RAS and FT is mean of 2 systems (Chapter 3). The value in RAS+USB is mean of two systems (Chapter 5). The fillet color grade varied from 1 to 3, 1 indicating the highest quality (Chapter 2 and 3). RAS-pond: outdoor pilot RAS with floating moving bed biofilter and septic tank installed in 1.8 deep pond (data from one trial without replication).

Based on the sustainability indicators, RAS+USB had the smallest environmental impact, using less water and discharging less wastes than the other striped catfish production systems studied. Energy consumption in RAS was high, but was considerably reduced moving to a

larger scale in the pilot RAS-pond trial, in which all water movement was created through airlifts.

The highest water use and waste discharge occurred in FT. These systems are no longer allowed under Vietnamese law, mainly due to environmental concerns (MARD, 2014c). Although ponds performed better than FT, survival, water use, chemicals use, antibiotics use, nutrients utilisation efficiency and wastes discharge in ponds could be further improved by applying RAS technology. Upscaling of RAS technology for striped catfish remains a challenge. The RAS-pond pilot system performed equally well on all sustainability indicators in RAS, except for water use and mortality. Considering the full production cycle, 419 l of water was consumed per kg fish produced. Mortality in the RAS-pond was higher than in FT, RAS and RAS+USB, because no pathogen free striped catfish fingerlings were available for stocking and the transport from the hatchery to the RAS-pond by accident happened to be very long, which most likely reduced the condition of the fingerlings at the moment of stocking. The mortality was high during the first month after stocking, but dropped to similar levels as in RAS and RAS+USB during the remainder of the culture period after treatment.

Water use and its consequences

For pond culture of striped catfish, large volumes of pond water are daily replaced through tidal exchange or active pumping. On most farms, influents and effluents are exchanged without treatment. Therefore, pond production and rearing conditions are influenced by the quality of the intake water, while the discharge of pond effluents affect the water quality in the river. More than 90% of the daily water exchange in ponds is used to maintain water quality, the remainder being used to compensate water losses due to evaporation and seepage. In our study, upstream ponds used much more water than downstream ponds, in spite of the fact that farms in the latter location depend more on pumping than on tidal/gravitational exchange.

The water quality in the Mekong river changes during the year. In addition, there is always a risk that the river carries high loads of nutrients, residual chemicals (Toan et al., 2013) and antibiotics (Rico et al., 2013; Rico and Van den Brink, 2014) originating from neighboring aquaculture or agriculture operations. Particularly, during the rainy season, runoff from large expanses of agricultural land in the upstream catchment area, negatively affects water quality.

The Mekong river carries heavy metals (Minh et al., 1997), persistent organic pollutants (Minh et al., 2007), pesticides and agriculture wastes (Toan et al., 2013). Intrusion of salt water during the dry season occurs today deeper inland than before, in part due to sea level rise (Anh et al., 2014; Nguyen and Savenije, 2006) and could reduce striped catfish production in the future (Renaud and Kuenzer, 2012). The Vietnamese government, recognizing lack of fresh water as a treat to the striped catfish industry, therefore prioritized the development of a semi-closed farming system (MARD, 2015). Production in RAS is considered a good option to reduce water use. Reliance on high water exchange increases the risk for horizontal disease transmission (Anh et al., 2010; Madsena et al., 2015; Phan et al., 2009). Although treatment of influent water to reduce, for instance trematode, infection in the striped catfish fingerling ponds has been proposed (Madsena et al., 2015), the large volumes exchanged in grow-out ponds make treatment of intake water costly and labor intensive. Treatment of influent and effluent water to and from striped catfish grow-out ponds is often propagated (Anh et al., 2010; ASC, 2012; BMP, 2009; Phan et al., 2009), but it is rarely applied. Applying RAS, the water use per kg fish produced was reduced 38 times compared to ponds. By exchanging smaller water volumes it becomes possible to remove or decrease pathogens from farm influents and effluents, reducing disease related mortality. Considering the fact that in Vietnam experts in RAS are few and investment costs are high, interest in recirculation technology in Vietnam is small (Ngoc et al., 2016a; Ngoc et al., 2016b). Treating influent water in a large reservoir that acts as a stabilization pond and passing effluents through a sedimentation pond was suggested as a cheaper and more practical solution (Anh et al., 2010), but is also not adopted, mainly because land along the Mekong river is expensive.

Mortality, chemical and antibiotic use

Pathogen free striped catfish fingerlings are practically unavailable in Vietnam. This in part contributes to the frequent occurrence of diseases in grow-out ponds, especially shortly after stocking (Phan et al., 2009). The highest amounts of antibiotics and chemical are applied to ponds at the start of the culture period (Phan et al., 2009). The daily water exchange is a route for re-infection, also after treatment, and disease reoccurrence is frequent, as also observed in our ponds study (Chapter 2). Diminishing water exchange might help in reducing disease related mortality. In RAS, disease related mortality occurred only during the first month of the grow out period and then stopped. In ponds, the mortality was highest during the first

months of the culture period, but during the rest of the culture period small numbers of dead fish were collected daily. The total disease related mortality in RAS was 6 times smaller than in ponds. According to Ngoc et al. (2016b) fingerlings represent about 10% of the total variable cost. Considering that in ponds the total fish mortality was 36 %, this represents 3.6% of the variable costs or a loss of 16,000 $US per ha per production cycle. Other variable costs include feed, transportation, chemicals, antibiotics and labor. Higher survival in RAS would reduce fingerling costs by 13,600 US$ (Chapter 3). By integrating a denitrification reactor in RAS, the cost for $NaHCO_3$ and NaCl would be further reduced by 50% (Chapter 5). About 20 antibiotics and 12 chemicals are commonly applied in striped catfish ponds (Rico et al., 2013). Per kg fish produced, the applied quantities are smaller than for other cultures (Chapter 2), however, because culture density in ponds is very high, and the amounts applied per unit surface area are high. Chemical and antibiotic application come with environmental risks (Rico and Van den Brink, 2014) and the possibility of development of antibiotic resistance (Rico and Van den Brink, 2014; Sarter et al., 2007b).

Energy consumption

In striped catfish pond culture, pumping is only needed when tidal water exchange is insufficient to maintain water quality and is thus relatively cheap. In RAS, the highest amount of electricity was consumed for pumping water over the trickling filter and for aeration of the moving bed reactor (Chapter 3). Because the cost for aeration in a moving bed reactor is less than for pumping water over a trickling filter, we recommended to use moving bed reactors for biofiltration in striped catfish RAS. In RAS, aeration in the moving bed biofilter kept the dissolved oxygen above 2 mg O_2 l^{-1} (Chapter 3) which was higher than in ponds (Chapter 2). Systems with moving bed reactors can be kept shallow, which is advantageous for striped catfish because the fish is then always close to the surface where it takes oxygen from the air (Lefevre et al., 2011b) and releases CO_2. In a pilot system with a moving bed biofilter and without trickling filter, the electricity consumption was 0.8 kWh kg^{-1} fish produced (Nhut et al., 2015). If biogas would be produced from the sludge collected in RAS then close to half of the energy needed for biofiltration can be recuperated (Chapter 4).

Solid waste reuse as valuable products

Solid waste collected in the RAS is a valuable resource to produce compost or bio-methane gas. Producing compost is cheap and simple, making it a good option to re-use part of the

nutrients otherwise lost during culture. In the Mekong delta, rice straw is available in large quantities, and farmers often burn it. Thus, using rice straw for making compost from solid wastes collected during striped catfish culture is a logical thing to do. The value of compost can compensate about 50% of the pumping costs in RAS. In addition, it can partially replace the huge amounts of inorganic fertilizer applied to rice fields and gardens in the Mekong delta (Hach, 2012). In addition, replacing inorganic fertilizer partially with compost would considerably reduce greenhouse gas emissions from rice farming (Favoino and Hogg, 2008). Methane yields from solid organic aquaculture wastes are small (Chapter 4), which is also the case for striped catfish production. So the amount of electricity produced is small (see section on energy consumption). However, RAS-sludge can be easily collected, so in cases where composting is not an option it can be considered. The ash remaining after digestion could still be a source of phosphorous.

Fish quality

Vietnam exported striped catfish already to more than 130 countries. The standard export market requires white and lean fillets that are pathogen free without traces of residual chemicals and antibiotics (Anh et al., 2010). Processing and exporting firms check striped catfish quality at farm gate. Criteria include fillet color, fillet dress out weight percentage, no traces of micro-microsporidian in meat, average body weigh equal to or above 700g, uniformity of individual weight, absence of off flavor and no traces of forbidden residual chemicals and antibiotics. Based on these criteria the price is negotiated. Fish not meeting export criteria fetch a lower price and can still be sold locally. Fish quality is considered a key indicator of economic sustainability (Verreth and Oberdieck, 2009). The quality of striped catfish is closely related to pond management and culture technology. For instance, the high water exchange during pond culture is believed to contribute to a high portion of white fillets in the population (Anh et al., 2010; Phu et al., 2014). A low dress out weight and high fat body content reduces fillet yield and increases the amount of waste remaining after processing. If not processed before disposal, high amounts of N, P, DM, BOD and COD contribute to pollution resulting from farming (Anh et al., 2010). The quality of striped catfish produced in RAS (Chapter 3) was better than in ponds (Chapter 2). In ponds, the quality of striped catfish produced is less predictable, as options to control water exchange and water quality are limited (Phu et al., 2014). In RAS, the farmer can control water quality making the farming outcome more predictable. It should be noted that in the flow through

systems (FT) a similar product quality was achieved as in RAS (Chapter 3), but the high water consumption ($14 m^3$ kg^{-1}fish) and waste discharge, makes this culture system not a sustainable option for the future.

Feed and nutrients utilization efficiency

Feed accounted for 87-91% of the total variable cost in striped catfish pond culture (Ngoc et al., 2016b). During grow out, diets containing 22 – 30 % protein are used, which is lower than for most other fish species produced in aquaculture. The average feed conversion ratio of the industry of 1.86 (Bosma et al., 2011; Phan et al., 2009) is high compared to carnivorous fish species, but in the same range as for omnivorous species and better than for crustacean culture (Pahlow et al., 2015). Using similar feeds in pond and RAS (Chapter 3), the feed conversion ratio in RAS was 19% lower than in ponds (Chapter 2). Most likely, by developing RAS feeds there is room to improve the feed conversion further.

Waste discharge

The amount of waste discharged per kg fish produced increased with the amount of water used (all Chapters): discharge of nutrients was highest in the FT systems and lowest in the RAS+USB. In ponds, 30-34% of nitrogen and 29-37% of dry matter supplied through feeding volatized into N_2 and CO_2, respectively, making them intermediate between the FT and the RAS for waste discharge. The use of sedimentation ponds (Anh et al., 2010; Anh and Mai, 2009; ASC, 2012; BMP, 2009), turning pond sludge into compost (Phung et al., 2009), discharge the sludge directly to gardens (Da et al., 2015), or anaerobic digestion of the sludge for biogas production (Chapter 4) or denitrification (Chapter 5) all have been suggested to diminish discharge of nutrients from striped catfish ponds. Because water volumes exchanged with the river are large, a large sedimentation pond is needed, which is expensive considering land prices along the river. In addition, most farmers would need to invest in extra pumping capacity, which is costly to install and operate. Therefore, the best option to minimize sludge discharge is to grow striped catfish in RAS+USB. Whether sludge collected from the USB reactor will also produce good quality compost, needs further research. It can be considered an advantage that once the sludge is collected, the scale of processing facilities can be adjusted to farm size.

6.2. Water quality

The water quality in the ponds, FT, RAS and RAS+USB is summarized in Table 6.2.

Overall, water quality in ponds was worse than in RAS. For instance, the oxygen concentration in FT, RAS, RAS+USB and RAS-Pond was much higher than ponds, and concurred with less disease related mortality, faster growth and better feed conversion ratios. Although production volumes of striped catfish are high, insight in optimal water quality for striped catfish grow-out is still limited, especially about interactions between different parameters, and more research on this aspect is highly recommended.

Table 6. 2:Summary of water quality in striped catfish culture systems

Parameter	Unit	Downstream pond	Upstream pond	FT	RAS	RAS+USB	RAS-pond
Temperature				31.7	28.7	28.1	30.1
- morning	^{o}C	30.4	29.5				
- afternoon	^{o}C	31.6	30.9				
pH				7.5	7.7	7.3	7.1
- morning	-	6.3	6.2				
- afternoon	-	6.6	6.7				
Oxygen				3.2	5.4	4.4	3.7
morning							
- 1m depth	$mg\ l^{-1}$	1.5	1.9				
- 2m depth	$mg\ l^{-1}$	1.2	1.6				
afternoon							
- 1m depth	$mg\ l^{-1}$	1.9	2.2				
- 2m depth	$mg\ l^{-1}$	1.5	1.8				
Transparency							49.8
- morning	cm	24.6	30.2				
- afternoon	cm	24.2	28.2				

Table 6.2 (continued- 1): Summary of water quality in striped catfish culture systems

Parameter		Downstream pond	Upstream pond	FT	RAS	RAS+USB	RAS-pond
Total Nitrogen	mg l^{-1}	9.9	7.1	4.9	58.8	65.2	53.7
TAN	mg l^{-1}	1.4	1.2	1.5	1	0.33	6.4
NO_2-N	mg l^{-1}	0.3	0.1	0.2	0.7	0.47	1.1
NO_3-N	mg l^{-1}	0.4	0.4	3.2	52.8	62.8	39.1
H_2S	mg l^{-1}	0.2	0.1	0.01	0.11	0.24	0.02
PO_4-P	mg l^{-1}	0.4	0.4	0.5	20.7		3.2
TP	mg l^{-1}	2.4	1.5	1.7	24.1	24.3	12.8
Alkalinity	mg l^{-1}	61.6	51.1	246.7	134.8	113.7	85.6
CO_2	mg l^{-1}	42.4	30.9	14.3	17.5		19.1
TC	mg l^{-1}	28.9	30.4	78.2	58.8		
TOC	mg l^{-1}	8.4	6.3	3.3	16.7		
TSS	mg l^{-1}	80.8	49.3	6.6	31.9	65.7	58.3
Chlorophyll-a	µg l^{-1}	114.3	100				11.3
COD	mg l^{-1}	19.4	19.4	8.8	32.2	31.8	25.2
BOD_5	mg l^{-1}	15.4	15	5	16.6		29.1
Salinity	ppt	0.4	0.06	0	2.6	0.4	1.3

FT: flow-through system; RAS: indoor recirculating aquaculture system; RAS+USB: indoor RAS integrated with a denitrification reactor; RAS-pond: outdoor pilot RAS with floating moving bed biofilter and septic tank installed in 1.8 deep pond (data from one trial without replication).

6.3. Nutrients mass balances in systems

By making nutrient mass balances on DM, N, P and COD[4] for the ponds, FT, RAS and RAS+USB (Chapter 2, Chapter 3 and Chapter 5) nutrient flows through different production systems could be compared and sustainability indicators calculated. Based on these data, the impact of production system on pollution and sustainability could be quantified. For instance, the fraction of nutrients in influents and effluents were high in the ponds and FT, while negligible in recirculation systems. However in RAS systems, more nutrients volatilize contributing to greenhouse gas emissions. Comparing effects of the different types of nutrient losses on global or regional scales was outside the scope of this thesis, but also needs further research. The detailed data sets provided in this thesis, provide a good basis to contribute to broader studies on sustainability, for instance through life cycle analysis (Bosma et al., 2011).

[4] COD mass balance was calculated for RAS, Flow through and RAS+USB, excluding for traditional ponds in this thesis.

6.4. Recommendations for future research

The thesis research showed that recirculation technology reduces the environmental impact of striped catfish production at farm level. By shifting from pond systems in which water quality is maintained through water exchange to closed systems based on RAS technology, striped catfish will become one of the most sustainably produced aquaculture products, satisfying consumer and market demands for environmental responsible produced farmed fish. In addition, the low water consumption in RAS would allow the industry to adapt to the anticipated future reduction in year round freshwater availability in the Mekong delta of Vietnam, caused by climate change. Currently, about 5000 ha of 4-6 m deep ponds are used for grow-out striped catfish production in the Mekong delta. These ponds are high value assets. Therefore, the industry is interested in developing RAS in deep pond first. However, developing RAS technology for 4-6 m deep pond is difficult, because it is expensive to create the water movement required for collecting sludge and maintaining water quality, while minimizing water use. RAS in shallow ponds or tanks would require much less energy to concentrate and remove sludge and maintain water quality, and therefore, is preferred above RAS in 4-6 m deep ponds. If and how the striped catfish industry and Vietnamese government can mobilize the resources necessary to change from deep to shallow ponds is a key question to be addressed with priority.

Developing policies supporting and facilitating adoption of RAS technology for striped catfish production in the Mekong delta should be considered. Striped catfish producers recognize that RAS technology will make farming success more predictable and bio-secure. However, besides high investment costs, lack of education and experience with recirculation technology and high energy consumption, while consumers are not willing to pay a premium price for sustainably produced striped catfish make that few producers believe the industry will adopt RAS technology quickly (Ngoc et al., 2016a). The Vietnamese government is more convinced, and already approved research and development programs for striped catfish culture in RAS (MARD, 2014b). The government could play an intermediate role in promoting sustainably produced striped catfish as a quality product deserving a premium price, by linking producers, processors and retailors in a platform to work with NGOs and governments to develop labels informing consumers.

The RAS technologies tested in this thesis project were only first trials, copying systems successfully applied with other fish species. The filter units installed and water flows applied were larger than required for striped catfish culture. Although production in the trials was satisfactory, energy and labor requirements were high. More research is now needed to adapt RAS technology to the species. Important to consider is domestication of striped catfish to RAS environments and development of genetically improved breeds. This effort is linked to the development of disease and pathogen free production lines of fingerlings to stock in grow-out ponds. Stocking healthy fingerlings in RAS will considerably reduce disease related mortality, and thus make the application of antibiotics and chemicals sporadic events compared to the common use today throughout the industry. Once the sector succeeds in establishing bio-secure striped catfish production in RAS, attention can also be given to fish welfare and product quality, important issues linked to consumer acceptance. Topic related to fish welfare include continuous provision of optimal water quality, noise and vibration free culture environments, and feeding and housing conditions which minimize stress. This also includes the development of RAS feeds for striped catfish, fulfilling fish nutrient and energy requirements while minimizing pollution. Finally, the development of finishing diets, to influence for instance texture, taste and DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) content (Bell et al., 2004) of fillets could also be considered.

6.5. Main conclusions

From a sustainability point of view, striped catfish culture in ponds compared well to other important aquaculture species (Chapter 2). Nevertheless, water, chemicals and antibiotics use, survival, and the amounts of waste discharged could be further reduced through recirculation and treatment of solid wastes (Chapters 3, 4 and 5). All RAS systems investigated were shallow systems. To merge recirculation technology into pond systems the 4-6 m depth makes it costly to generate flows required to pass, concentrate and treat organic wastes in the water treatment units that are part of the RAS. Abandoning deep ponds and producing in shallow ponds with biofiltration and sludge collection and treatment will be costly and thus challenging. However, if the necessary investments could be made, in combination with a further fine-tuning and cost reduction, producing striped catfish in RAS might become as cost effective as producing in deep ponds. Additional advantages of producing in outdoor low water exchange systems, is that pollution will become minimal and that stocking disease free fingerlings will become economically advantageous.

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APPENDICES

Summary

Acknowledgements

Curriculum vitae

Training and supervisor plan

SUMMARY

Summary

Intensifying aquaculture production while meeting societal and consumer demands for sustainable production is challenging. Technically it is possible to culture fish without discharging nutrients, but this is most often not done due to the costs involved. The aim of this thesis was to document possible improvements in sustainability of striped catfish production through the application of recirculation technology and waste treatment techniques. To be able to document improvements in sustainability, a set of sustainability indicators which can be measured on all types of farm was compiled. Twenty sustainability indicators were measured including environmental, economic and social dimensions, and considering the use of fingerlings, water, diesel oil, electricity, labor, chemicals and antibiotics. Also, indicators related to nutrient utilization efficiencies and wastes discharge were monitored. In addition, a sampling scheme, necessary to measure organic matter, nitrogen, phosphorous and chemical oxygen demand (COD) mass balances during a full production cycle, applicable in different production systems, was developed.

Striped catfish is the most important aquaculture species in the Mekong delta of Vietnam. It is produced in 4-6 m deep ponds along the Mekong river. During this thesis project, besides measuring sustainability indicators and nutrient mass balances in ponds, striped catfish production in flow-through tanks and in recirculating aquaculture systems (RAS) with or without denitrification, was monitored during a full grow-out production cycle. In addition, the yields of methane and compost from solid wastes collected from ponds and RAS without denitrification were compared. By measuring or calculating the effects on sustainability indicators and nutrient mass balances the room available to improve sustainability of striped catfish production was explored.

In ponds, water use per kg fish produced was 2.8 m^3 in downstream and 7.1 m^3 upstream ponds. This water exchange rate was sufficient to maintain favorable water quality during production. However, daily water exchange with the river is also a route for entrance of pollutants or diseases. To contain disease related mortality, 0.1 to 0.2 g antibiotics were applied per kg fish produced. Still, mortality was 24 – 49%, with the bulk of mortality occurring during the first months of the production cycle. Considering pond diets with 26 –

30% protein were used, the realized dry matter utilization efficiency of 28 – 30% was good. For nitrogen, the utilization efficiency was 40 – 44 % and for phosphorous 17.6 – 17.7 %. Per kg fish produced, the combined discharge through exchange water, drainage water and sludge removal was 357 – 415 g dry matter, 19.8 – 20.1 g nitrogen and 17.0 – 17.7 g phosphorous. Due to denitrification and fermentation occurring at the bottom in the 4 – 6 deep ponds, 29 – 37 % of the dry matter (DM) and 30 – 34 % of nitrogen applied with the feed volatilized **(Chapter 2)**. In flow-through tanks (FT) and RAS, these losses were reduced by trapping solid wastes from the water flow leaving the fish tank in the swirl separator (**Chapter 3**). Better water quality in RAS and FT concurred with better survival (93-96%) and feed conversion efficiencies (FCR 1.3) than in ponds (FCR 1.6). Water consumption in RAS was 100 and 19 – 49 times smaller than in FT and ponds, respectively. Similar amounts of solid waste were trapped in the swirl separators of RAS and FT, but in RAS, a considerable fraction of solid waste not trapped in the swirl separator accumulated under the biofilter, where passive denitrification occurred. Therefore, nitrogen, DM and COD discharge in RAS were significantly lower than in FT. Overall, sustainability indicators were better in RAS than in ponds. In addition, the solid waste collected from the swirl separator in RAS or from the bottom in ponds, was used to produce either compost or methane gas (**Chapter 4)**. Although on a weight basis six times more sludge was collected from ponds (1200 g dry matter per kg fish produced) than from RAS (200g dry matter per kg fish produced), the amount of organic matter collected per kg fish produced was similar in RAS and ponds. The quality of compost produced from RAS-sludge was better than for compost based on pond-sludge. Considering methane (CH_4) production, 125 and 201 L CH_4 per kg COD was produced from pond and RAS sludge, respectively. The amount of methane produced was comparable with other types of aquaculture, but was lower than for example for pig manure.

Using solid waste collected in the swirl separator as carbon source in a denitrification reactor, the goal was to further reduce water use while maintaining the nitrate concentration below 100 mg per L **(Chapter 5).** For each kg of feed applied in RAS, 11.2 g NO_3-N, 12.3 g total nitrogen (TN), 145 g COD and 145 g DM was removed in the denitrification reactor, while 53 g $CaCO_3$ alkalinity was produced. The latter allowed to reduce the amount of sodium bicarbonate required to maintain the pH in RAS. The water use in RAS with denitrification

dropped to 38 L per kg fish produced, while the NO_3-N concentration in the fish tank remained below 100 mg per L. Adding a denitrification reactor to RAS did not affect fish growth, nutrient retention efficiencies and the quality of the fish fillets produced. In consequence, integrating a denitrification reactor in RAS further improved the sustainability of striped catfish farming.

Overall (**Chapter 6**), from a sustainability point of view, striped catfish culture in ponds compared well to other important aquaculture species. Nevertheless, water, chemicals and antibiotics use, survival, and the amounts of waste discharged could be further reduced through recirculation and treatment of solid wastes. All RAS systems investigated were shallow systems. To merge recirculation technology into pond systems the 4-6 m depth makes it costly to generate the flows required to move wastes towards water treatment units of the recirculation system where it is concentrated and can be collected for further treatment. Abandoning deep ponds and producing in shallow ponds with biofiltration and sludge collection and treatment is however costly. Further cost reduction in outdoor RAS systems is possible through the development of special RAS feeds and striped catfish breeds, a further reduction of the feed conversion ratio to below 1.0, shortening the grow-out period, and valorization of compost or energy produced on solid wastes. Combined, these improvements could make production of striped catfish in RAS more cost effective than producing in deep ponds. Additional advantages of producing in outdoor low water exchange systems, is that pollution will become minimal and that stocking disease free fingerlings will become economically advantageous.

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Without tremendous contributions of dedicated supervisors, colleagues, friendship, sponsors and my family, this thesis would never have been completed. Huge works during six years for this research were achieved by your contributions. I am truly grateful to all of you. I particularly would like to thank Prof. Johan Verreth, Dr. Marc Verdegem, Ir. Ep Eding, Dr. Roel Bosma and Dr. Nguyen Van Hao who helped me developing knowledge on recirculation technology and apply it with the ambition to improve the sustainability of striped catfish culture. Extra thanks go to Prof. Johan Verreth who allowed me to do this work in the context of a PhD study at the Wageningen University. The PhD study on striped catfish culture was not easy because it involved numerous dimensions, which were for this thesis mainly technical, but while also considering economic and social dimensions.

Each experiment in this thesis took a long time, with frequent and labor intensive sampling, which was made possible with the help of dedicated research assistants. Thank-you, Mr. Nguyen Hong Quan, Mr. Le Ngoc Hanh, Mr. Nguyen Van Huynh and Mr. Duong Dinh Nam and the lab-mates at the Research Institute For Aquaculture No 2 who all supported my work. Specially thank to my supervisors Dr Marc Verdegem and Ep Eding who went to Vietnam frequently to support me with techniques and data analysis during the different experiments. Also, I will never forget the contributions of Mr Rene Remmerswaal from the Aquaculture Consultancy & Engineering (ACE) company for participating in the design of the outdoor recirculation system trials.

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CURRICULUM VITAE

About the author

Nhut Nguyen was born on 30th June, 1976 in Camranh City, Khanh- Hoa province, Vietnam. He obtained a BSc degree in aquaculture in Nhatrang University of fisheries in 2000.

His scientific career began in 2000. He lives in Ho Chi Minh city, Vietnam where he works in the field of aquaculture at the Research Institute for Aquaculture No2 (RIA2), Vietnam. He obtained his MSc degree in aquaculture at Ghent University, Belgium in 2005. Following an exchange student program between the Wageningen and the Ghent University, he studied several courses at the Wageningen University, the Netherlands. Specially, the recirculating aquaculture system (RAS) course was interesting. He did his MSc thesis on the "Design and performance of RAS integrated with a denitrification reactor for African catfish culture" at the Aquaculture and Fisheries Group (AFI) of Wageningen University. He believed that RAS could be adopted to improve sustainability of striped catfish culture in the Mekong Delta, Vietnam. So, he started in 2010 to work on the SuPa project for developing recirculation technology for striped catfish culture when he also received a sandwich PhD grant from Wageningen University.

During his PhD, he participated by oral presentations at international conferences. Now, he is one of key researchers in RIA2. Also, he has been carrying out national projects on RAS for eel culture, shrimp production systems, shrimp (white shrimp and tiger shrimp) domestication, and fresh water prawn and striped catfish production systems in the Mekong delta, Vietnam. He is a vice-director of Experimental Biology Department of RIA2.

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List of publications

Referred scientific journals

Nhut, N., Hao., Bosma, R.H., Verreth, J.A.V., Verdegem, M.C.J., Eding, E.H. Nutrient mass balance, water quality and water use in striped catfish (*Pangasianodon hypophthalmus, Sauvage, 1878*) pond culture: down-stream versus up-stream. Aquaculture Environment Interactions, *under revision for re-submission*.

Nhut, N., Quan, N.H., Hao, N.V., Verreth, J.A.V., Verdegem, M.C.J., Eding, E.H. Nutrient mass balances, water quality and water use of striped catfish (*Pangasianonodon hypophthalmus,* Sauvage, *1878)* in flow-through and recirculation systems. Aquaculture, *under revision to re-submision.*

Nhut, N., N.V. Hao., Bosma,R.H.,Verreth, J.A.V., Verdegem, M.C.J.,Eding.E.H. Biogas production and compost composition of sludge from striped catfish (*Pangasianodon hypophthalmus, Sauvage, 1878*) ponds and recirculating systems. Clean Production, *Submitted.*

N. Nhut., N.H.Quan., N.V. Hao.,Bosma, R.H., Verreth, J.A.V., Verdegem, M.C.J., Eding, E.H.Effect of an upflow-sludge-blanket denitrification reactor on environmental sustainability of striped catfish production (*Pangasianodon hypophthalmus, Sauvage, 1878*) in recirculating aquaculture systems. *In preparation for Aquaculture*.

Conference proceedings and abstracts

Nhut Nguyen, Hao Nguyen Van, Roel Bosma, Ep Eding, Johan Verreth, Marc Verdegem. Nutrients and water budgets in pangasius, Pangasianodon hypophthalmus, ponds in Mekong delta, Vietnam. In book of abstracts of International Fisheries Symposium - IFS2012 on 6-8th December 2012 in Cantho, Vietnam.

Nhut Nguyen, Hao Nguyen Van, Roel Bosma, Ep Eding, Johan Verreth, Marc Verdegem. Waste production from pangasius, Pangasianodon hypophthalmus, raised in ponds, flow-through and recirculating aquaculture systems. In book of abstracts of Asian Pacific Aquaculture (APA13), Hochiminh city, Vietnam on 10 -13th December 2013 in Hochiminh city, Vietnam.

Nhut, N., Quan, N.H., Hao, N.V., Verreth, J.A.V., Eding, E.H., Verdegem, M.C.J., 2015. Effects on sustainability of pangasius (Pangasianodon hypophthalmus) production in flow-through and recirculation compared to pond farming. Conference: European Aqua-culture Society, Rotterdam, The Netherlands, 20–23 October 2015.

N. Nhut., N.H.Quan., N.V. Hao.,Bosma, R.H., Verreth, J.A.V., Verdegem, M.C.J., Eding, E.H. Effect of an single sludge denitrification reactor on growth and environmental sustainability of striped catfish production (Pangasianodon hypophthalmus, Sauvage, 1878) in recirculating aquaculture systems. In book of abstracts of Asian Pacific Aquaculture (APA16) on 26 -29th April 2016 in Surabaya, Indonesia.

Training and supervision plan

Education and training	ECTS
The basic package	**3**
WIAS introduction course:	
Ethics and philosophy in life sciences	
Scientific exposure	**9.7**
Participation & 3 oral presentations in international conferences:	
-International Fisheries Symposium -IFS2012 on 6-8th December, 2012 in Cantho, Vietnam (1 oral presentation)	
-Asian Pacific Aquaculture (APA13) on 10 -13th December 2013 in Hochiminh city, Vietnam (1 oral presentation)	
-Asian Pacific Aquaculture (APA16) on 26 -29th April 2016 in Surabaya, Indonesia (1 oral presentation)	
-WIAS science day, Wageningen, the Netherlands, in 2015 (1 poster)	
Kick-off meeting SUPA project on 18-19th January 2010	
-1st, 2nd, 3rd , 4th SUPA progress meeting in 2011, 2012, 2013 , 2015 (4 presentations)	
-1st Vietnamese pangasius research day on 5th December 2012 (1 presentation)	
In-depth studies	**6**
Recirculating aquaculture system	
Environmental impact assessment of livestock systems	
Statistic for life sciences course	
Professional skills support courses	**12**
Getting papers published peer reviewed-journals	
Project and time management	
Preparing own PhD research proposal	
Building and running a large scale of RAS for striped catfish culture	
Didactic skills training	**4**
Supervision of one MSc major student	
Preparing and tutorship of cyprinid fish & physiology of reproduction course	
Education and training total	**34**

Colophon

The research described in this thesis was financially supported by the bilateral Vietnam-Netherlands Public Private Partnership Fisheries coordinated by the ministry of Economic affairs, Agriculture and Innovation (EL&I) from the Netherlands, the Vietnamese Ministry of Agriculture and Rural Development (MARD) and the WIAS Graduate School of Wageningen University. The printing of this thesis was financially supported by Wageningen University.

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